THE ZONATION OF COASTAL DUNE PLANTS IN RELATION TO SAND BURIAL, RESOURCE AVAILABILITY AND PHYSIOLOGICAL ADAPTATION

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ABSTRACT

When considering the large amount of work done on dune ecology, and that a number of the classical ecological theories originate from work on dunes, it is apparent that there remains a need for physiological and mechanistic explanations of dune plant phenomena.

This thesis demonstrated that in the extreme coastal environment dune plants must survive both high rates of burial (disturbance), and low nutrient availability (stress). The ability of four species to respond to these two factors corresponded with their position in a vegetation gradient on the dunes.

A low stem tissue density was shown to enhance the potential stem elongation rate of buried plants, but reduced the maximum height to which a plant could grow. Such a tradeoff implies that tall light-competitive plants are able to survive only in stable areas, while burial responsive mobile-dune plants are limited to areas of low vegetation height. This stem tissue density tradeoff was suggested as the mechanism determining the zonation that species show within the dune vegetation gradient present at various sites in South Africa.

Finally, detailed investigations of dune plant ecophysiology found that: 1) The resources used in the response to burial derive from external sources of carbon and nitrogen, as well as simple physiological and physical mechanisms of resource allocation. 2) The leaves of dune plants were found to be operating at one extreme of the photosynthetic continuum; *viz* efficient use of leaf nitrogen at the expense of water loss. 3) Contrary to other ecosystems, the environmental characteristics of dunes may allow plants to occupy a high disturbance, high stress niche, through the maintenance of lowered competition. 4) At least two mobile-dune species form steep dunes, and are able to optimise growth, on steeper dunes, such that they have to grow less in response to burial than plants that form more shallow dunes.

In this thesis, it was shown that the link between the carbon and nitrogen economies of dune plants was pivotal in determining species distributions and survival under extreme environmental conditions. As vast areas of the world's surface are covered by sand dunes these observations are not just of passing interest.

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Chapter 1

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“By burying the past, the dune offers to plant life a world for conquest, subject almost entirely to existing physical conditions.” pp. 96, Cowles (1899)

What determines plant growth?

A typical kilogram of plant tissue is made up of roughly 810g H_2O, 88g C, 10g N, and by comparison, a relatively small mass of other essential elements: Ca, K, Mg, and P (Foulds, 1993; Schlesinger, 1991; Shipley and Vu, 2002). The environment and the physiology of the plant limit the acquisition of these primary building blocks of tissue. Consequently, the production of new biomass and the fitness of a plant are largely determined by the availability of these three components. Characteristic of this environment is the need for a high rate of carbon fixation for the plant to produce sufficient growth to survive burial by sand. The coastal dune environment has an unusual combination of a high environmental demand for carbon fixation and high availability of water, but a soil with extremely low nitrogen content. For this reason, the dynamics of plant carbon fixation and the interrelationship with nitrogen in the abiotic and biotic components of the coastal dune ecosystem are of great interest.

What is known about dune ecosystems?

Dune ecosystems have been studied in great detail since the turn of last century (Cowles, 1899). Succession, the change of vegetation over time, was particularly apparent in this ecosystem (Oosting and Billings, 1942). The study of succession has led to a large amount of work on the autecology and synecology of lacustrine and coastal dune ecosystems. However, a number of physiological and ecological issues have not been addressed:

- A fundamental “plant’s eye view” of burial remains to be described. A detailed description of the spatial heterogeneity, periodicity, and the short- and long-term nature of burial has not been determined for a species or site.
- There is a need for a synthesis of the nutrient, osmotic, and water dynamics of coastal dunes. In particular estimates of the ecosystem-level pools, flows, and biological uses of these factors require more attention.
- A number of current hypotheses explain dune species’ zonation and succession as a result of important environmental gradients (e.g. burial: Maun and Perumal, 1999; salt spray: Wilson and Sykes, 1999; mycorrhizae in combination with plant soil pathogens: van der Putten *et al.*, 1993 and Little and Maun, 1996). One suggestion is that burial acts as a filter for species, and is a major cause of zonation on coastal and lacustrine dunes. However, the general adaptations allowing this to occur have had less attention (but see Dech and Maun, 2006 and Maun, 1998). Species’ zonation is thought to form when a species has an ability to survive one extreme of an environmental gradient but not another. These inverse trait correlations underpin much of our modern understanding of zonation and succession (McCook, 1994), but this idea has not been applied to coastal dune zonation. Related to this idea, the observed burial responses of species provide only half of the zonation explanation: an unknown factor must prevent coloniser species from dominating the stable-dunes. Soil pathogen complexes were suggested as an explanation of this aspect of dune zonation (van der Putten *et al.* 1993).
- Many studies have shown burial or nutrient dynamics to be important in coastal dune ecology, but only one, by Zhang (1996) investigates the interaction between these two factors for *Cakile edentula* in a greenhouse. Thus, there is a need for a study of the *in situ* responses of dune species to these two factors in combination. More broadly, there is need for the study of plant responses to the interactions between environmental factors. These types of studies are more likely to explain dune zonation than single factor studies.
- Species may have different effects on soil nutrient content. In particular, nitrogen-fixing species may change local nutrient dynamics in this nitrogen-limited ecosystem. The effect of nitrogen-fixers on other species remains to be

investigated for the South African coastal dune ecosystem (but see Shumway, 2000, for an example of such a study in another region).

- The physiological explanations of dune plant “reactive” growth in response to burial and nutrient addition remain unclear. The quantitative contribution of the numerous suggested adaptations allowing plants to survive burial have not been compared in single experiments. A holistic view of the carbon and nitrogen sources of growth after burial is needed.
- The low aboveground biomass of many coastal dune plants has generally not been considered as an adaptation (Ripley and Pammenter, 2004a). This trait could potentially explain aspects of the fast growth rates of these species, under persistently low nutrient conditions.
- Implicit in many studies of plant growth, subsequent to burial, is the assumption of vertical growth being optimal. Indeed, vertical growth is the only parameter measured. However, growth normal to the dune surface is theoretically the shortest growth necessary to maintain the shoot above the sand surface, and thus optimal growth depends on the slope of the dune surface. There is a need for a study of plant growth geometry in relation to this problem, as optimal growth will minimise the plant’s use of valuable carbon and nitrogen resources.

All of these issues are centred on the growth and survival of plants in this ecosystem. The need for high growth, with limited resources, is first described, and then hypotheses are tested, linking carbon and nitrogen physiology.

As the coastal ecosystem is moderately diverse, this study has focussed on a smaller number of species. Many of the chapters in this thesis include: *Arctotheca populifolia*, a potentially interesting ruderal species that combines fast growth with high photosynthetic rates and relatively high efficiency of nitrogen use; *Scaevola plumieri*, which displays an unexplained inducible response to burial and is the dominant dune stabilising species in many subtropical dune areas; *Myrica cordifolia*, a non-leguminous nitrogen-fixer that dominates many stable-dune areas, and thus is potentially interesting in this nitrogen limited ecosystem; *Metalasia muricata*, an abundant stable-dune shrub. Where generalisation was necessary, analysis of these four species has been augmented with other common dune species.

The majority of this research was conducted at a protected site at the mouth of Old Woman's River (OWR) in the Eastern Cape Province, South Africa. A number of surveys were also conducted at the extensive dune fields near Kleinemonde and Kasouga and at various sites near Cintsa, north of East London.

Finally, on a global basis, psammophilous plants and sand dunes are important as these areas make up a considerable amount of the land surface of the world. While coastal dunes are less extensive, these will be the first land-ecosystem directly affected by the inevitable sea-level changes of the future (currently 3 mm rise in mean sea level per year; Church and White, 2006). Thus, the adaptations of plant life to these ecosystems are important and applicable to such wide fields as: dune reclamation, the identification of novel adaptations allowing more efficient nutrient use, and have implications for ecological theory through to desertification.

THESIS OUTLINE: AIMS AND QUESTIONS

The intial aim of this study was to determine the major environmental factors that affect the survival and growth of coastal dune species. This information allows the formulation of hypotheses of how these factors influence the zonal distribution and ecophysiology of these plants. Finally, hypotheses of specific adaptations to survive the extreme environmental conditions present on coastal dunes could be tested. The global aim of the thesis was to answer: Where and how plants survive in the coastal dune environment.

In relation to these aims the thesis has been divided into three sections of research content. **SECTION 2** is an introduction to an array of abiotic factors influencing dune plant ecology. **SECTION 3** considers the causes of species' zonation. **SECTION 4** describes a number of the adaptations that allow dune plants to survive in this harsh environment. Each section starts with an introduction to the important concepts of the section, and is comprised of one to four chapters developing different ideas relevant to the section as a whole. The final section presents a synthesis of these ideas and results.

SECTION 1: INTRODUCTION

- **Chapter 1 –** Introduction to current questions in dune ecology, including:
    - An introduction to the study sites,
    - A description of the zonal distribution of species growing on these coastal dunes, and
    - A description of the major species present.

SECTION 2: ABIOTIC FACTORS IN DUNE ECOLOGY

This section combines a detailed literature review with experimental data for the following abiotic factors, while asking the following questions:

- **Chapter 2.1** - How much, when, and where are coastal dune plants buried by sand? Is burial predictable from prevailing wind patterns?
- **Chapter 2.2** - Do dunes in the Eastern Cape have similar nutrient contents to other dunes? Are dune plants nitrogen limited?

- **Chapter 2.3** - Are osmotic stress related factors: salt spray, salinity, and inundation by seawater, important factors for dune plant ecology in the Eastern Cape?
- **Chapter 2.4** - Is the growth of coastal dune plants limited by water?

SECTION 3: ZONATION OF COASTAL DUNE SPECIES

Ecophysiological explanations of dune zonation: Testing whether differences in species' carbon and nitrogen economies can result in zonation of species on coastal dunes in the Eastern Cape. This section included:

- **Chapter 3 –** Introduction to ecophysiological explanations of zonation.

And asked:

- **Chapter 4 –** Could the inverse correlation between plant burial responses and the ability to grow tall result in the zonation of dune vegetation?
- **Chapter 5 –** Is there an interaction between the nutrient limitations and burial responses of coastal dune plants?
    - Do plants found in different areas of the dune zonation gradient have different responses to burial and nutrients?
    - Which carbon and nitrogen related adaptations allow these species to respond to burial?
    - Do these patterns relate to species' zonation?
- **Chapter 6 –** What is the relative contribution of *M. cordifolia*, which symbiotically fixes nitrogen, to the nutrient dynamics of the stable-dune zone?

SECTION 4: ADAPTATIONS THAT ALLOW PLANTS TO SURVIVE ON COASTAL DUNES

Investigations of specific traits allowing species to survive on coastal dunes. This section was aimed at:

- **Chapter 7 –** Estimating a budget of the carbon and nitrogen requirements of *Scaevola plumieri*, necessary for this species' inducible response to burial. Investigating:
    - The physiological and functional adaptations that allow *Scaevola plumieri* to survive high rates of burial.

- **Chapter 8** - Synthesising the important aspects of the interaction between the carbon and nitrogen metabolisms for coastal dune species, and quantifying the efficiency of the photosynthetic allocation of nitrogen in *Arctotheca populifolia* and *Scaevola plumieri* by comparison with other species. Specifically this chapter was aimed at:
    - Considering and expanding the concepts of whole plant nitrogen use efficiency.
    - Determining the contribution of different photosynthetic mechanisms to the photosynthetic nitrogen use efficiency of dune plants.
    - Comparing the whole plant and photosynthetic nitrogen use efficiency of coastal dune plants.
- **Chapter 9 –** Determining the implications of having a low biomass on dune plant growth. A model of dune plant growth is proposed, concerning the negative effects of having a high amount of competing biomass. Specifically, this chapter was aimed at:
    - Formulating a mathematical model of this hypothesis, derived from the technique of growth analysis.
    - Validating a number of assumptions made by this model.
    - Testing the model with a variety of greenhouse and *in situ* experiments.
- **Chapter 10 –** Determining whether dune plants have optimal growth geometries during partial burial. Specifically,
    - What are the theoretical predictions for the geometry of plant growth under burial conditions?
    - Is the growth of measured plants optimal in relation to the theoretical growth geometry?

SECTION 5: SYNTHESIS

- **Chapter 11 –** A synthesis of the research presented in the thesis, including a description of the abiotic factors affecting dune plants, the causes of the zonation of species, and review of a number of adaptations that allow dune plants to survive extremes of the coastal dune environment.

Growth, fitness and survival

This thesis attempts to describe the dynamics of plant production in relation to resource limitation. The survival of dune plants is determined by the response of a plant to burial, thus growth under nutrient limitation relates directly to survival and fitness in the long-term, the ultimate criteria by which these dynamics should be judged (Cahill, 2002). There is a clear fitness advantage for large perennial dune plants to be able to respond to burial. Particularly when reproduction is a positive function of plant size and age, as is the case for many of the species studied here.

Statistics

A substantial review of some the statistics used in this thesis is presented in Appendix 1. The appendix includes details on why and how: ANCOVA, ordinary least squares regression, standardised major axis regression, and allocation statistics were used.

STUDY SITES

Old Woman’s River

The majority of this study was conducted on the dune field to the west of Old Woman’s River (OWR) mouth (27^{o}08’49”E, 33^{o}28’59”S), Eastern Cape Province, South Africa (Plate 1.1 and 1.2a and c). The mean annual precipitation of 618 mm falls mostly in summer with some winter rainfall occurring. The mean maximum and minimum temperatures for summer are 24.6 and 17.8ºC, and for winter 20.2 and 10.2ºC. The site consists of a long (*ca*. 800m) stretch of stable coastline. The shore-to-inland vegetation gradient generally consists of a large vegetated incipient foredune of mobile sand above the high tide mark, a densely vegetated depression and a stable relict dune that leads to dune thicket 20 to 80 metres from the high tide mark. The headland at the Fish River mouth shelters this bay from westerly sand sources, but does not affect the influence of westerly winds on other aspects of dune ecology (e.g. salt spray). An un-vegetated dune field lies to the east of Old Woman’s River mouth, and represents a large sand source.

Dune field southwest of Cintsa West

The dune field southwest of Cintsa West (Plate 1.1 and 1.2b and d; 28^{o}06’55”E, 32^{o}51’27”S) was used for a number of surveys of *Scaevola plumieri* growth, and the zonation of species. This site has a large number of individuals of *S. plumieri* and excellent examples of speciose natural dune zonation. The site also lacks the exotic nitrogen-fixers: *Casuarina equisetifolia* and *Acacia cyclops*, which may affect the nutrient dynamics and species composition of the ecosystem. The general site description and the vegetation zonation are very similar to Old Woman’s River. East London (34 km distant) has a mean annual precipitation of 860 mm, the majority of which falls in summer. The mean maximum and minimum temperatures for summer are 24.0 and 16.5ºC, and for winter 21.7 and 12.0ºC (Müller, 1982).

Glengariff

The dunes at Glengariff (Plate 1.1; 28^{o}05’31”E, 32^{o}53’28”S) were used as an accessible site to make the gas exchange measurements presented in Chapter 8. This site is close to the study site at Cintsa West (~4 km), and shares many of the same characteristics. Due to the accessibility of this site, a high amount of human activity is present, and thus was not used as a site representative of dune zonation.

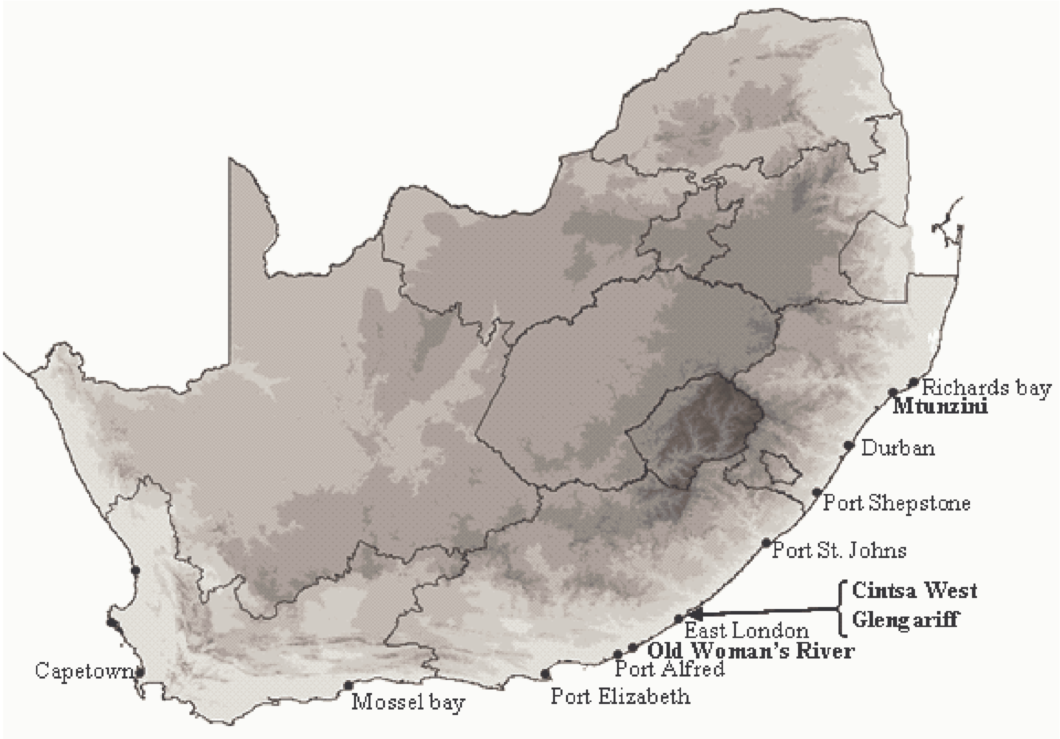


Plate 1.1: Location of the Old Woman’s River, Cintsa West, and Glengariff study sites, the prograding successional dunes at Mtunzini, and the major coastal cities of South Africa.

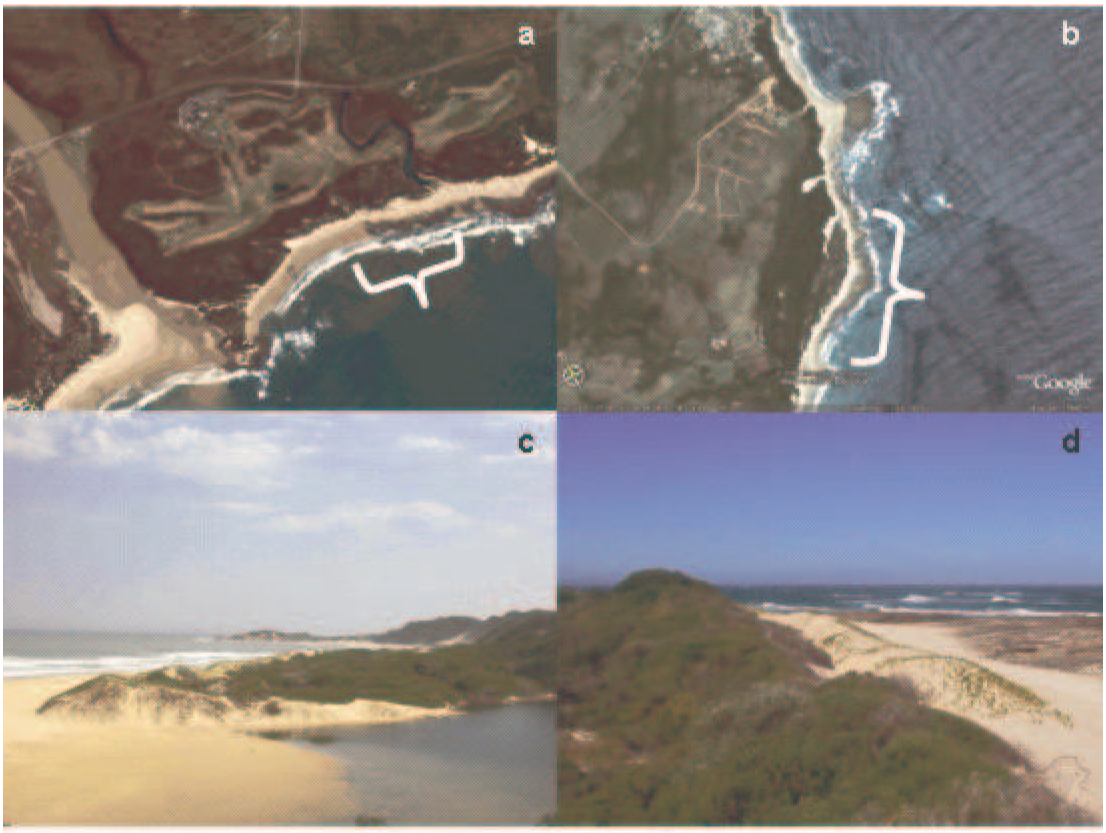


Plate 1.2: Google Earth© and site photographs for Old Woman’s River (a, c) and the dune field site southwest of Cintsa West (b, d). Brackets indicate the extent of the dunes studied (approximately 800 metres).

THE ZONAL DISTRIBUTION OF DUNE SPECIES

The spatial separation of species along a gradient is termed zonation (Smith and Huston, 1989). In the case of Eastern Cape coastal dunes a number of environmental gradients extend from the sea to the thicket or forest, and these are associated with distinct zonation of species (Lubke and van Wijk, 1998). Generally, these zones are explained by differential abilities of species to survive environmental or biotic gradients (McCook, 1994). Brown and McLachlan (1990) gave a list of these gradients: increasing soil organic content, nitrogen, plant cover, diversity, canopy height, insects, vertebrates, interstitial fauna, biological interactions, and specialisation with distance from the sea, but decreasing sand transport, sand $CaCO_3$, pH, salt spray, salinity, and temperatures with distance from the sea. These gradients can be categorised as physical and biotic stresses and disturbances, with both stress and disturbance generally decreasing with distance from the sea. Biological interactions, and plant biomass, may be seen as related to stresses that increase with distance from the sea.

Are species distributed within zones? Which environmental gradients determine the zonation of species? And, which plant traits allow species to have differential abilities to survive the important disturbances and stresses? In order to answer these questions it is necessary to describe the zonation of species.

Five belt transects, at OWR and Cintsa West, were used to assess the distribution of species within the dune zonation. Each transect consisted of a linear series of adjacent 2 by 2 m quadrats starting at the first vegetation landward of the high tide mark and ending two metres into the dune thicket or forest. The presence or absence, and the maximum height of each species were recorded for each quadrat. To aid comparison between the transects of different lengths (20 to 50 metres), the distance from the start of the vegetation, to the dune thicket was normalised to 100. The gradient of sand burial was measured over a year along the five transects at OWR using erosion pins spaced at 2 m intervals (see Chapter 2.1 for more details).

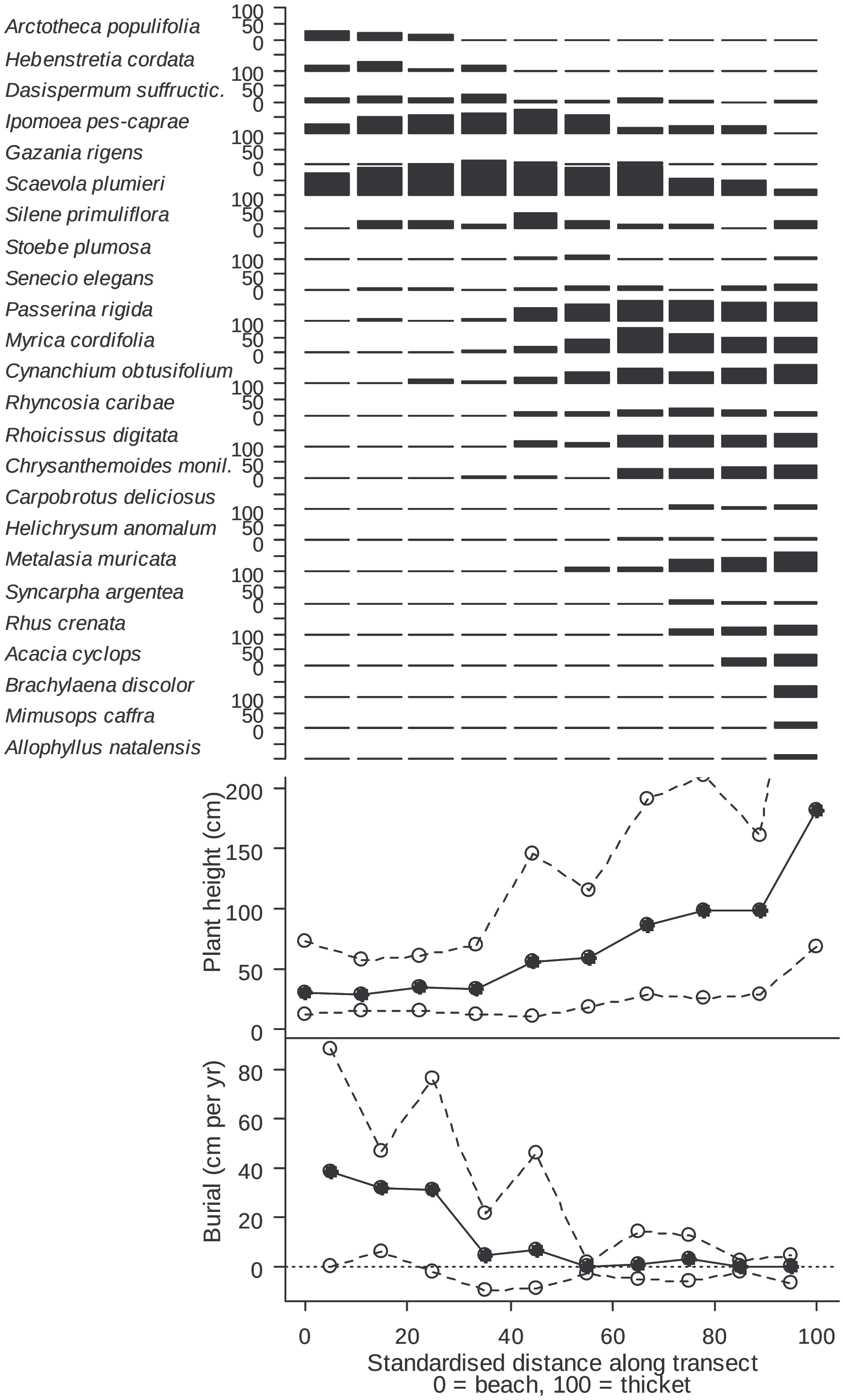


Figure 1.1: Zonation of species and the distribution of the abundance of mobile and stable dune species in ten standardised transects from OWR and Cintsa West. The height of the bars represents the percentage of quadrat's with a species present. The solid circles represent the average plant height or rate of burial in quadrats, and the open circles the maximum and minimum measured value. Burial was measured for a year period along the 5 transects at OWR.

Species' average ranks along transects were highly correlated between the two sites (Spearman's rank order correlation coefficient for species common to both sites, $R_{Spearman}^2 = 0.87$, n = 11 species, $P<0.05$), suggesting that zonation data was comparable between sites, and subsequently the data from both sites were combined.

The clear shift from areas with low vegetation and high burial to tall vegetation and low burial, coincided with a shift from dominance of a few herbaceous species to many woody species (Figure 1.1). The correspondence of this environmental change with a change in the vegetation can be used to define a mobile-dune zone and a stable-dune zone (the hyphens indicating that it is the dunes that are mobile and not the plants). Species typical of the mobile-dune area were not limited to this area, being found in the stable-dune area, some with high cover and abundance. The stable-dune species were not an important component of mobile-dune vegetation (Figure 1.1).

The dune zonation typically begins with sparsely distributed clumps of tidemark species such as *Arctotheca populifolia*, and seaward growing shoots of the creeper *Ipomoea pes-caprae*. Landward of this the perennial, *Scaevola plumieri*, forms large dunes and dominates a 5 to 40 metre section of the zonation. A number of common, small herbaceous species occur within more stable areas of the *Scaevola plumieri* dunes, but make up little of the total biomass or cover (e.g. *Senecio elegans*, *Silene primulifolia* and *Dasispermum suffructicosum*). Landward of the open-canopy *S. plumieri* dunes, shrubs and spreading plants form dense stands which vary in height. *S. plumieri* also forms an important part of this zone, but is limited to areas of low or open vegetation. Of the many species characteristic of this zone the following are the most important in terms of total cover: the generally low and spreading *Myrica cordifolia*, the tall shrub *Passerina rigida*, the dense canopy shrubs *Metalasia muricata* and *Chrysanthemoides monilifera*. In some areas, this zone is backed by dune forest or thicket, which has species generally limited to this final zone, e.g. the trees *Mimusops caffra* and *Brachylaena discolor*.

Additional mobile-dune species present at the sites, but not present in the transects are *Thinopyrum distichum* (syn. *Agropyron distichum*, OWR, Cintsa West), *Hydrophylax carnosa* (Cintsa West), *Mesembryanthemum aitonis* (Cintsa West), and *Tetragonia decumbens* (Cintsa West). The subtropical dune grass, *Sporobolus virginicus*, was present at both sites, and in localised areas can stabilise medium sized dunes. The other common grass species, *Ehrharta villosa*, is locally common to sheltered areas, such as river mouths, and areas of open sand within the stable-dunes. In contrast to temperate dunes in the Northern Hemisphere, grasses are only dominant dune stabilisers in local areas, the woody *S. plumieri* being the dominant dune stabiliser along the eastern and south eastern coastline of Africa (Doing, 1985). S*ideroxylon inerme* (OWR, Cintsa West) was present at both sites and is a codominant tree in the dune forest/thicket. The forest/thicket communities at both sites are diverse (>49 species, Burns, 1986, from a survey of coastal dune forests near Cintsa), but were not sampled in this study.

Similarity of the species' zonation at these sites and the dune succession at Mtunzini

The coastal dunes at Mtunzini, on the northern coast of KwaZulu-Natal, are extending seaward at a rate of 2.4 m yr^{-1} (Weisser and Backer, 1983). As a result, the plants growing on the dunes at Mtunzini show a clear succession towards forest over time. This successional sequence is strikingly similar to that found for the dune zonation sequences for OWR and Cintsa West. A comparison was made between the positional rank of species on static zonations at OWR and Cintsa West (Figure 1.1), and the spatial rank of species on successional dunes at Mtunzini. The rank correlation coefficient between the zonation and succession sequences was high and significant ($R_{Spearman}^2 = 0.81$, $P < 0.05$, 11 species were shared between sites, and the ranks for Mtunzini were assigned in the order reported by Weisser and Backer, 1983). This commonality suggests that similar processes, or factors, may determine the sequence of plant species found on sandy beaches along a large proportion of the coast of southern Africa.

SPECIES DESCRIPTION

Four species were selected based upon their importance in the dune ecosystem (high abundance and cover relative to other species) and their position along the burial/ zonation gradient (Figure 1.1). The nomenclature of all plant names was as per Lubke and van Wijk (1998).

Arctotheca populifolia (Berg.) Norl. (Asteraceae) is a short, pauciennial, semi-succulent, large-leafed and fast growing species of prostrate habit (see Plate 1.3a). This species often forms the first vegetation encountered above the high tide line, and represents the ephemeral/perennial tidemark community of Doing (1985), it is however, also found landward of the tidemark, occurring in disturbed areas devoid of vegetation.

Scaevola plumieri (L.) Vahl. (*syn. S. thunbergii*; Goodeniaceae) is a semi-succulent large-leafed perennial species (see Plate 1.3b) that forms large dunes with dispersed stems connected underground (Barker *et al.,* 2002). This species is the dominant dune binding/foredune former along much of the moist coasts of tropical and subtropical Africa (but see Peter *et al*. 2003), and regions of Florida and Mexico (Doing, 1985).

Myrica cordifolia L. (Myricaceae; Plate 1.3c) is a nitrogen-fixing woody shrub (Van Ryssen & Grobbelaar, 1970) that grows from the start of the stable-dune zone to the dune thicket or forest. This species forms prostrate (sometimes buried) stems that root adventitiously. Short vertical growth from these horizontal stems allows an individual to dominate large areas of the stable- and semi-stable-dunes. Recent taxonomic work on the genus has suggested that the name of the majority of species in this genus should be *Morella* (e.g. Parra, 2003). This new name has not been used in the dune ecology literature yet, and for this reason *Myrica cordifolia* is used here. This species may have a similar ecology as its congener from the north east coast of the United States; *Myrica pensylvannica*, which facilitates the growth of other species due to its nitrogen-fixing ability (Shumway, 2000).

Metalasia muricata (L.) D. Don (Asteraceae) is an ericoid small woody shrub growing up to two metres in height (see Plate 1.3d). This species is limited to stable-dune areas, where it has the highest cover and total biomass. *M. muricata* shrubs often grow within stands of *M. cordifolia* where the latter species is locally out competed due to the higher height and cover of *M. muricata*.

Plate 1.3: Species photographs: *A. populifolia* growing at the high tide mark (a), *S. plumieri* showing stem elongation in response to burial of the lowest leaves (b), *M. cordifolia* growing with *S. plumieri* stems (c), and *M. muricata* (d).

SECTION 2: ABIOTIC FACTORS IN DUNE ECOLOGY

Chapter 2

A detailed description of the abiotic environment of the coastal dunes of the Eastern Cape

Description of important environmental factors — What is the nature of sand burial? — Nutrient dynamics — Salt spray, salinity, and seawater inundation — Is the dune environment an arid, water stressed, ecosystem?

2.1. WIND AND SAND BURIAL

INTRODUCTION

The use of the term "coastal *sand dunes"* implies that sand transport and burial are dominant processes in this ecosystem. Burial results in species' zonation (Maun and Perumal, 1999; Dech and Maun, 2005; Martinez *et al*., 2001), and the requirement for a plant to respond to burial in order to survive in this environment. Due to the importance of burial, the burial responses of plants have been widely studied (summarised in Maun, 1998), but less attention has been given to a complete description of the nature of burial for a site. A large number of studies do measure burial, and successfully relate this to plant growth (e.g.; Eldred and Maun, 1982; Greipsson and Davy, 1996; Harris and Davy, 1986; Martinez *et al*., 2001; see Table 2.1.1 pp. 42 for more). However, few of these studies include the detailed temporal and spatial description of burial that is necessary to determine the regime of burial relevant to plants surviving on these dunes (see list of burial characteristics below). A "plant's eye view" description of burial is vital, as these burial characteristics determine which adaptations are needed in order for plants to survive. It is possible that contrasting adaptations may be present in plants from adjacent areas with different burial characteristics. Therefore, a detailed description of burial must be completed prior to generating hypotheses of how dune plants survive burial. Such a survey was attempted for the study site at Old Woman's River.

Consider the following characteristics of sand burial and possible associated plant traits:

1) Magnitude and duration – a quick burial of small magnitude may result in the death of mosses, while taller plants can survive greater burial depths (Maun and Perumal, 1999). If burial events were of short duration many plants would be able to tolerate a period of darkness, until re-exposure (Sykes and Wilson, 1990b). To this author's knowledge, no field measurements have been undertaken of the proportion of buried plants that are subsequently re-exposed, nor of the duration of burial.

2) Partial burial – the effect of the magnitude of burial is dependent on the height of the plant, and the distribution of leaves. A tall habit, or bare stem above the sand surface, can be viewed as a possible adaptation to minimise photosynthetic losses due to burial. Thus, whether a plant encounters complete burial, or partial burial, is related to a combination of the magnitude of burial relative to the characteristics of the plant. Indeed, in coastal environments such as the Eastern Cape, partial burial (0-40cm yr^{-1} depth) of plants (0-200cm height) may be more prevalent than complete burial (pers. obs.).

3) Stochasticity and periodicity of burial events – large burial events require a quick response to burial, and may be followed by a period of stability (recovery period) or repeated burial. Therefore, the predictability and periodicity of burial are important characteristics. Storage of carbohydrates would enable buried plants to have reactive growth in response to burial. However, environments in which burial is continuous or random may allow little recovery period for the plant to re-store used carbohydrates. Longer periods between burial events may allow a quick growing ruderal plant to complete its lifecycle in between burial events. This strategy may be particularly important for plants growing in the inter-dune “pavements” in the large dune fields found along this coast (McLachlan *et al*. 1996).

4) Gradual burial – requires stem growth to constantly exceed the burial rate. In this case neither height nor storage are beneficial characteristics, as the need for constant growth does not allow the plant any time to regenerate lost height, or build up carbohydrate reserves.

5) Seasonality – burial may coincide with the growing season or with the period in which the plants are dormant e.g. *Ammophila breviligulata* is dormant during the winter storms of the Great Lakes, which provide much of the yearly burial (Maun, 1985). In Iceland, *Leymus arenarius* was exposed to burial during the vital summer growing months, but not during winter (Greipsson and Davy, 1996).

6) Yearly differences in burial – year to year variability may have large effects on the establishment of individuals or species. As the effect of burial is dependent on plant size, yearly variability may allow invasion of new species into dune areas where that species was not able to establish previously. The responses and vulnerability of seedlings to burial may prevent establishment, but adult plants may be able to survive.

7) Spatial homogeneity of burial – results in all plants and all shoots of a plant requiring resources simultaneously, while heterogeneous burial may result in adjacent connected shoots having different burial states, dependent on scale. Clonal plants or interconnected shoots may be better able to survive heterogeneous burial conditions (desert herbs and grasses: Yu *et al*. 2002, Yu *et al*. 2004). In the case of nutrient dynamics, enhanced survival due to physiological integration of clones is dependent on the scale of the heterogeneity (Hutchings and Wijesinghe, 1997). Physiological integration of water, nutrients and carbohydrates has been shown to be important for dune plant survival (Evans, 1991).

8) Erosion (episodic or gradual) – may result in dune collapse, in exposure of roots, or a lack of support resulting in stem mechanical problems.

9) Climatic regions – regional differences in climate influence the prevailing wind strengths and directions, and thus burial. It is envisaged, that differing regional burial regimes may limit plant distribution. Climatic variation in burial was not further considered in this thesis.

*****Important note to readers: The World Meteorological Organisation defines winds by the direction of origin. Thus, an easterly wind blows from the east to the west, and transports sand in a westerly direction*****

Mechanisms of sand transport in relation to wind

Sand movement is determined by the speed and direction of the wind (Bagnold, 1941). Sand deposition is influenced by physical dune characteristics (dune aspect, slope, and surface roughness), and vegetation cover (Hesp, 2002). Burial is likely to be proportional to sand movement rates, as large dunes of *Scaevola plumieri*, or other dense vegetation, trap a large percentage of the sand entering the vegetation (Hesp, 2002, or see Arens, 1996 for a comparable situation). Buckley (1987) demonstrated that a 17% cover of small short spreading plants results in >80% reduction in sand movement at near gale force winds. Plant characteristics, such as cover, height, and frontal area index (the ratio of vertical cover to horizontal cover), determine the effect the plant has on slowing sand bearing winds, and thus amounts of deposition (Hesp, 2002).

Bagnold (1953, cited by Lancaster, 1985) suggested that winds have the following qualities determining sand movement: 1) there is a velocity threshold below which sand is not transported (4.5 m s^{-1}; Lancaster, 1985), and 2) the amount of sand transported by a wind is proportional to the velocity minus wind threshold raised to the power of three. Sand transport equations including the velocity to the third power, and a threshold term most accurately estimate measured transport rates (Sarre, 1988). The water content of the surface layers of sand also has a large influence on the amount of sand transport (Sherman *et al.*, 1998; Wiggs *et al.*, 2004). These general characteristics of sand transport, allow a rudimentary model of the relative sand transport to be generated from detailed wind information for any site. The total amount of sand transport predicted from such models is called drift potential (*DP*) (Pearce and Walker, 2005). This value does not take into account the antagonistic effect of winds of opposite direction, thus the resultant drift potential (*RDP*) was developed to represent the resultant vector of net sand transport (Pearce and Walker, 2005). This technique has been termed the Fryberger model (Pearce and Walker, 2005), although the exact equations of the Fryberger model differ slightly from those outlined above. A large number of studies use variations of the original Fryberger model to model dune movement, and the vegetation present on dunes (e.g. Illenberger and Rust, 1988). There is also a large variation in the equations used for calculating sand transport, despite these equations having a similar form and results (e.g. Sherman *et al.*, 1998). The equation adopted in this thesis is outlined above, and retains simplicity, and was recommended by Bagnold (1941), the original author in this field.

Other effects of wind

Wind also results in: sand abrasion, physical damage, decreased leaf boundary layer conductance, and increased transpiration rates. Salt spray is also proportional to wind speed (Boyce, 1954). The amount of salt spray transported by a wind has no minimum wind speed threshold, although a considerable increase in salt spray occurs from 5 to 7 m s^{-1} (Boyce, 1954).

AIMS

The surveys reported here, were aimed at describing the characteristics of sand burial for plants growing along the Eastern Cape coastline, by:

1) measuring *in situ* rates of sand burial at OWR,
2) modelling the potential sand movement (drift potential) dynamics of the coastal dunes at OWR from wind speed and direction, and
3) comparing this drift potential model with two years of measured sand transport rates for the dominant dune building species *S. plumieri*.

The characteristics of sand burial were assessed by two methods: 1) changes in sand level were monitored for 1 to 2 year periods, and 2) the relationship between burial and wind was established, and other characteristics of burial were inferred from detailed wind data.

METHODS

Measurement of sand burial

Net rates of sand burial were measured in three experiments at OWR. The experiments were designed to determine typical burial regimes for the: dominant dune building species (*S. plumieri*), a range of four species from different zones on the dune gradient, and transects leading from the storm water mark to the dune thicket. In all cases sand movement was measured by monitoring changes in sand level relative to erosion pins (50-100 cm long high tensile wire pins of 3 mm diameter) buried up to half their length. During the course of the three surveys the height of pins was measured, on average, every 40 days.

Burial regime of a dominant dune building species

The burial regime of *S. plumieri* was surveyed for a two-year period at OWR (30 May 2005 to 4 May 2007), by placing 65 erosion pins on and around five distinct *S. plumieri* dunes. An erosion pin was placed in the centre of each dune, with two pins evenly spaced from this pin in N, S, E and W directions. Four of these dunes had the greatest fetch from an eastern direction, while the fifth was protected on the east, but not the west. These orientations were representative of the majority of *S. plumieri* dunes at OWR.

Burial regime of four species from different zones on the dune gradient

Erosion pins were established on 7 January 2004 in homogenous stands of *Myrica cordifolia* (*n* = 24) or individual shrubs of *Metalasia muricata* (*n* = 17) and monitored over a 656 day period. The data of *Scaevola plumieri* dunes (above) were used for this comparison. The highly disturbed foredune environment of *Arctotheca populifolia* resulted in a lack of long-term burial data for this species. As an alternative, the burial in 30 to 40 clumps of *A. populifolia* was measured for two short periods of 29 and 100 days during winter and summer, respectively.

Burial gradient with distance from the sea

Erosion pins were placed at two metre intervals along the five transects used in the vegetation survey (Chapter 1) on the 1 April 2006. This experiment was monitored on eight occasions over a one year period. The results were reported previously in Figure 1.1.

Spatially explicit measurements of burial

A spatially explicit study of burial on dunes at OWR was done by Ketelaar (1998), for four similar sized dunes from the 29 November 1996 to 19 January 1997. Ketelaar laid a 6 by 6 metre grid of 36 erosion pins across an un-vegetated dune, one *Sporobolus virginicus* dominated dune, and two *S. plumieri* dunes. The net amount of sand accumulation for these four dunes over the 50 day measurement period was used by the current author to generate spatial maps of burial. In order to assess the spatial heterogeneity of burial, the spatial coordinates of the measurements pins were plotted on an x-y graph, with a 1m^2 block representing each pin. The range of recorded burial depths was divided into 15 categories, such that each block could be coloured on a 15 point scale from dark (highest rates of burial) to light (net erosion). To further emphasise areas of net burial a 5 cm net burial depth contour was plotted encompassing all areas that had a net burial greater than 5 cm (as interpolated using the algorithms of the *lattice* package of R; Sarkar, 2007).

Weather data

Weather data was kindly supplied by Garth Sampson and Coleen de Villiers of the South African Weather Service. Wind speed, direction, and rainfall were recorded according to the international standards of the World Meteorological Organisation, for the airport at Port Alfred. Data included hourly measurements for the period (January 2003 to June 2007). The Port Alfred airport is exposed to similar coastal winds to the OWR site, and is the nearest long-term digitised weather station to the study site (28 km). The weather station recorded the average wind speed, and direction (precise to 10 degrees) at hourly intervals. Twenty year wind roses exist for the Great Fish Point Lighthouse, prior to 1980 (Schultze, 1980). As this site is nearer to OWR (5 km), than Port Alfred (28 km), all Port Alfred based results were compared to the Great Fish Point Lighthouse data.

Weather model of sand transport

A qualitative sand burial model was developed to predict relative amounts of net sand movement in different directions for the study site. This model was similar to the model suggested by Fryberger (details in Tsoar, 2005, and Pearce and Walker, 2005), where the net direction and relative amount of sand movement, were calculated as the resultant of a wind rose transformed to represent sand drift potential (this model is illustrated in Figure 2.1.1). Specifically, the model applied the following rules to hourly weather data for the 704 day period during which sand movement was measured: 1) wind velocities below an assumed threshold (4.5 m s^{-1}, Lancaster, 1985) were removed from the analysis, 2) days on which greater than 3 mm of rain fell were excluded as this was assumed to be sufficient to stabilise the sand, a threshold of 3 mm was used to include days of dew formation in the analysis, 3) the remaining velocities were transformed to be proportional to sand movement α (velocity - threshold)^3, based upon equation 2.1 from Buckley (1987), and Lancaster (1985), and 4) the transformed velocities were summed for each direction interval of 10 degrees. The model that was used differed from the original formula of Bagnold (1941), and the one used by Fryberger, by removing all assumed constants, apart from the threshold velocity. But since the form of the equation remained the same, the results of this formula and Bagnold’s are perfectly linearly and isometrically related. Thus, the absolute numbers produced by this formula lack meaningful units, but have exactly the same relative magnitudes as the results from a Fryberger model.

In order to analyse the data that this model produced, the following steps were taken to further summarise the data (summarised in Figure 2.1.1): 1) the average hourly drift potential of each of the two dominant winds (easterlies and westerly's) were summed within a range of 60^o, 2) the total drift potentials for winds from other directions than the dominant winds were summed, 3) the total hourly *DP* for each set of directions (W, E and other directions) were summed by day, month, burial measurement interval, or for the total period of weather measurements, and 4) as W and E winds account for the majority of the *DP*, and are antagonistic, these two daily totals were subtracted (E-W) to determine the net resultant drift potential, for days or months. These calculations allowed the following to be compared: 1) the resultant drift potential of a measurement period to the measured burial for that period, 2) the directional distribution of the average annual drift potential in the form of a "wind" rose (a sand rose?), 3) the contribution of daily wind events to the total drift potential of a period, and 4) the monthly seasonal change in drift potential, including shifts between the two dominant wind directions.

The predicted drift potential is generally an overestimate of actual sand transport rates (Sherman *et al.*, 1998), due to the effects of soil moisture, and lack of available sand, amongst other reasons. Sherman *et al.* (1998) found that for the beach and foreshore at a site with onshore winds and 200 days of precipitation per year, soil moisture largely determined sand transport rates. At OWR the dominant wind directions are parallel to the shore and the site has half the precipitation of the previous study. Thus, it is likely that soil moisture plays a smaller role in determining sand transport rates at OWR, in comparison with south western Ireland. Pearce and Walker (2005) found that this type of analysis is biased by the interval settings for direction. As the wind directional data for the current study was strongly bimodal, the possible interval setting bias should have no effect on these results. The mean hourly wind speed and direction were used in this analysis avoiding the bias introduced by using a summary wind rose for the calculations (Pearce and Walker, 2005). The directions of the dominant winds shift with season along the Eastern Cape coastline (Stone *et al*. 1998), and therefore the calculated drift potentials of winter and summer were compared in different analyses. For this purpose summer and winter were defined as the periods between the average solar equinoxes, the 22 March and 21 September. When monthly averages were used, winter was defined by the months ending closest to equinoxes: from the 1 April to the 31 September, and summer from the 31 September to the 1 April.

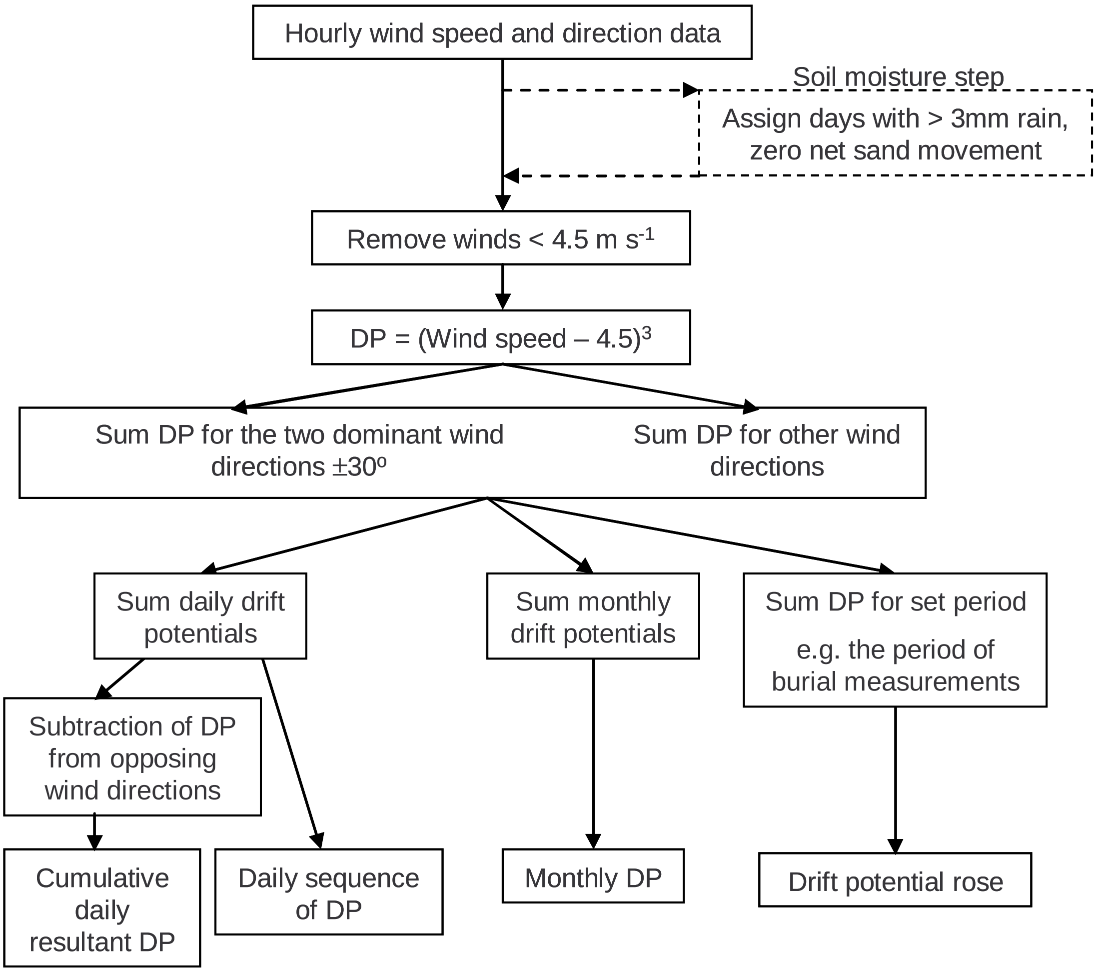


Figure 2.1.1: The procedure used to model sand transport (drift potential, *DP*) from hourly wind data. Burial was assumed to be proportional to sand transport as it has been shown that the majority of transported sand is trapped by dense vegetation (see Arens, 1996). The inclusion of the soil moisture step did not have a large effect on the magnitude of the result, and this was excluded from most analyses. The equation for *DP* was derived from Bagnold (1953; cited in Lancaster, 1985), this was found to be most representative of sand transport (Sherman *et al.*, 1998). The equation was simplified by removing the constants used to estimate mass transport. Thus, the *DP* reported here is theoretically proportional to sand transport (m^3 m $width^{-1}$ $hour^{-1}$), and lacks units.

RESULTS

The characteristics of burial

The average burial rates of the four species differed greatly (Figure 2.1.2). These results were consistent with the decreasing gradient of burial with distance from the sea, and these species' zonal distributions (Figure 1.1). The two stable-dune species encountered no net sand movement and the pioneer species, *A. populifolia*, had highly variable positive rates of sand accumulation.

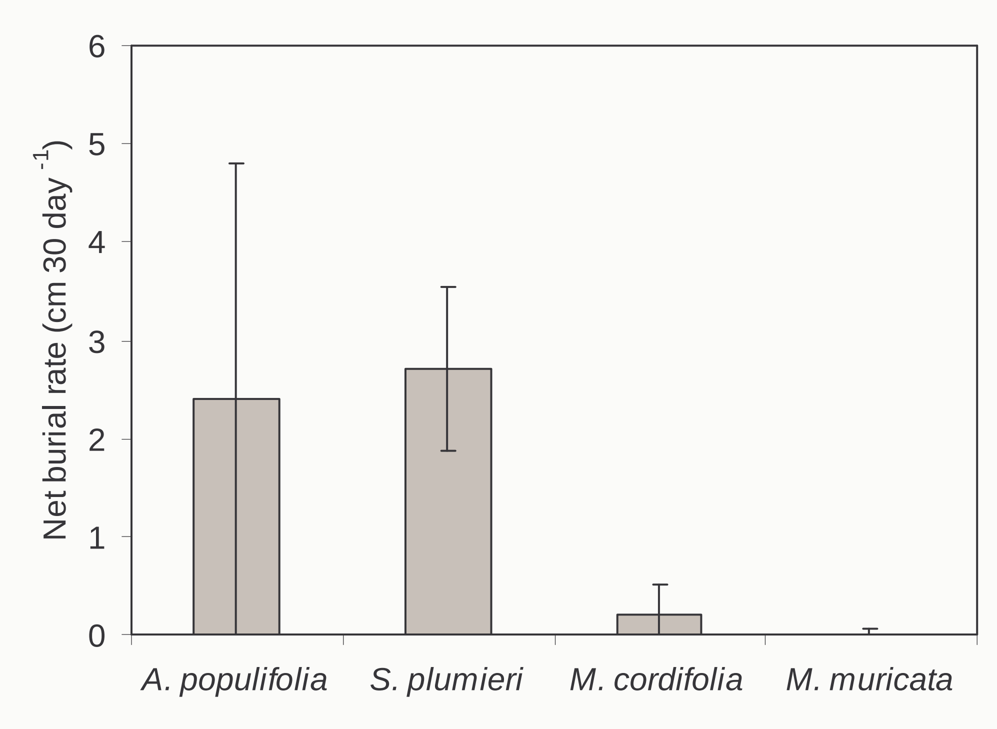


Figure 2.1.2: The net rates of burial measured for four species of dune plants**;** *A. populifolia* and *S. plumieri* (mobile-dunes), *M. cordifolia* and *M. muricata* (stable-dunes). Error bars represent 95% confidence intervals for the mean.

Dunes formed by the dominant dune building species, *S. plumieri*, had a range of different types of burial. Three of the five dunes studied showed a seasonal net increase in dune height in the centre of the dune (Figure 2.1.3). Over some periods, some of the dunes showed similar decreases in sand levels (A in Figure 2.1.3), and during other periods a single dune showed large fluctuations in sand level (B in Figure 2.1.3). The unstable crest of this particular dune is shown in Plate 1.3b.

The maximum net burial for a measured point on these *S. plumieri* dunes was 65 cm (3 cm per month), and the maximum sand accumulation rate over a single measurement period was 43 cm month^{-1}. In the long term, the dunes had a net positive sand accumulation, significantly different from zero, although erosion did occur during some measurement periods, and net erosion occurred for some erosion pins (Figure 2.1.4). The average burial rate for all measurement periods was 2.0 cm month^{-1} ± 0.73 (95% confidence interval of the mean). For all measured points, over all time intervals, burial events occurred 83% of the time. The lack of weekly measurements of sand level prevent the direct testing of hypotheses of the short-term periodicity of burial. However, the short-term periodicity of burial may be indicated by the short-term changes in drift potential calculated from wind characteristics.

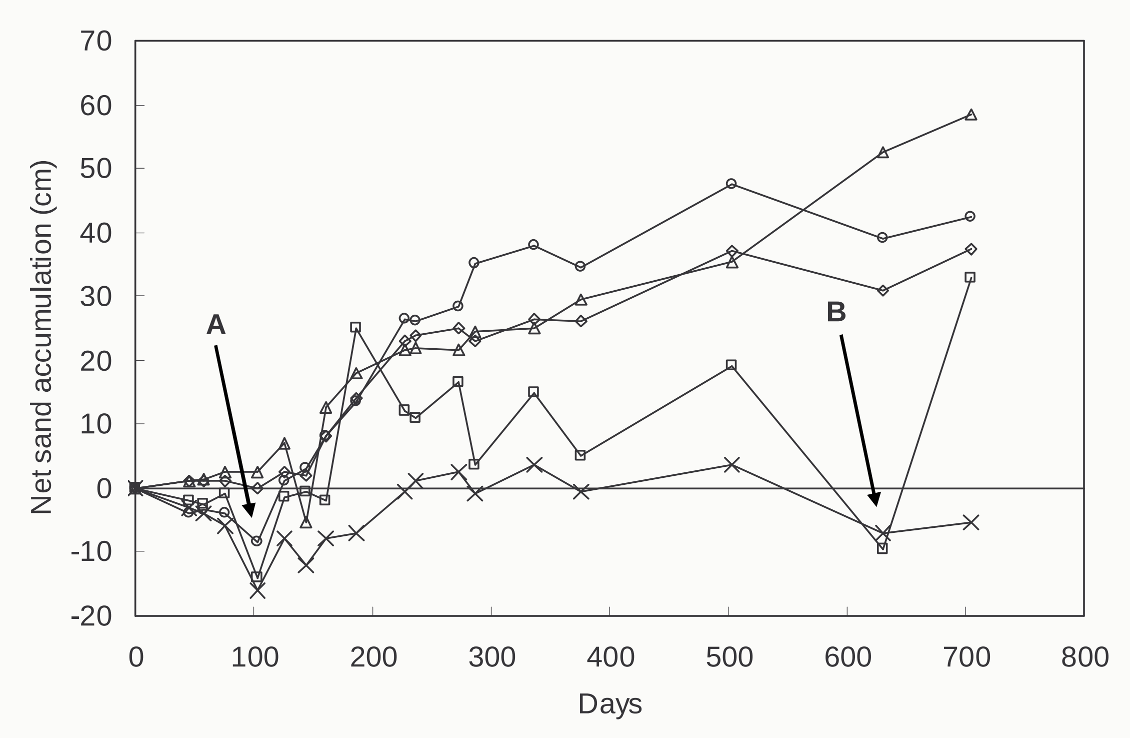


Figure 2.1.3: Illustration of the different net sand accumulation patterns measured in the centre of five *S. plumieri* dunes. Large burial or erosion events occur, for example affecting three dunes (A), or a single dune (B).

The spatial heterogeneity of burial was not measured directly in this study, but burial along transects, and the burial on different sides of dunes was measured. The burial rates measured along transects decreased sharply with distance from the sea, reaching zero net accumulation where stable-dune species were present (Figure 1.1). The eastern faces of *S. plumieri* dunes showed a consistent high increase in sand level, with the southern face, and centre area showing smaller increases in sand level. The western and northern sides of dunes showed variable sand accumulation rates, both positive and negative (Figure 2.1.4). The data of Ketelaar (1998) allowed some measure of the spatial heterogeneity of burial for OWR (Figure 2.1.5). At a 1 metre spatial scale, burial at a particular point was often associated with burial at adjacent erosion pins 1 metre away (Figure 2.1.5). The un-vegetated dune showed the most homogenous burial conditions (Figure 2.1.5). Hence, burial for all dunes was uniform at scales of 1 metre and greater, indicating that heterogeneity of burial occurs at quite large scales.

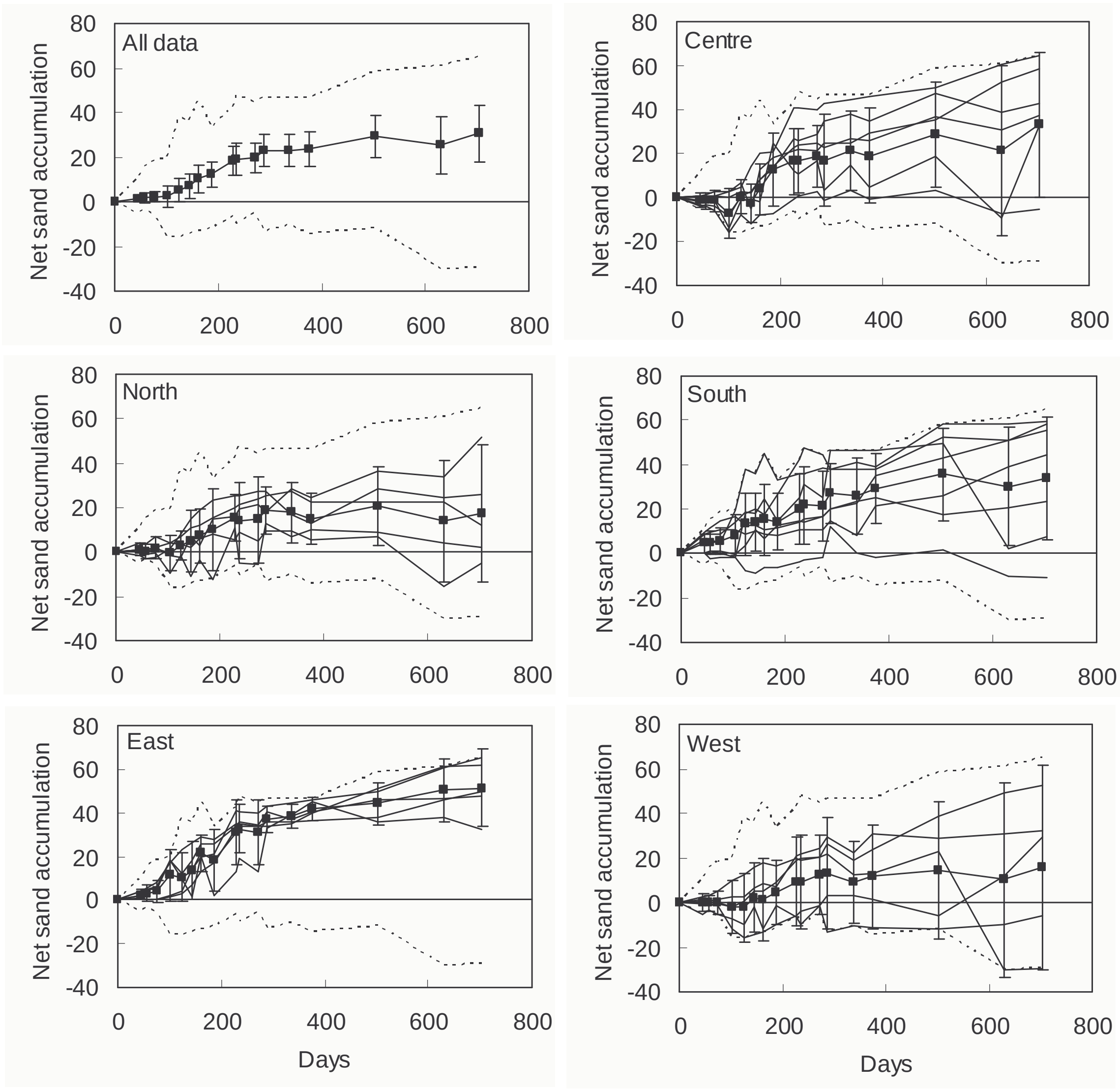


Figure 2.1.4: Net sand accumulation of five *S. plumieri* dunes measured at different positions (centre or two pins evenly spaced from the centre in each of N, S, E and W directions). For each dune aspect, the two most extreme sand accumulation rates for the entire data set are shown as dotted lines. Vertical bars represent the 95% confidence interval of the mean.

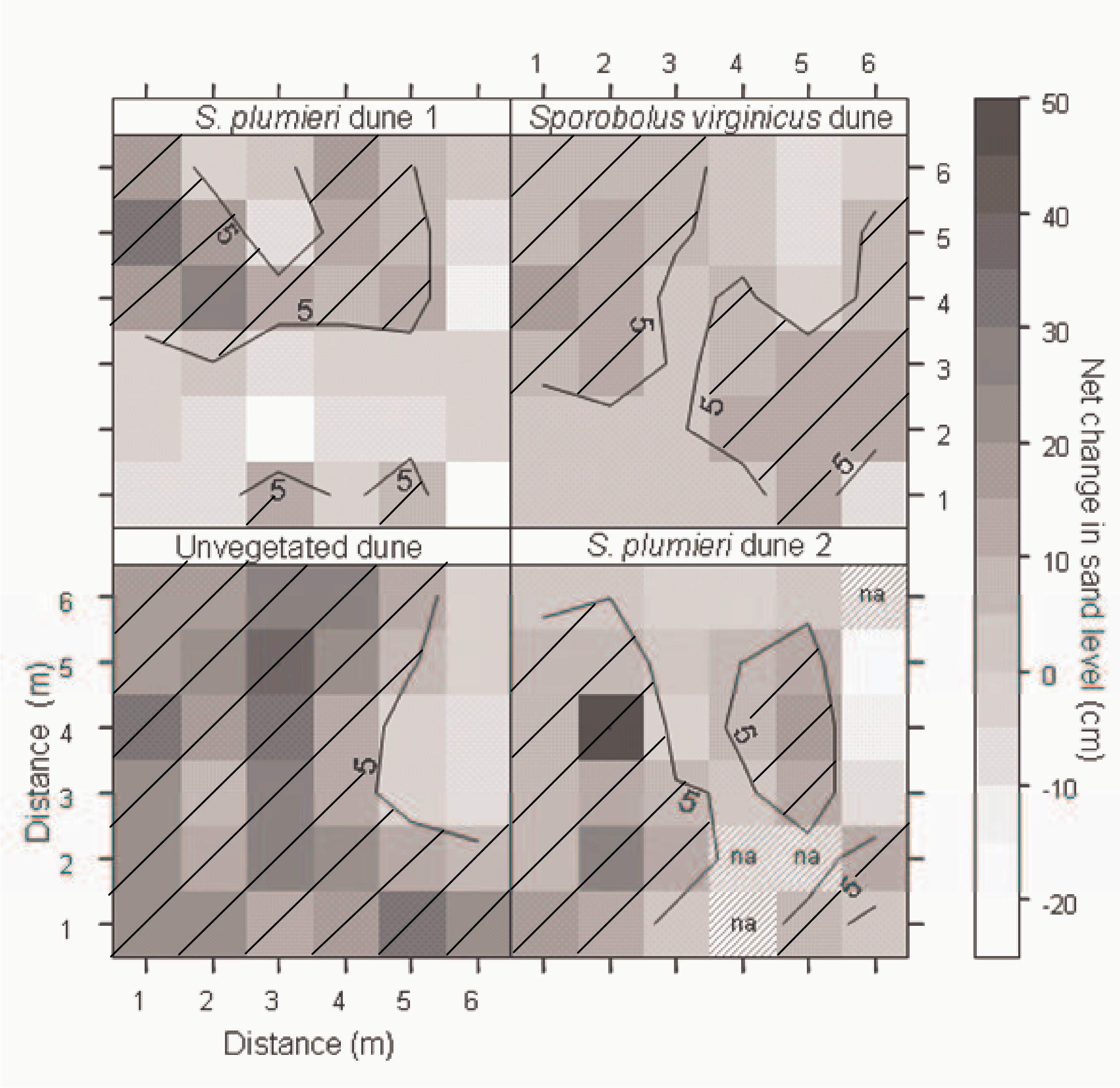


Figure 2.1.5: The net change in sand burial mapped for two *S. plumieri* dunes, one *Sporobolus virginicus* dune and an unvegetated dune, for a 50 day period of the summer of 1997, at OWR (raw data from Ketelaar, 1998). Lines enclose and fill the area of the dunes that had greater than 5 cm net sand accumulation. Labels are positioned on the side of increasing sand accumulation. Cells with na represent missing data.

The effect of wind on burial

The highest rates of sand burial were associated with summer easterly winds, and closely related to the cumulative easterly drift potential (Figure 2.1.6). Little net sand accumulation occurred during winter periods when westerly winds were strongest. Large significant increases in net sand level occurred after periods of strong easterly winds (Figure 2.1.6). The net sand level and the cumulative resultant drift potential corresponded well, with clear seasonal effects of drift potential on burial (Figure 2.1.7). These two parameters had a strong correlation ($R^2 = 0.91$), although the cumulative nature of these parameters biases correlative types of analyses. The inclusion of the soil moisture correction (no sand movement on days following >3mm of rain), had little effect on the values in this model (data not shown). Hence, no soil moisture correction was used in subsequent analyses.

As this data supported the hypothesis that drift potential largely determines sand burial, wind was used as a predictor of the seasonality, short-term periodicity, and regional nature of burial.

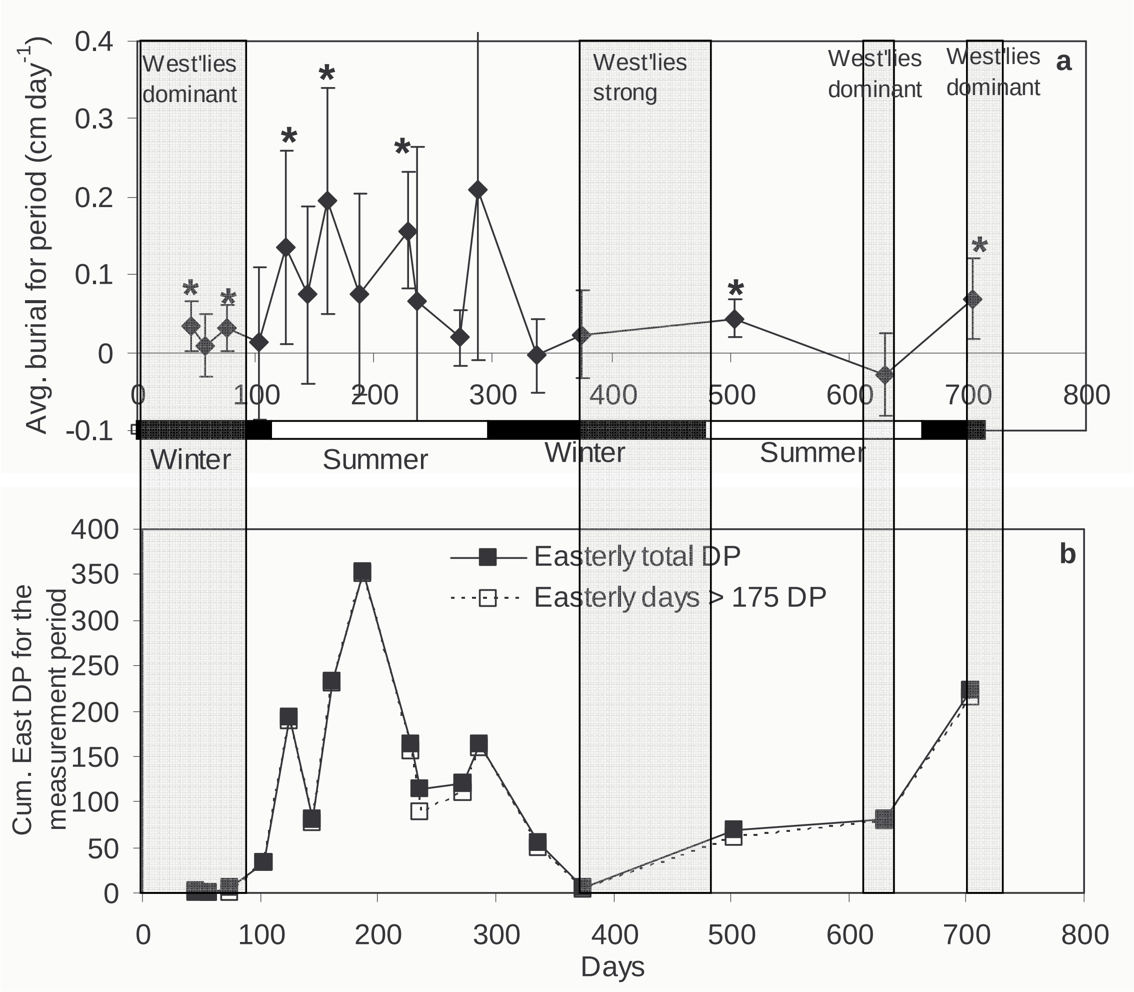


Figure 2.1.6: The average rate of burial for 27 erosion pins placed at different directions on five *S. plumieri* dunes, for 17 periods over 704 days (a), and the Drift Potential calculated for all easterly winds (solid line, closed squares), or easterly winds occurring on 16% of days where *DP* > 175 (dotted line, open squares) (b). The error bars represent 95% confidence intervals of the mean burial rate, burial rates significantly greater than zero are marked with an asterisk.

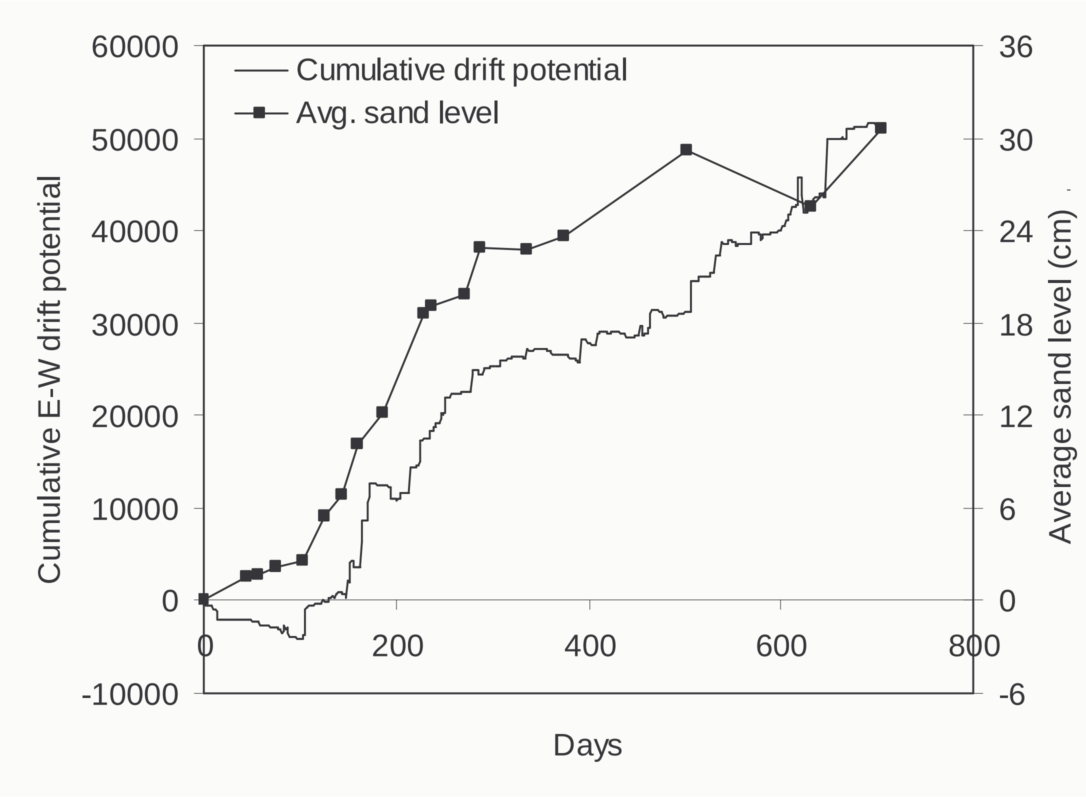


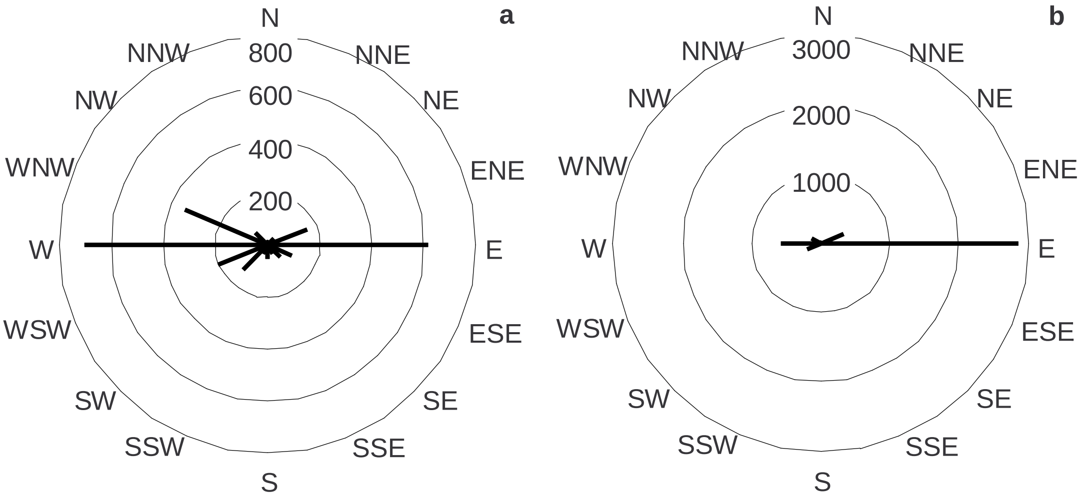
Figure 2.1.7: The relationship between the resultant drift potential and sand accumulation. The resultant drift potential was calculated as cumulative easterly *DP* minus the cumulative westerly *DP* and indicates the net direction and magnitude of sand transport as calculated from wind data.

Wind model of sand transport: directional movement of sand

Port Alfred has a strongly bimodal westerly and easterly wind pattern (Figure 2.1.8a). Although the winter westerly winds make up the greatest proportion of the total amount of wind, the summer easterly winds have a greater occurrence of winds with speeds above the sand movement threshold. Thus, the westerly winds have little drift potential in comparison to the easterly winds (Figure 2.1.8b).

Wind model of sand transport: Periodicity and stochasticity

A few very windy days account for the majority of the total drift potential (54 days account for 88% of *DP* of the 704 days during which burial was measured; Figure 2.1.9). Thus, it is of interest to ask: What is the periodicity of the days that largely contribute to the total drift potential? But, different weather systems may alter the periodicity of windy days at different times of the year, therefore, the periodicity of high winds during winter and summer were compared (Figure 2.1.10). For a four year sequence of wind data, 16% of windy days contribute 90% of the total *DP*, these days had a total daily drift potential of 175 or more. There was little difference in the lengths of the time between windy days between summer and winter for this four year period (Figure 2.1.10). Seventy-eight percent of windy days (daily *DP*>175) were followed by a second high wind event within 10 days, regardless of season. Twenty two percent of windy days were followed immediately by a second windy day.



Average cumulative wind speed per month calculated from hourly averages($\mathrm{m\ s^{-1}\ month^{-1}}$)

Average cumulative sum of *DP* per month (Dimensionless)

Figure 2.1.8: Wind rose diagrams of the average monthly sum of hourly measured wind speed (a), and the average monthly sum of drift potential (b), for the period of burial measurements (704 days).

Is the sequence of windy days and calmer days random? Or is there a periodicity to this sequence? This was determined using a runs test, which calculates the observed number runs (sequences of similar data), in comparison with the number of runs expected from a random pattern (Sokal and Rohlf, 1981). The runs test found that the sequence of days with and without high *DP* was significantly different from a random sequence for winter ($P = 0.008$, but the significance of this test was highly sensitive to a small change in the number of runs), while summer was not different to a random sequence ($P = 0.25$). A more sensitive test is necessary to further elucidate the periodicity of winds with high drift potential.

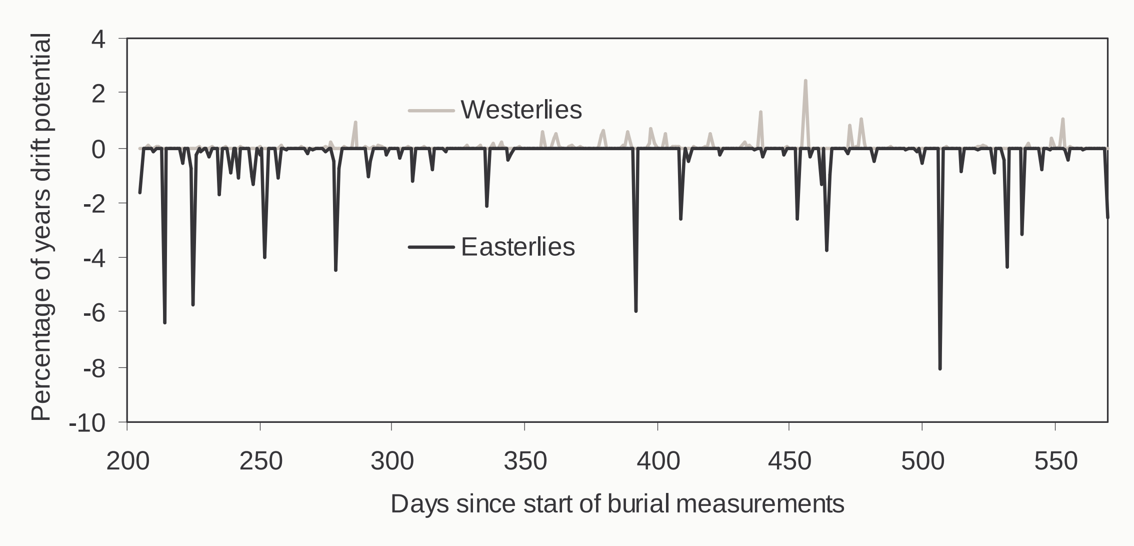


Figure 2.1.9: An example of drift potential calculated for a year, taken from the period during which burial was measured. Easterly and westerly winds are antagonistic and were assigned opposite signs for this reason (negative and positive, respectively). For this time period, winds on 54 days (all greater than > 175 daily *DP*) account for 88% of the total drift potential, and easterlies account for 84% of the total drift potential.

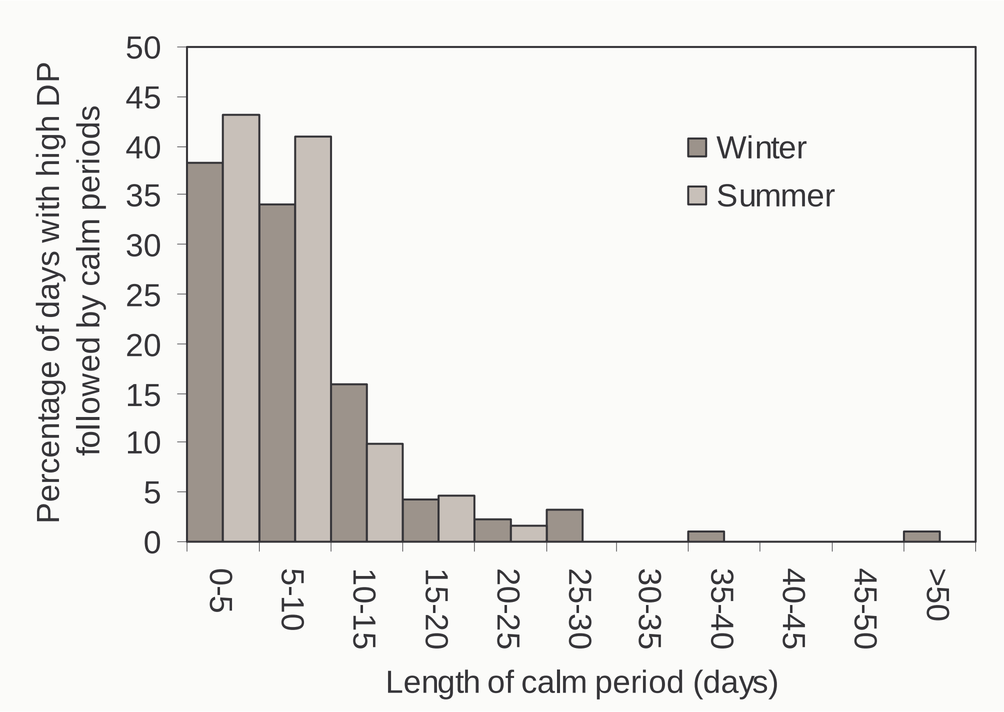


Figure 2.1.10: Frequency distribution of the length of calm periods between days with high drift potential (>175). Days of *DP* greater than 175 correspond to 90% of the total *DP* for Port Alfred from 2003 to 2006, but only make up 16% of days.

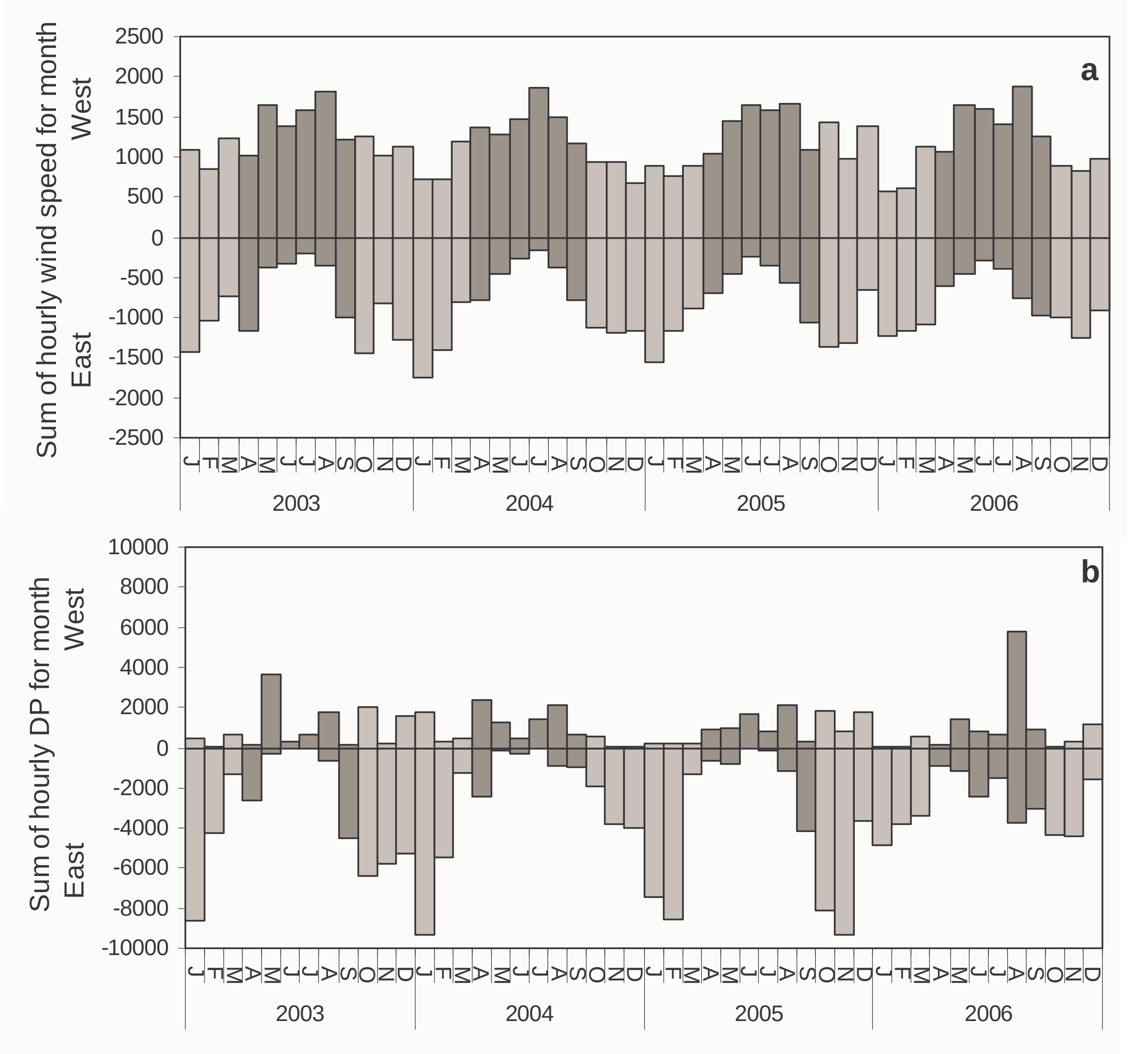


Figure 2.1.11: Seasonal shifts in the sum of hourly wind speed measured for the month (a), and drift potential for the two dominant wind directions (b). Easterly and westerly winds are antagonistic and were assigned opposite signs for this reason (negative and positive, respectively).

Wind model of sand transport: Seasonality

The seasonal shifts from westerly dominant winds to easterlies, and relative strengths of each for Port Alfred (Figure 2.1.11), are partly confirmed by the data presented in Lubke and Sudgen (1990). These wind roses were made by Schultze (1980, cited in Lubke and Sudgen, 1990), for the Great Fish Point Lighthouse for a twenty-year period leading up to 1980. Summer easterly winds were stronger than winter westerly winds, although the winter westerly winds had a greater proportion of total *DP* than those calculated from the Port Alfred data. As this weather station is near OWR (~5 km), the data from it partly confirms that the weather patterns observed for Port Alfred are indicative of those at OWR, but that there may be some negative bias for westerly winds.

DISCUSSION

Is there a zonation of burial?

Maun and Perumal (1999) demonstrated that Lake Huron dune species found in areas of high burial are able to survive higher amounts of burial than stable-dune species. However, few other studies correlate species' zonation with actual measured burial rates (Wilson and Sykes, 1999). Species' zonation was similar across three sites in southern Africa (Chapter 1), and this common zonation sequence corresponded very well with measured rates of burial (Figure 1.1). These results suggest that the action of burial on species' zonation is best described as forming two zones, a mobile-dune zone, and a stable-dune zone, rather than a burial gradient.

Characteristics of burial: burial measurements

During the course of two years, the dominant dune building species, *S. plumieri*, had an average net increase in sand levels of 33 cm (Figure 2.1.4). This is consistent with the year-long monitoring of burial along 5 transects, where 40 cm of burial was recorded for the seaward areas (Figure 1.1). Given an average net increase in sand levels of 33 cm per year this equates to a net annual deposition of 495 kg of sand per square metre! (Assuming a sand bulk density of 1.5 g cm^{-3}). Four species were monitored for various extended periods, confirming the results of the transect data, namely, that species found landward of the first foredune encountered low rates of burial. *A. populifolia* had the most variable burial rates (Figure 2.1.2), consistent with this species position as the most exposed seaward species (Figure 1.1). These results do not confirm the two years of net erosion measured for *S. plumieri* at this site, by Knevel (2001). However, measuring burial or erosion is clearly dependent on dune aspect (Figure 2.1.4).

Some erosion pins had large fluctuations in sand level, often associated with short-term movement of the dune crest (B in Figure 2.1.3 corresponds with the dune shown in Plate 1.3b). In these circumstances *S. plumieri* was generally able to survive under the sand, or emerge from burial, during these short term events. This pattern of short term burial was only evident for one dune, suggesting that net burial, without subsequent erosion was more common.

The spatially explicit burial data of Ketelaar (1998) allowed an analysis of the spatial heterogeneity of burial at a one metre scale (Figure 2.1.5). Clearly, for all dune types, burial at one point was associated with burial at adjacent points. *S. plumieri* has belowground interconnected shoots at a scale of approximately 50 cm to 150 cm (see Plate 1.3b, where all visible shoots are likely to be connected). Thus, it is suggested that inter-stem allocation of resources could occur from unburied stems to connected buried stems. But, if large areas of dune were buried as demonstrated in Figure 2.1.5, then such resource allocation may have importance only along the boundaries of the area affected by burial.

Modelling burial from wind data

Monthly measurements of burial are of great importance as these are representative of the long-term net burial which plants must survive. In order to determine the short-term characteristics of burial, a link was made between long-term burial measurements and wind data for the measurement periods. A rudimentary model of burial based upon wind speed and direction, was used to allow an insight into regional, short-term, and seasonal changes in burial.

A clear relationship was found between the strength of easterly winds, and the net burial on *S. plumieri* dunes for OWR (Figure 2.1.6). The easterly influence was ascribed to two reasons: the small sand source to the west of the dunes (Plate 1.2c), and a westwards resultant drift potential due to dominant easterly winds (Figure 2.1.8b). The cumulative resultant drift potential calculated with the wind model demonstrated that the seasonal changes in drift potential corresponded with changes in measured burial (Figure 2.1.7). It is noted that the western sides of *S. plumieri* dunes have the greatest range of positive and negative sand accumulation, consistent with some erosion (Figure 2.1.4). This result may imply that westerly winds are erosive, lacking sufficient available sand for transport to occur from the west of this site. Thus, it is difficult to determine whether the largely unimodal wind model fits the burial data due to a true causal link, or whether the wind data is biased for westerly winds, and corresponds to the burial data due to the lack of an upwind sand source to the west.

The similarities between the wind model of drift potential and the measured burial rates may validate the use of this model as a proxy for burial characteristics of which no measurements were possible. A large number of physical and empirical models of sand transport have been proposed, and these describe actual sand transport with varying degrees of success (Sherman *et al*. 1998). The inclusion of the influence of vegetation in these models adds an additional degree of complexity to an already chaotic system (Baas, 2002). In the current study, a simple model of sand transport was found to be representative of the average burial conditions for this site, although on a point to point basis, no model could account for a large proportion of the variation. An R^2 of 0.42 was found for the minimum adequate model fit for all the points measured on *S. plumieri* dunes, using a variety of predictor variables. A distinction should be made: that the *measurement* of point to point burial characteristics is indicative of actual conditions, while the lack of a good wind model for point to point burial does not reduce the validity of the measured burial rates. This consideration limits the predictions of Fryberger-type wind models to the prediction of the general characteristics of burial for a site. Having stated this, Fryberger-type wind models do give a good estimate of the maximum sand transport for a site, given that certain conditions hold (Tsoar, 2005). The type of wind model used in this study is often used for modelling dune formation (Lancaster, 1985), movement (Illenberger and Rust, 1988; Blumberg and Greeley, 1996), and the success of vegetation on these dunes (Tsoar, 2005), and thus is a widely accepted way of estimating general sand transport characteristics.

Limitations of the Fryberger technique

An important limitation of the Fryberger method is that the use of a threshold velocity for sand transport to occur, introduces a large degree of sensitivity into the model. If winds of 3 to 6 m s^{-1} are frequent for a weather station, but on the dunes the wind speed is consistently 1 m s^{-1} more or less than the weather station, then this sensitivity will have a large effect on the calculation of *DP* and *RDP*. It is possible that this could be the case for the unexpected dominance of easterly winds at Port Alfred (Figure 2.1.12). The daily sum of the hourly wind speeds measured at Port Alfred were highly correlated with those measured for the same period at East London and Port Elizabeth (data not shown). However, the wind speed at Port Alfred was generally lower. Whether this is the case for the winds on the dunes, is difficult to determine. The westerly *RDP* at Port Alfred may or may not be representative of actual dune wind conditions: for instance the 20 year wind roses for January and July (pre 1980) at the Great Fish Point Lighthouse show the summer ENE winds are the most frequent and strongest, although for these two months west winds have a greater *DP*.

Characteristics of burial: wind data

A large percentage of potential sand movement (90%) occurred on a few very windy days (16% of days). Over a two year period this results in seemingly gradual change in sand level (Figure 2.1.7). However, it is suggested that burial occurs in a series of wind events, and it is the timing of these events that determines what type of response would be beneficial to a plant. The weather processes generating these winds are classically chaotic, and therefore not random (Gleick, 1987). For the plant, there seems little predictability of when large wind events will occur. However, two broad generalisations may be made about when a large wind event may occur: 1) it is likely to occur within 10 days of a previous high wind event (Figure 2.1.10), and 2) the magnitude of the wind events (and burial) is seasonal, summer having the most burial and strongest winds (Figure 2.1.6, and 2.1.11).

There are few long-term studies that relate sand movement to wind in the Eastern Cape, north east of the Alexandria dune fields, which have been extensively surveyed (Illenberger and Rust, 1988). Alexandria has a consistent westerly wind that transports sand eastwards (Illenberger and Rust, 1988). Fraser (2003) presents sand movement data for a site at Kenton-on-sea (30 km south west of Port Alfred), for just over a year period. Easterly and westerly winds, measured at Port Alfred, were important indicators of dune movement for the site at Kenton-on-sea (Fraser, 2003). However, opposite to the current study at OWR, the existence of a vegetated area to the east of that site, reduced the influence of easterly winds. A summer maximum sand transport was reported for this study, with a net westwards movement of sand (Fraser, 2003). Other studies have focused on dune movement for short periods further east along the coast (Port Alfred: Lubke and Sudgen, 1990, for winter during two months, westerly winds were dominant; Kleinemonde: Avis and Lubke, 1985, winter, 94 days, westerly winds dominant; OWR: Ketelaar, 1998, summer, 50 days; Knevel, 2001, two years, net erosion for *S. plumieri*, but presented no data relating wind to burial). Thus, there is a lack of long-term studies of sand burial and related wind patterns for sites along the eastern part of this coastline.

A number of these studies found a net eastward movement of sand, although dunes were found to reverse under prevailing winds (Lubke, 1983). The current study contradicts this idea, possibly because of two reasons: 1) Old Woman's River has an easterly sand source, but little westerly sand source, resulting in net deposition of sand on vegetated dunes on the eastern sides of the dunes, under easterly conditions, 2) this site lies more than halfway from Port Elizabeth to East London, and East London does not have as strong dominant westerly winds as Port Elizabeth (Stone *et al*. 1998, citing Schulze, 1980). Indeed data from the Port Alfred airfield shows that while equal amounts of easterly and westerly winds are present, the summer easterlies are of greater velocity and thus have greater sand movement potential. Thus, the influence or lack of sand sources, and data for weather stations close to a site are vital considerations in modelling sand transport, and burial. These conditions result in the majority of burial occurring in a westerly direction during the summer easterly winds (Figure 2.1.6). This is a clear difference to the data for Alexandria, and Port Elizabeth (Illenberger and Rust, 1988).

Implications of burial for dune plants

Burial at OWR occurs often (weekly), but with a seasonal decrease in burial during the winter, and results in a general net increase in sand level. The burial is dependent the on aspect of the dune, the exposure of the dune to sand transport, and the distance of a point from the start of vegetation. While burial is heterogeneous at the scale of a dune, at a 1 metre scale it is quite homogenous.

These burial characteristics form an environmental context in which adaptations related to burial can be assessed. For instance, at OWR a minimum height to the first leaves (>30cm) would allow plants to maintain photosynthetic leaf area, under the majority of the measured burial conditions. For the period of measurements (2004-2007) little erosion was evident at the site, in contrast to the net erosion measured for this site by Knevel (2001). It is not known whether year-to-year variation, experimental biases, or other factors such as changing vegetation dynamics or storms could account for this difference. The unpredictability of the occurrence of the ~20%

days that potentially result in significant sand transport, may have significance for the growth of plants. Thus, long enough recovery periods needed to allow replenishment of depleted carbohydrate reserves may be limited to less exposed areas of the dunes. An alternative explanation of reactive growth to burial, the interconnectivity of shoots, may also be limited to areas of spatially heterogeneous burial. A clear shift was found for coastal dunes, from annual species with high relative cover at medium disturbance sites, and laterally spreading herbaceous perennials at higher disturbance sites (Fahrig *et al.,* 1994). This shift is attributed to laterally spreading perennials being faster invaders of disturbed areas, than seed dependent annuals (Fahrig *et al*. 1994). The benefits of this habit are clearly related to the heterogeneity of burial for a site.

With this data-set it was difficult to determine how often buried plants are re-exposed, or the duration of burial. For periods of longer than a month, there was little evidence to suggest that plants were re-exposed. It is suggested that for plants exposed to sand transport, burial consists of unpredictable partial covering of the shoot, and in most cases not complete burial. Consistent net burial requires that a shoot responds to partial burial, or complete burial may occur.

Comparison with other sites

As with this study, the characteristics of burial vary widely between and within other sites (Table 2.1.1). Gradual burial, erosion, and the large influences of storms were all characteristic of different points within relatively un-vegetated English dunes (Ranwell, 1958). Similar to data found for this study, Ranwell (1958) found that a particular gale resulted in the accumulation of 40 cm of sand in three days. These dunes had less seasonal burial than the dunes at OWR, with the period of maximum burial varying from summer to autumn (Ranwell, 1958). Dunes along the Gulf of Mexico experienced high amounts of northerly gales (>22 m s^{-1}) during the winter season (Martinez *et al*. 2001). As these gales were associated with little rain, they resulted in net erosion on dune arms and slopes, but accumulation on dune crests. Interestingly, after four years the vegetation on these dunes reached a threshold cover (~30-60%) and subsequently the dunes were stable (Martinez *et al*. 2001). Lake dunes in Michigan displayed net burial for a year, with a subsequent lack of net burial for three years (Bach, 2001). The dune fields at Alexandria had a seasonal decrease in sand transport, but no large switch in wind direction (Illenberger and Rust, 1988). The lack of vegetation in this large (120 km^2) dune system was attributed to a low rainfall, high wind regime, single dominant wind direction, and large source of sand. A list of the magnitude of burial characteristics is given for various other sites and dune species (Table 2.1.1).

Most of the mentioned dune systems have two characteristics in common, namely, that burial is seasonal, and periods of high wind speeds contribute disproportionately to burial (Table 2.1.1). Considering that sand transport is proportional to wind speed raised to the third power, the influence of storms is hardly surprising. However, this relationship indicates that the majority of burial, in almost all ecosystems, must be associated with high wind “events” rather than being a daily feature of dune life. The periodicity and predictability of these events are vital factors in determining the burial regime of a site. Many of these references have noted that burial occurs on the front (van der Valk, 1974), or rear (Ranwell, 1958, Lubke and Sudgen, 1990), or sides (Martinez *et al*. 2001, this study, Figure 2.1.4) of the first foredune. Thus, spatial heterogeneity of burial on the scale of a vegetated dune is a common occurrence. Plants that are physiologically integrated at this scale may have an advantage in being able to subsidise growth of buried shoots from unburied shoots.

The most extensive study of burial dynamics is that of Levin *et al*. (2006), for partially vegetated dunes in Israel. Levin *et al*. (2006) showed that burial was spatially heterogeneous when at a large scale (>50 m). The areas of highest burial were the coastal foredune zone, followed by actively accreting dune slip faces (Levin *et al*. 2006). Levin *et al*. (2006) present similar data to Figure 2.1.6 demonstrating the correlation between drift potential, resultant drift potential, and sand movement. Ketelaar (1998) measured extremely high rates of burial and erosion in the short-term (extrapolated to a year in Table 2.1.1), while actual rates of burial and erosion were an order of magnitude lower in the long-term (Knevel, 2001, and this study; Table 2.1.1). This difference demonstrates that the short-term fluctuations in sand levels of windy days do not consistently act on the dunes. Instead burial is best viewed as the net long-term outcome of these counteractive processes.

Table 2.1.1: Sand burial characteristics reported in this study, and by other authors. Where two numbers are given these represent a range, and where a single number is given this represents a mean burial rate. A number of references were taken from the similar table by Maun (1998).

Study	Site	Net effect	Vegetation	Period of study	Seasonal?	Spatial heterogeneity?	Net erosion?	Strong winds?
This study	Old Woman's River	-10 to 35 cm yr^{-1}	*Scaevola plumieri*	2 yr	summer	y dune [a]	burial	y[c]
		0 to 60 cm yr^{-1}	*Arctotheca populifolia*	120 d	-[f]	-	burial	-
		-5 to 90 cm yr^{-1}	Mobile-dunes	1 yr	-	y dune	burial	-
		-10 to 15 cm yr^{-1}	Stable-dunes	1 yr	-	y dune	inter.[b]	-
Ketelaar, 1998	Old Woman's River	-180 to 330 cm yr^{-1} [d]	*Scaevola plumieri*	50 d	-	y>>1m	-	
		-50 to 120 cm yr^{-1} [d]	*Sporobolus virginicus*	50 d	-	y>>1m	-	
		-70 to 250 cm yr^{-1} [d]	Un-vegetated dune	50 d	-	y>>1m	-	
Knevel, 2001	Old Woman's River	-8 cm yr^{-1}	*Scaevola plumieri*	3 yr	-	-	eros.	-
Lubke & Sudgen, 1990	Port Alfred	-	Partially vegetated	7 m	-	y dune	inter.	y
Dahl *et al*. 1975 [e]	Gulf of Mexico	80 to 120 cm yr^{-1}	*Panicum amarum*	-	-	-	-	-
Martinez *et al*. 2001	Gulf of Mexico	-	Dune community	8 yr	highly	y dune	-	y
Eldred & Maun, 1982	Lake Huron	-5 to 25 cm in winter	*Ammophila breviligulata*	1yr 2 m	winter	y1.4m	burial	-
Bach, 2001	Lake Huron	0 to 5 cm yr^{-1}	Dune community	4 yr	-	-	burial	-
Maun, 1985	Lake Huron	9 cm yr^{-1}	*Calamovilfa longifolia*	2.5 yr	winter	-	burial	-
Olson, 1958 [e]	Lake Michigan	30 cm yr^{-1}	*Ammophila breviligulata*	-	-	-	-	-
Barbour *et al.*, 1985	Pacific coast NA	32 cm yr^{-1}	*Elymus mollis*	-	-	-	-	-
		44 cm yr^{-1}	*Abronia umbellata*	-	-	-	-	-
		57 cm yr^{-1}	*Ammophila arenaria*	-	-	-	-	-
van der Valk, 1974	Atlantic coast NA	20 to 30 cm in winter	Dune community	1 yr	winter	y dune	wave	-
Zaremba & Leatherman, 1984 [e]	Atlantic coast NA	-78 to 102 cm yr^{-1}	*Ammophila breviligulata*	-	-	-	-	-
Harris and Davy, 1986	England, Atlantic	<-30 and >30 cm	*Elymus farctus*	1 yr 3 m	winter	y	both	-
Ranwell, 1958	England, Atlantic	30-90 cm yr^{-1}	*Ammophila arenaria*	3 yr	winter	y dune	inter.	y
Greipsson & Davy, 1996	Iceland, South Coast	19-34 cm yr^{-1}	*Leymus arenarius*	1yr 2 m	summer	-	-	-

[a] spatial heterogeneity based upon comments or results, the scale of heterogeneity is given. Dune represents different sides of dunes have different burial. [b] significant net erosion over dunes (eros.), significant net burial over dunes (burial), or intermediate amounts of both erosion and burial (inter.) [c] strong winds represent the effects of single or repeated burial events, such as gales. [d] a short study period. [e] cited in Maun (1998). [f] – represents unknown or not applicable.

Special cases

In general, little sand is deposited on the stable-dune zone (Figure 1.1). However, burial does occur in these areas when a blow-out, or physical forcing of wind results in a local area of erosion and deposition (e.g. Weller, 1989). Often these cases are associated with the formation of a steep slip face which buries the vegetation below it. A number of these areas are present at OWR (pers. obs.), and may contribute to the presence of burial tolerant species in the otherwise stable-dunes.

The large poorly vegetated transgressive dune fields found from the Sunday's River, to the Kei River, may have different dynamics from those presented here for vegetated dune fields. A number of the mobile-dune species found at OWR are present in these dune fields (McLachlan *et al.,* 1987). The wet relatively stable slacks, in between the dunes, often contain these species. Two types of burial occur in this environment: 1) the deposition of sand within or next to clumps of vegetation, resulting in hummock or shadow dune formation (Hesp, 1989); and 2) the movement of a large transverse dune over a plant (McLachlan *et al.* 1996). The latter burial can occur either as the shallow slope of a dune moves over the plant, or more commonly as a slip face rapidly moves over the plant (7 m yr^{-1} eastwards for Alexandria dune field, McLachlan *et al.*, 1987). The survival of the plant depends on the plant growing faster than the dune accretion. These environments may be better modelled by wind patterns as there is less influence of vegetation on dune form (Baas, 2002). The year round dominant easterly winds at the Alexandria dune field, may result in little respite for plants being buried. While further east, dune plants being buried in this manner, may have seasonally shifting wind directions, allowing some respite from burial (data not shown). Thus, it is predicted that for dune fields in the western part of the Eastern Cape, dune slack plants will be limited to short-lived species, capable of completing their lifecycle in the period between dune movement across a point (~ 5 years, an average of 30-40 m distance between dunes, and 5-7 m yr^{-1} movement of dunes eastwards, Burkinshaw, 1990, cited by McLachlan *et al.,* 1996). While further east, dune slack plants may be able to survive longer periods, and possibly outgrow moving dunes. An adaptation allowing survival in these particular circumstances is investigated in Chapter 10.

2.2. NUTRIENT DYNAMICS

INTRODUCTION

A universal characteristic of dune soils are extremely low levels of nitrogen (see the literature summarised in Table 2.2.1). Phosphorus is less widely studied, but is often considered to be the secondary limiting nutrient to plant growth (Pakeman and Lee, 1991a and b). A lack of organic matter results in little adhesion and retention of free nutrients, combined with high potential leaching resulting in low soil nutrient levels (Kellman and Roulet, 1990). Salt spray (and seawater) is rich in K^+, Na^+, Cl^-, Ca^{2+}, and other micronutrients (Rozema *et al*. 1985). Seawater in comparison with Hoagland's plant growth solution contains 22000 times more Cl^-, 1.7 times more K^+, 460 times less N, and 1000 times less P (adapted from Rozema *et al*. 1985). This dichotomy potentially results in dual limitation of early zonation species by low levels of N and P, and inhibiting levels of Na^+, Cl^- and other salts.

At a number of dune sites there is a drop in soil inorganic P with distance from the sea (barrier islands off the coast of Virginia: Day *et al*., 2001; Veracruz, Mexico: Kellman and Roulet, 1990; Lake Michigan: Lichter, 1998). Soil nitrogen increases with distance from the shore (Lichter, 1998). Thus, across a dune zonation these changes in nutrient availability may result in differing nutrient limitations with distance from the sea.

Table 2.2.1: Nitrogen and phosphorus contents of dune soils from various studies.

Reference	Site	Situation	Total N μg g^{-1}	P μg g^{-1}
Harte and Pammenter, 1983	Range	Various	980-4900	3.1-301
Harte and Pammenter, 1983	Durban, South Africa	Dunes	14-140	3-6
Ripley, 2002	OWR, South Africa		16	3.2
Fay and Jeffrey, 1992	Ireland		0.4	-
Ernst, 1983	Netherlands		172	7.4
van der Putten *et al*. 1993	Netherlands		47-263	-
Skiba and Wainwright, 1984	Lincolnshire, UK		22-27	-
Boorman and Fuller, 1982	Norfolk, UK		1135-1163	22-25
Imbert and Houle, 2000	Hudson Bay, Canada		10-182	-
Cain *et al*., 1999	Texas, USA		1.1-3.4	-
Sykes and Wilson, 1991	New Zealand		-	2.8-4.6
Hunter and Hoy, 1983	New Zealand	Forested dunes	98-196	2.8
Baldwin and Maun, 1983	Lake Huron, Canada	Lake dunes	-	0.9-5.0
Maun, 1985	Lake Huron, Canada		-	0.9
Sun, 1999	Lake Huron, Canada		48-137	84-118

Nutrient limitation: Method of measurement

The N:P mass ratio of plant tissue has been shown to relate to the relative limitations of these two nutrients (Güsewell, 2004). Generally, an N:P ratio of <10 indicates nitrogen limitation, while >20 P limitation, for a wide range of species and ecosystems (Güsewell, 2004). Regardless of the actual N:P values and limitation thresholds, it is logical that a shift in this parameter must indicate a change in soil or plant nutrient dynamics. Alternative methods of determining nutrient limitation, involve the addition of single or multiple nutrients to the dune soil, and monitoring the growth response of plants. This technique, although widely used, is limited due to the differential abilities of species on nutrient gradients to respond to fertiliser. The nutrient addition method has been criticised for dune plants, for a variety of reasons (Barbour *et al*., 1985). One problem is that long-term changes in community composition may not reflect short-term changes in the growth of a single species (Chapin *et al.*, 1986). An example is the nutrient addition experiment of Willis (1963), where dune grasses became more dominant after nutrient addition. The observed increase in biomass can thus, be attributed to either the change in vegetation from mosses to grasses, or the nutrient limitation of production (Willis, 1963). Thus, it is uncertain whether the change in abundance of the mosses was due to altered competition, or whether the mosses responded to the increased nutrient supply. Community responses may differ from those of component species; further examples are the experiments of Boorman and Fuller (1982). Therefore, nutrient addition experiments should be conducted in monospecific stands, if an individual species response is to be studied (Chapin *et al.*, 1986). Nevertheless, nutrient additions remain the most direct way of determining whether nutrients are limiting to plant growth, and if adaptations providing additional nutrients would have beneficial consequences for plant growth.

Nutrient limitation

Plants of most life-forms have been found to be nutrient limited on coastal dunes: dune annuals (Ernst, 1983; Pemadasa and Lovell, 1974), grasses (Willis, 1965; Hester and Mendelssohn, 1990; Pavlik, 1983), shrubs (Valverde *et al.*, 1997), trees (Hunter and Hoy, 1983). Kachi and Hirose (1983) found that pot and field grown plants had different limiting nutrients. Those authors ascribed this difference to the higher rates of nitrogen mineralisation and lack of leaching, in pots, resulting in sufficient nitrogen, but a lack of phosphorus (Kachi and Hirose, 1983). Other studies have consistently shown nitrogen to be the most limiting element, followed by phosphorus (England: Willis, 1963, Willis, 1965; Japan: Kachi and Hirose, 1983). Hester and Mendelssohn (1990) found that along the South Eastern coast of North America, *Panicum amarum* and *Uniola paniculata*, had increased growth in response to a combined macronutrient addition, but no positive effect was found for micronutrient addition. The fine root production of Virginian barrier island plants was shown to be nitrogen limited, at three different ages of dune succession (Stevenson and Day, 1996).

Valverde *et al.*, (1997) demonstrated that six species of Mexican dune plants showed a large amount of phenotypic plasticity in response to nutrient addition. Plasticity in response to nutrient addition is a characteristic that may not be evident in species from other oligotrophic environments (Chapin *et al.*, 1986). Most studies on dune plant nutrition indicate some degree of plasticity, suggesting that these plants are opportunistic in their uptake of nutrients. However, in the experiment of Valverde *et al.*, (1997), predictable nutrient pulses consistently resulted in greater total leaf areas in comparison to plants treated with the same quantity of nutrients, but at random intervals. Few published nutrient addition studies have been conducted on dune plants in South Africa.

Nutrient limitation and salt spray

Van der Valk (1977) suggested that dune grasses are presented with a Catch-22 situation (Heller, 1961). On dunes, plants need greater soil nutrient contents for optimal growth, but the addition of more nutrients may increase the susceptibility of the plants to salt spray (larger less tolerant leaves). Van der Valk (1977) found no evidence of increased growth due to absorption of salt spray nutrients by the leaves. These findings were in contrast to those of Seneca (1972), whose experiments were less representative of coastal dune conditions than those of van der Valk (1977). *Cakile maritima* and *Salsola kali*, two highly salt tolerant species had a different response to salt spray, where increased growth was found, indicating that the absorption of salt spray ions was beneficial to these nutrient limited plants (Rozema *et al.*, 1982).

An alternative view is that dune plants require nitrogen to tolerate salt spray (Pakeman and Lee, 1991a and b). Highly nitrogen rich osmoticants, protective proteins and enzymes form a part of many dune species tolerance to salinity (Rozema *et al.*, 1985). Thus, dune species, that avoid osmotic stress, and plants that substitute nitrogenous osmotic protectants with carbon-based mechanisms will have a dual advantage over plants that suffer osmotic stress and are nitrogen inefficient.

Nitrogen budgets

The nitrogen resources available to dune plants are determined by the inputs and outputs of nitrogen, and the standing plant biomass of a dune ecosystem. Fog, salt spray (Boyce, 1954), wet deposition in the form of rain, and dry deposition in the form of dust (and sand burial?), biological nitrogen fixation, accumulation of algal wrack (McLachlan and McGwynne, 1986), and litter fall are the major components of the nitrogen entering dune soils. Leaching, denitrification, and loss of litter and sand, by wind, are the major components of nitrogen loss.

Given the importance nitrogen limiting dune plant growth, and the large number of references relating to this, it comes as a surprise to find relatively few studies that measure nitrogen mineralisation rates in dune soils (Fay and Jeffrey, 1992; Kachi and Hirose, 1983), and a similar lack of measurement of available nitrogen, or rates of decomposition. The work of Kellman and Roulet (1990), Cain *et al.*, (1999), Fay and Jeffrey (1992), Ripley (2002), Sun (1999), Skiba and Wainwright (1984), and Gerlach (1993), are some of the studies of nutrient fluxes and nutrient pools within dune soils. None, however, demonstrate a complete picture of the biogeochemistry of nitrogen in dune soils for a particular site.

The major conclusions of these studies are summarised here: Dune soils have heterogeneous spatial and temporal, nitrogen and phosphorus fluxes (Cain *et al.*, 1999). There is a high potential mineralisation of soil nitrogen, and the wet intertidal shore may be the source of the increased nitrogen found in freshly blown sand (Fay and Jeffrey, 1992). *Ammophila arenaria* may use this extra nitrogen for a “reactive” growth response to burial (Fay and Jeffrey, 1992). The dune zonation forms a biomass gradient, potentially resulting in different amounts of available nitrogen at different places along the dune zonation (Ripley, 2002; Ripley and Pammenter, 2004a). Litter fall sequesters nutrients for long periods, with times for total decomposition, ranging from one year to more than three (Sun, 1999; McLachlan and van der Merwe, 1991).

A relatively large amount of nitrogen cycling literature exists for the intertidal zone of sandy shores in the Eastern Cape (McLachlan and Gwynne, 1986; Cockroft and McLachlan, 1993). Surprisingly, the sandy shore intertidal zone recycles a large amount of nitrogen, either in the form of quickly decomposing algal wrack (470gN m^{-2} yr^{-1}; McLachlan and Gwynne, 1986), or nitrogen cycling in the interstitial spaces of the sand (19.7gN m^{-2} yr^{-1}; calculated from Cockroft and McLachlan, 1993, assuming that 590gN recycled by interstitial processes per metre of coastline was for a 30m intertidal zone). Koop *et al.*, (1982) estimated that interstitial bacteria incorporate 94% of the nitrogen released by decomposing kelp, with very little nitrogen returned to the ocean on a short timescale. Thus, the saline interstitial spaces of the shore, and the potentially saline areas of storm debris represent large accumulations of nitrogen, to which dune plants may or may not have direct access. While the large wrack deposition measured by McLachlan and Gwynne (1986) may be considerably higher than those at OWR, this nitrogen source is orders of magnitude higher than any other potential nitrogen source for dune plants.

AIMS

The aim of this section was to synthesise the literature on nutrient dynamics of dunes, and provide a brief description of the nutrient dynamics of the dunes at OWR. The following results are presented for the four selected dune species:

- leaf nutrient contents, and
- soil nutrient levels.

A number of nutrient addition experiments, either with full nutrients or nitrogen were applied to these plants, and are reported elsewhere in the thesis:

- the interaction between burial and nutrient limitation (Chapter 5),
- the effect of dune position, and nitrogen-fixers and on the nutrient limitation of neighbour species (Chapter 6), and
- the source of nutrients used in the burial response of *S. plumieri* (Chapter 7).

METHODS

Shoots of seven individuals of each species (*Arctotheca populifolia, Scaevola plumieri*, *Myrica cordifolia* and *Metalasia muricata*) were harvested at the start of a year-long monitoring experiment. Leaf nutrient contents were measured on the youngest fully expanded leaves (nitrogen: Kjeldahl analysis; phosphorus: Bray extraction and molybdenum blue colorimetric analysis; potassium: ammonium acetate extraction with atomic absorption spectroscopic analysis; Matrocast Laboratories, Capetown). Three samples of soil were collected from the wet sand layer at 10 to 40 cm depth, below homogenous stands of each species. The soil was homogenised and large organic matter removed by sieving using a 1 mm mesh. The soil was analysed for total nitrogen, phosphorus, and potassium in the same manner as the leaf tissue. A sub-sample of this soil was placed in ceramic dishes and weight loss measured after 3 hours at 360^{o}C. It was not expected that the sand contained significant amounts of structural water (associated with clay; Mook and Hoskin, 1982, cited by Craft *et al.*, 1991), or that this relatively low furnace temperature would release carbonates (Davies, 1974, cited by Craft *et al.*, 1991). This estimate was taken to represent the upper limit for soil organic matter content.

RESULTS

Plant and soil nutrients

The soil nutrients measured under stands of each species ranged from: N = 35-39 μg g^{-1} (in comparison to a diverse range of soils: 980-4900 μg g^{-1}), P = 1.7-2.7 μg g^{-1} (diverse range of soils: 3.1-301 μg g^{-1}), K = 16-28 μg g^{-1} (diverse range of soils: 3.9-390 μg g^{-1}) (Table 2.2.2). Harte and Pammenter (1983) compiled the range of nutrient contents for diverse soils. Thus, the dune soils have exceedingly low nitrogen and phosphorus contents, with low potassium contents comparable to other soils. These low nutrient contents corresponded with low soil organic matter contents (Table 2.2.2). The phosphorus contents of the leaves varied between mobile- and stable-dune species, largely resulting in different N to P ratios for these species.

Table 2.2.2: Sand and leaf nutrient contents for un-manipulated stands of four species.

	Sand nutrient content (*n* = 3)				Leaf nutrients (*n* = 4)			
	Organic matter (mg g^{-1})	N (μg g^{-1})	P (μg g^{-1})	K (μg g^{-1})	N (mg g^{-1})	P (mg g^{-1})	K (mg g^{-1})	N:P -
A. populifolia	6.6 ± 1.2ns	37.7 ± 5.1ns	2.3 ± 0.6ns	28 ± 10.4ns	9.1 ± 1.4 ab	1.3 ± 0.4 a	8.6 ± 2.7 b	7.3 ± 1.2 b
S. plumieri	5.4 ± 1.8ns	38.7 ± 3.1ns	1.7 ± 0.6ns	26.7 ± 9.8ns	10.3 ± 2.9 ab	1.3 ± 0.4 a	13.1 ± 2.2 a	9.3 ± 6.3 b
M. cordifolia	7.7 ± 0.7ns	34.7 ± 0.6ns	2.7 ± 0.6ns	19.3 ± 10.2ns	11.6 ± 1.1 a	0.5 ± 0.1 b	5.1 ± 1.2 b	25.9 ± 3.8 a
M. muricata	5.9 ± 0.8ns	36.3 ± 3.1ns	1.7 ± 0.6ns	16.3 ± 3.8ns	7.7 ± 1.1 b	0.4 ± 0 b	5.9 ± 1 b	20.5 ± 1.5 a

Different letters in each column represent significant differences at $P<0.05$, for either one-way ANOVA or Kruskal-Wallis ANOVA by ranks, depending on whether variances of data or transformed data were homogenous (Levene's test). ns, $P\geq0.05$

DISCUSSION

The soils measured under each species had extremely low amounts of nitrogen and phosphorus in comparison with normal soil levels (Table 2.2.1 and 2.2.2). Based upon generalisations of N:P ratios, and the nutrients that limit plant growth in different ecosystems (Güsewell, 2004), the different N:P ratios of the four species (Table 2.2.2), suggest that the mobile-dune species are likely to be nitrogen limited (N:P <10), while the stable-dune species are likely to be limited by phosphorus (N:P > 20). As soil nutrient contents were similar between the areas, in which the species grew, and the species are likely to be limited by different nutrients, this indicates that the species have different nutrient dynamics.

The leaf and phosphorus concentrations of most of the dune species were well below the thresholds of 15 mg N g^{-1} and 1 mg P g^{-1} dry leaf for nutrient deficient crop plants (Chapin and Van Cleve, 1989). A phylogenetically broad study of Australian vegetation of oligotrophic soils, gave average leaf nitrogen and phosphorus values of 10 and 0.8 mg g^{-1}, respectively (Foulds, 1993). These values are comparable to those measured for the dune plants (Table 2.2.2). More broadly, the dune species fall within the lower half of the worldwide leaf economics spectrum of Wright *et al.* (2004). While dune plants may have low leaf nitrogen and phosphorus concentrations, by comparison with highly productive species, in general, the leaf concentrations are not exceedingly low. Other chapters of this thesis further test for nutrient limitation of the four selected species.

2.3. SALT SPRAY, SALINITY AND SEAWATER INUNDATION

INTRODUCTION

Osmotic stress occurs in response to the contact of seawater with a plant's leaves and roots. Thus, three types of osmotic stress may affect dune plants: salt spray, soil salinity, and seawater inundation. Dune soils often have very low soil salinities, comparable to most terrestrial soils (Kearney, 1904). However, decreasing gradients in soil salinity have been reported with distance from the sea (Ishikawa *et al.*, 1995; Greaver and Sternberg, 2007). Thus, the tidemark community (Doing, 1985) may be the most affected by long-term high soil salinity. At shorter time scales extreme tides and storm surges may temporarily increase the salinity of soil, or allow direct contact of the plant with seawater. Osmotic stress, due to salt spray, occurs more frequently, and is correlated with wind speed (Boyce, 1954; de Jong and Barbour, 1979). In general, the adaptations that allow plants to cope with osmotic stress may be similar whether salt is applied as salt spray, salinity, or by physical contact (Rozema *et al.*, 1985). Contrary to this suggestion are the findings of Sykes and Wilson (1989), who found little correlation between salinity and salt spray tolerance. Tolerance of soil salinity may be due to a number of specific adaptations, i.e. salt exclusion mechanisms in roots (Rozema *et al.*, 1985). This section reviews the topic of osmotic stress, and presents limited data on the effects of these factors on Eastern Cape dune plants.

Salt spray

Coastal dunes are subject to high loads of salt spray, which can affect vegetation far inland from the high tide-mark (Avis and Lubke, 1985: 20% of the total salt spray deposited at five sites was deposited at a site 200 m from the high tide mark). According to Boyce (1954), the amount of salt spray is greatly increased at wind speeds exceeding 5 m s^{-1}. This phenomenon has been attributed to the transition between laminar and turbulent flow of wind across the surface of the ocean (Boyce, 1954). This suggestion makes the assumption that salt spray originates predominantly from the open ocean, and not heavily breaking waves, which may be somewhat independent of immediate wind speed. Thus, the salt spray load is dependent, on wind speed, distance from the ocean, direction of the wind, orientation of the plant and the leaves (Boyce, 1954), and the occurrence of dissipative shores (shores with breaking waves).

Oosting and Billings (1942) and Oosting (1945) found a correlation between the salt spray tolerance of a species and the position of the species on a dune zonation. Thus, it was suggested that salt spray is the dominant factor in determining species' zonation. Since the work of van der Valk (1974), burial has also been viewed as an important factor in determining species distributions (Maun and Perumal, 1999; Moreno-Casasola, 1986). Unfortunately, both factors are highly correlated (Donnelly and Pammenter, 1983), presumably due to the similar influence of wind on both factors. Sykes and Wilson (1989, 1990a) measured the responses of a broad range of New Zealand dune species to burial, soil salinity, and salt spray. Species' ordination was significantly correlated to burial and salt spray, with different importances for each factor at different sites (Sykes and Wilson, 1990a; Wilson and Sykes, 1999).

Salinity

Boyce (1954) measured soil salinity at 1 cm and 15 cm depths on coastal dunes in the United States. Salt was accumulated at the sand surface, and was leached out after rains, while at the 15 cm depth, no fluctuations in salinity were observed. There was a small decrease in the salinity of the sand surface with distance from the ocean (Boyce, 1954). Thus, it has been suggested that the majority of the soil column has little salinity, apart from salt spray accumulation on the soil surface, and possibly higher salinity at depth (e.g. Figure 6.2, Ripley, 2002). The most extensive study on soil salinity is that of Sykes and Wilson (1989). These authors found that ordination of vegetation from areas with high precipitation was unrelated to the salinity tolerances of the component species. In drier areas, salinity tolerance contributed to the structure of species ordinations. This effect was attributed to higher rates of leaching in areas of high precipitation (precipitation ranged from 515 to 3455 mm yr^{-1}, between sites). Grasses were the dominant group of halophytes surviving more than 1% salinity (Sykes and Wilson, 1989).

Greaver and Sternberg (2007) found that during the dry season, dune plants (including *I. pes-caprae* and *S. plumieri*) had significant amounts of water in their xylem with an oceanic water isotoptic signature. It remains unclear how this ocean water arrives in the plant, as sea spray seems an unlikely source of up to 52% of xylem water as suggested by Greaver and Sternberg (2007, pp. 219). This result seems at odds with the minimum leaf water potentials measured by Ripley and Pammenter (2004a) for South African *I. pes-caprae* and other dune species. This species had a minimum leaf water potential of -0.98 MPa, well below the expected maximum of -1.25 MPa if the xylem contained 52% seawater (the osmotic pressure of sea water is -2.5 MPa for undiluted seawater; Sperry *et al.*, 1988). Long roots of *I. pes-caprae* and *S. plumieri* extend to below the high tide mark (pers. obs.). Could these roots be accessing interstitial nutrients (McLachlan and McGwynne, 1986), at the expense of taking up some saline water?

Seawater inundation

Seawater inundation is a daily event for saltmarsh and mangrove plants, but occurs rarely for most sand dune plants (Rozema *et al.*, 1985). However, the severity of these extreme events may have a large role in structuring the dune ecosystem. Apart from the physical effects of seawater inundation, the temporary increase in soil salinity, and contact of the plant with seawater, these events may cause damage to plants otherwise unaffected by osmotic stress.

From the 17 to the 19 of March 2007, a large cut off low pressure system resulted in large waves and exceptionally high storm surges along the eastern coast of South Africa (Hunter, 2007). These surges resulted in many instances of waves penetrating the first dune ridge and inundating vegetation to the rear, at Glengariff near Cintsa (pers. obs.). Such overwash events are unusual, and were the most severe in the seven years that the author has been monitoring the coastline at Glengariff. The stable-dune vegetation was severely affected by the overwash events, with many species showing signs of dieback, or death. The damage was associated with unknown combinations of mechanical damage, increases in soil salinity, and the direct effects of salinity on the inundated plants. As the foredunes dominated by *S. plumieri* took the brunt of the physical damage caused by these events, the damage in the stable-dunes was thought to be largely osmotic. These overwash events represented a unique opportunity to determine the differential effects of seawater inundation on many species of dune plants.

A number of studies have observed vegetation changes in response to large overwash events on the barrier islands off the eastern coast of the United States (Schroeder *et al.*, 1979), and England (Harris and Davy, 1986). These events can be so severe that physical destruction, and sand burial (or erosion) by metres are the major causes of vegetation change (Schroeder *et al.*, 1979, and references therein). In these cases, the physical effects of seawater inundation appear to outweigh the potential osmotic effects.

Adaptations to osmotic stress

Numerous adaptations, such as: hypertrophy, succulence, or salt dilution (Rozema *et al.*, 1985), the transport of Cl^- to senescing leaves (Ripley, 2002), salt bladders, epicuticular wax layers (Rozema *et al.*, 1985), root exclusion mechanisms, osmotically active metabolites, protective enzymes and proteins (Hasegawa *et al.*, 2000), and CAM metabolism (Luttge, 1993; El Haak *et al.*, 1997), result in tolerance of higher levels of salt spray or soil salinity for coastal dune species.

Salt avoidance and tolerance have been described for species of CAM plants (Luttge, 1993). Salt avoidance is typified by the presence of root exclusion mechanisms, while tolerance is associated with metabolic and osmotic adjustments (Luttge, 1993). In the coastal dune species, *Euphorbia paralias*, CAM is induced by salinity while salt spray has less of an effect (El Haak *et al.*, 1997). Salt avoidance and tolerance are not restricted to CAM plants: sand dune grasses may fall into the former category (Rozema *et al.*, 1985), potentially explaining the dominance of grasses on temperate dunes, and in areas of moderate salinity (e.g. Sykes and Wilson, 1990a).

Rozema *et al.* (1982) found that two species of sand dune plants were more sensitive to soil salinity than salt spray, terming these plants aerohalophytes. Under some of the salt spray treatments an increase in growth was found, indicating that the absorption of salt spray ions was beneficial to the nutrient limited plants (Rozema *et al.*, 1982; but also see van der Valk, 1977, and Seneca, 1972). The accumulation of methylated quaternary ammonium compounds and other osmotic solutes were suggested as the reasons for the dune species ability to tolerate osmotic stress (Rozema *et al.*, 1982).

Kearney (1904) was one of the first authors to consider that many of the unique characteristics of coastal dune plants may be xerophytic, and not halophytic. At the same time Kearney was well aware of the abundant water status of dune sands: “although [dune plants are] growing in a soil usually amply supplied with water and in an atmosphere often nearly saturated with moisture” (Kearney, 1904). He made the following important points: 1) the characteristics of halophytic and xerophytic plants overlap, and 2) some classical halophytic plants are found on the strandlines of both coastal and lake shores e.g. *Cakile edentula*, and *Salsola kali*. Thus, other reasons must be found to explain why these “halophytic” plants are successful on lacustrine dunes. An important analysis would be to compare the contribution of halophytic plants to the flora of lacustrine and coastal dunes. It is possible that the examples of lascutrine “halophytes” may be exceptional species.

AIMS

In summary, osmotic stress tolerance appears to be an important general adaptation for coastal dune plants, and a prerequisite adaptation to growing on exposed areas of the dunes. The role of osmotic stress in determining succession and species distributions, relative to other factors, seems less certain. This section aims to provide a brief description of salt spray conditions at the OWR study site, and an investigation into the effects of the overwash events of March 2007.

METHODS

Seawater inundation

A survey was made of plants affected by overwash events along two kilometres of coastline north of Glengariff, a month after the overwash event occurred (the event occurred on 19-20 March 2007, and was measured on the 22-23 April 2007). Eight sites were identified, where seawater had penetrated beyond the incipient foredune. All plants that were visibly mechanically damaged were not considered in the survey. Debris from the overwash event was used to assess the extent and severity of the sea water inundation at a site. Plants were surveyed along the edges of this debris, where the physical effects of the surge did not affect plants. It was assumed that the effects of the surge were due to a combination of increased soil salinity and leaves coming into contact with seawater. At each site individuals of each species were surveyed for damage on a 4 point scale: 0 = no visible effect on the plant; 1 = slight effect, dead shoots visible; 2 = major dieback occurred, but new growth visible; 3 = individual dead. An index of the susceptibility of a species to the overwash event was calculated as the mean damage to a species across all sites and individuals (i.e. range = 0-3; 0 = unaffected; 3=dies after overwash event). Species with succulent, or hypertrophy of older leaves were also noted.

Salt spray

Square wooden frames holding a sheet of porous muslin fabric (25 by 25 cm) were placed at a height of 50 cm above five monospecific stands of the four dune species; *A. populifolia*, *S. plumieri*, *M. cordifolia* and *M. muricata*. These salt spray traps were left overnight, for a period of 30 hours on the 30 and 31 August 2004. During this period strong winds (>6 m s^{-1}) blew from both of the predominant wind directions (perpendicular to the orientation of the traps) for ca. 6 hours each, thus these measurements are representative of moderate salt spray conditions. Two salt spray traps were placed on the exposed beach adjacent to the other traps, allowing an estimate of relative salt spray loads across species.

The salt laden fabric was placed in sealable plastic bags, and transported back to the laboratory. A Eutech chloride-ion electrode (EC-CLO-03) was used to determine chloride ion concentration, by soaking the fabric in 100 ml dH_2O for 24 hours, at room temperature. The chloride concentration of unexposed dH_2O and fabric were measured and all data corrected for initial chloride content. The Eutech electrode was calibrated repeatedly during the measurements, and showed no calibration drift.

The leaf production, loss, and areas of the four species for which salt spray loads were measured, were monitored for a year adjacent to the salt spray measurement site (for methods and detailed data see Chapter 8). The production and loss of leaves allowed an estimate of the average leaf longevity for three of the species. The leaf area and longevity data calculated in this manner were used to calculate the salt spray load for leaves of each species. This calculation was based upon the assumption that the salt spray distribution measured over the two days was typical for those sites in the long-term.

RESULTS

Seawater inundation

Severe damage was observed on plants which had been totally immersed in seawater, on plants which were partially submerged, and on plants immediately adjacent to areas of inundation.

There was a highly significant association between the zonation position of a species and the susceptibility of a species to overwash effects (Spearman’s rank order correlation coefficient: $R^2_{spearman} = 0.64$, $n = 14$ species, $P<0.001$). The susceptibility of a species to the effects of salinity was significantly associated with a species ability develop hypertrophy of all, or just older, leaves (Wilcox rank sum test, $W = 110$, $n = 19$ species, $P<0.034$).

Table 2.3.1: Species responses to the osmotic effects of seawater inundation relative to the mean position of the species on transects, and the ability for species to develop leaf hypertrophy.

				Osmotic effect of overwash			
Species	Position on transect[a]	Hyper-trophic leaves?[b]	Effect of overwash (mean)	0: No visible effect	1: Slight effect	2: Dieback with new growth	3: Dead
Species present in transects at OWR and Cintsa West:							
Arctotheca populifolia	12	yes	0.0	5[c]			
Sporobolus virginicus	19	yes	0.3	2	1		
Dasispermum suffructicossum	37	yes	1.0		1		
Ipomoea pes-caprae	39	yes	0.8	3		2	
Scaevola plumieri	42	yes	0.2	4	1		
Isolepis cernua	46	no	0.0	1			
Passerina rigida	71	no	2.0		2	1	2
Cynanchium obtusifolium	72	no	0.7	1	2		
Rhoicissus digitata	78	no	2.0			2	
Chyrsanth. monilifera	80	yes	1.7		1	2	
Carpobrotus deliciosus	84	yes	1.7		1	2	
Metalasia muricata	85	no	2.5			2	2
Rhus crenata	89	no	2.0			1	
Mimusops ovata	97	no	3.0				5
Species not present in transects:							
Hydrophylax carnosa	na	yes	2.0			1	
Plantago crassifolia	na	yes	0.0	1			
Cyperus natalensis	na	no	0.5	1	1		
Stenotaphrum secundatum	na	no	0.0	1			
Juncus kraussii	na	no	1.0		1		

[a] ten transects, 0 = start of vegetation, 100 = thicket/forest; [b] species that show ability to form succulent leaves, diluting intracellular salt concentrations; [c] the number of individual plants suffering from a category of osmotic stress

Salt spray

Salt spray traps placed within stands of stable-dune species received 18% of the salt spray that mobile-dune species received (Table 2.3.2). Assuming the salt spray collected on the traps was representative of leaf Cl^- deposition, then daily Cl^- deposition corresponds to 1-5% of the leaf mass. If these results are extrapolated to deposition over the leaf lifespan, then considerably more Cl^- is deposited on the leaves of stable-dune *M. cordifolia*, than the mobile-dune species (Table 2.3.2). Leaf longevity was not calculated for *M. muricata*, as this species has tiny ericoid leaves. The methods for calculating the leaf longevity of species are further presented and discussed in Chapter 8.

Table 2.3.2: Chloride ion deposition at points on dunes corresponding to species position. Deposition rates where measured for 5 points within homogenous stands of each species, over a 30 hour period, with both prevailing wind directions blowing for half that period. Data are shown as means with the standard error of the mean. Letters represent significant differences between species, given by Tukey HSD *post hoc* tests.

		Cl^- deposition		
Position	Species	μmol cm^{-2} day^{-1}	Cl^- mass $leaf^{-1}$ day^{-1} as % of leaf mass	Total mass of Cl^- accumulated on a leaf over its lifespan as a % of leaf mass
Mobile-dunes	*A. populifolia*	19.1 ± 2.0 b	4.2 ± 0.6	479 ± 52
	S. plumieri	26.4 ± 2.7 a	4.19 ± 0.4	829 ± 83
Stable-dunes	*M. cordifolia*	5.3 ± 1.1 c	1.3 ± 0.3	1599 ± 341
	M. muricata	2.8 ± 0.2 c	na	na
	Exposed beach	29.7 ± 0.5	na	na
Mobile/Stable-dune species =		18%		

DISCUSSION

Seawater inundation

Individuals immediately adjacent to the overwash event were often dead or damaged, indicating that the effects of the overwash event were not entirely associated with the direct effects of inundation, but possibly with changes in soil salinity. The susceptibility of species to the overwash event correlates strongly with the position that species occupy on in a dune zonation ($R^2_{spearman} = 0.64$). The susceptibility index was also correlated to the ability of a species to produce hypertrophic leaves (Table 2.3.1). Thus, this trait appears to be important for dealing with a number of salt related factors: soil salinity, inundation by seawater, and salt spray (Rozema *et al.*, 1985).

The movement of Na^+ and Cl^- ions through the transpiration stream is partially limited by the active transport necessary for ions to cross the root endodermis (Hasegawa *et al.*, 2000). Grasses may have effective exclusion mechanisms in the roots, and as a consequence no dune grasses may need to develop leaf hypertrophy. Many salt tolerant dicot's have highly succulent older leaves, due to an accumulation of Na^+ and Cl^- in the vacuoles. However, this adaptation requires the formation of new vacuoles, and thus higher growth rates (Hasegawa *et al.*, 2000). A large number of nitrogen containing compounds are needed to tolerate osmotic stress: glycine betaine, proline, and dimethyl sulfonium compounds (Hasegawa *et al.*, 2000). A sizable proportion (7-8%) of the total nitrogen in osmotically stressed plants can be made up of glycine betaine (Pakeman and Lee, 1991b). Thus, for this nitrogen limited environment, a large proportion of nitrogen may be invested in mechanisms allowing salinity tolerance. Nitrate has been shown to increase the growth of plants under saline conditions (Ehlting *et al.*, 2007). Possibly compounding the nutrient limitation, the intake of K ions into cells is limited at high Na ion concentrations (Hasegawa *et al.*, 2000). However, this effect is not apparent in studies aimed at detecting limitation of plant growth by potassium (Willis, 1963, etc), possibly as potassium is relatively abundant in seawater, and dune soils (Rozema *et al.*, 1985).

These results indicate that the zonation of species on South African coastal dunes is likely to be related to more complex dynamics than simple burial related tradeoffs. However, the unusual nature of this overwash event, and the lack of determination of what specific factor caused damage, limits the interpretation of these results.

Salt spray

Salt spray loads measured above stands of stable-dune species were lower than those measured for species growing on the mobile foredune (Table 2.3.2). This relative pattern is consistent with other studies (Table 2.3.3), although the absolute magnitude of salt spray was low by comparison. Therefore, the calculation of accumulated salt spray over the lifespan of a leaf is necessarily tenuous (Table 2.3.2). However, this calculation illustrates a potentially interesting point concerning salt spray regimes on coastal dunes. Namely: mobile-dune species had 88% shorter leaf longevities than the stable-dune species, *M. cordifolia*. Therefore, all else being equal, *M. cordifolia* must receive less than 12% of the amount of salt spray that the mobile-dune species receive in order for the leaves of this species to encounter less salt spray over the lifespan of its leaves. Measurements over a windy 30 hour period found that stable-dune species received 23% of the salt spray that mobile-dune species received. If this proportion is representative of longer term salt spray distributions, *M. cordifolia* would receive more salt spray over the lifespan of its leaves than mobile-dune species. Donnelly and Pammenter (1982) measured salt spray under similar dune conditions finding a ratio of 5 to 18% depending on the amount of wind. A number of studies find similar ratios (van der Valk, 1974; see Table 2.3.3), and others lower ratios (Avis and Lubke, 1985; Wilson and Sykes, 1999). Therefore, a number of studies have stable- to mobile-dune salt spray ratios, with which mobile-dune species would accumulate less salt on their leaves than stable-dune species. Considering the lack of long term measurements of salt spray, in this study, and the effect of rain on leaching salt off leaves, the values given for the amounts of accumulated salt spray over a leaf lifespan are not likely to be representative. These values serve to illustrate the point that salt spray accumulation must be considered relative to the lifespan of the leaf.

Table 2.3.3: Salt spray loads measured at various sites, by a number of authors. The large variation in the absolute amount of salt spray is likely to be due to differing wind conditions and measurement procedures. However, the calculation of the relative difference in salt spray loads between stable and foredune habitats allows for proportional comparisons.

Reference	Site	Situation	Cl^{-} [e] μmolcm^{-2}day^{-1}	Stable/Mobile %
This study	OWR, SA	Mobile-dune	19-26	18%
		Stable-dune	2.8-5.3	
Donnelly and Pammenter, 1982	Durban, SA	Front of foredune	170-7400[a]	18%-5%[a]
		Rear of foredune	30-400[a]	
van der Valk, 1974	Cape Hatteras, USA	Front of foredune	16.0-18.6[b]	22%
		Back of foredune	2.6-4.9[b]	
Avis & Lubke, 1985	Kleinemonde, SA	Front of foredune	762000[c]	9%
		Dune slack	69000[c]	
Wilson & Sykes, 1999	Cole Creek, NZ	Foredunes	-	3.4%[d]
Greaver, 2005	Florida, USA	Foredune	43.3	26%
		Backdune	11.2	

[a] averages for a calm and windy day, respectively

[b] no units are given for the measurement period thus these units are μmol cm^{-2} time^{-1}

[c] there was an obvious unit problem in the data reported by Avis and Lubke (1985), the reported units are 6.9-76 mol Cl^{-} dm^{-2} day^{-1}, however this problem should not influence the relative salt spray of stable to foredune.

[d] measured as salinity of runoff from gauze traps for an entire year period.

[e] Cl^{-} content of salt spray was calculated for Greaver (2005) and van der Valk (1974) as a ratio of 1:0.68, Cl: Na, as reported by Donnelly and Pammenter (1982).

The factors determining leaf salt spray loads are complex. The accumulation of salt spray on the leaves of different species is determined by leaf size, length to width ratio, orientation, and position in the canopy (Boyce, 1954). It is surprising that of all the studies referenced (Table 2.3.3), only that of Greaver (2005) measured salt deposition on intact leaves, rather than with a salt spray trap. Few studies, including this one, measure salt spray loads for periods longer than a single day (but see Wilson and Sykes, 1999). No studies measure the amount of accumulated salt absorbed by the leaf, or removed by precipitation. These factors limit the extrapolation of local results to determine the effect of salt spray on dune plants. Clearly more work is needed assessing patterns of long term salt accumulation in the leaves. In particular, there is not necessarily a strong link between accumulation of salts in senescing leaves and salt spray. What proportion of leaf salt accumulation derives from saline soils, or alternatively salt spray?

Is osmotic stress an important factor for determining plant distribution on coastal dunes?

If it is: Does osmotic stress have differential effects on coastal dune species? Or: Is tolerance to osmotic stress a general prerequisite for survival on coastal dunes? While osmotic stress is clearly important, there is little unequivocal data that compares this factor to the importance of other factors, such as burial. The data that does exist is generally from species-poor greenhouse studies. Exceptions are the speciose data sets of Wilson and Sykes (1999). A brief overview of our current knowledge of the influence of osmotic factors on plant distribution is reviewed here.

A number of studies find that species position on dune zonations correlate with the salt tolerances of species (salinity, Ishikawa *et al.*, 1995; salt spray, Oosting, 1945). Ishikawa *et al*. (1995) found that water availability increased, salinity, and evaporative demand decreased with distance from the shore, corresponding to the zonation of salt tolerant and less tolerant species. Sykes and Wilson (1990a) found two informative PCA axes combining 10 salinity, salt spray, and burial related factors, for 29 New Zealand dune plant species. The first osmotic-related PCA axis explained 33% of the variation in the zonation of species, while the second burial-related PCA axis explained 19% of the variation. Potentially, the strong salinity/salt spray axis could result in zonation of dune species with distance from the sea. But, as with burial, such an explanation of zonation lacks a reason for the exclusion of tolerant species from areas distant to the sea.

Is tolerance to osmotic stress a general prerequisite for survival on coastal dunes?

Barbour and de Jong (1977) found that 10 out of 12 coastal dune species have greater than three times the tolerance to salt spray of a non-coastal species, indicating that Californian dune species may have a general tolerance to salt spray. In contrast, the species studied by Oosting (1945) displayed a range of tolerances to salt spray. Sykes and Wilson (1989 and 1990a) found that only half of New Zealand dune species had greater tolerance to salinity, or salt spray, than a control glycophyte; *Triticum* (wheat). Wilson and Sykes (1999) studying sites of different rainfall, found site-specific evidence for the influence of salt spray and salinity on the distribution of New Zealand coastal dune plants. The importance of osmotic stress is dependent on the species selected for study, and is site specific due to variation in: physical protection from salt spray (e.g. headlands), salinity gradients, rainfall, and the potential for extreme overwash events to influence stable-dune vegetation. The link between plant physiology (termed osmotic stress) and soil salinity, salt spray and inundation by sea-water deserves more attention (Rozema *et al.* 1985).

The relative importance of salt spray, salinity, and sand burial in determining zonation on South African coastal dunes

The following points would indicate that salt spray, or salinity, are important, but not primary causes of zonation on coastal dunes in South Africa:

1) If salt spray is the primary cause of zonation on the coast of South Africa, then it would be expected that rocky headlands would have equivalent species' zonation to adjacent dune areas. Along the South African coast there are many rocky areas with little elevation, but complete sand stabilisation, such that thicket vegetation grows immediately adjacent to the storm watermark. The proximity of this thicket to the heavy salt spray generated by the rocky shore may result in this area having higher salt spray loads than adjacent sandy bays. Many of these salt tolerant thicket species are common to dune thickets. This distribution would suggest that thicket canopy species have appreciable

tolerance to salt spray, but are limited to landward areas on the dunes due to some other factor,

2) This study finds that the leaves of seaward foredune species may be exposed to less cumulative salt spray than landward species, due to these species having considerably shorter leaf life spans. This finding suggests that the leaves of foredune species may effectively have to deal with less salt loads than landward species, as a consequence of fast growth rates,
3) Many of the species considered are found well above any zone of inundation. However, the zonation of some species does correspond to species tolerances of to osmotic stress related to overwash events.

Regardless of the relative importance of burial or salt spray in determining zonation on coastal dunes, an important aspect of dune ecology is the mechanism of salt tolerance of coastal dune species. A holistic viewpoint is advocated by many authors, where numerous factors co-limit dune species distributions (Maun and Perumal, 1999; Barbour and de Jong, 1977). Certainly, Eastern Cape dune species have adaptations that allow them to survive osmotic stress, and the vulnerability of species to seawater inundation relates to species’ zonation.

2.4. WATER AVAILABILITY AND STRESS

INTRODUCTION

A number of authors have suggested that the low soil water contents characteristic of dune sands are limiting to plant growth (e.g. Kearney, 1904; Salisbury, 1952). More recent literature has clearly established that adult plants growing on many temperate and subtropical dune systems are not water limited (de Jong, 1979; de Jong and Barbour, 1979; Pavlik, 1985; Ripley, 2002; Ripley, 2004a and b). Water suction curves (soil water potential at a range of soil water contents) indicate that while sands have low water contents relative to other soils, much of this water is available to plants at normal water potentials (Rundel and Jarrell, 1989). Models of soil water budgets demonstrated that even relatively arid areas (615 mm mean annual rainfall at OWR) have sufficient water to supply the low leaf area index dune plants with water in dry years (Ripley and Pammenter, 2004a). Thus, the trend for coastal dunes to have a low biomass (e.g. Barbour and Robichaux, 1976), may result in greater water availability for established plants than previously thought (Ripley and Pammenter, 2004b). It remains to be assessed whether stable-dune areas of higher biomass have a greater water use, and thus show water stress. This idea is reasonable, as Peter *et al.* (2003) demonstrated that the distribution of *S. plumieri* was limited by the amount of seasonal rainfall.

AIMS

The aim of this section was to investigate the *in situ* water status of typical mobile- and stable-dune species and assess whether these species have hydraulic characteristics capable of withstanding water stress.

METHODS

***In situ* plant water status**

Predawn (<5:30AM) and midday (14:00-16:00) water potentials were measured on five shoots of two mobile- (*Arctotheca populifolia* and *Scaevola plumieri*) and two stable-dune species (*Metalasia muricat*a and *Myrica cordifolia*). Measurements were made on 7 February 2004, using a custom built Schölander-type pressure bomb. Soil water content samples were collected at 25 cm depth, adjacent to the plants sampled for water potential measurements. Soil (>200 g) was placed in sealable plastic bags and transported to the laboratory in cool containers. Soil water content was calculated as:

$$SWC = \frac{\left(W_f - W_d\right)}{W_d}$$

where, W_f was the fresh weight of the soil, and W_d was the dry weight of the soil after desiccation in a 60°C oven for three days.

Pressure volume curves

Shoots adjacent to those measured for predawn and midday water potentials were harvested, re-cut under water, and re-hydrated in water in cool containers until laboratory pressure volume curves could be made (overnight). Once in the laboratory, each shoot was dried off, and weighed (fresh weight). The weight of each shoot was measured before estimating a corresponding shoot water potential. Each shoot was measured repeatedly (instantaneous weight), after periods of drying, such that a time series of decreasing shoot relative water contents, and corresponding water potentials, were measured. Upon completion of a measurement at -3 to -3.5 MPa the shoots were dried in a 60°C oven until constant mass, and reweighed (dry weight). The shoot relative water content (*RWC*) was calculated as:

$$RWC = 1 - \frac{\left(W_f - W_i\right)}{\left(W_f - W_d\right)}$$

where, W_f was the fresh weight of the shoot before measurements were taken, W_i the instantaneous weight of the shoot immediately prior to a water potential measurement, and W_d the dry weight of the shoot.

Pressure volume curve analysis

Pressure volume curves establish a relationship between the water potential (Ψ) of a shoot and the relative volume of water that the shoot has lost (Koide *et al.,* 1989). Pressure volume curves allow the calculation of the changing osmotic and tension pressures that a shoot experiences with increasing drought stress (Koide *et al.*, 1989). The pressure volume curve may show that a plant has hydraulic and osmotic characteristics that allow the plant to avoid turgor loss.

The reciprocal of the measured water potential plotted against RWC results in a linear relationship for low values of RWC, and corresponds to the osmotic component of the pressure volume curve (Koide *et al.*, 1989). A line ($1/\Psi = a*RWC+b$) was fit to the linear portion of this relationship, normally the 3 to 8 points with the lowest *RWC*. Osmotic potential at full turgor (π_i), where *RWC* is 1, can be solved for using this line as it represents osmotic potential (π). Hence:

$$\pi = \frac{1}{a*RWC+b}$$

The turgor potential (*P*) can be calculated for each measured water potential by:

$$P=\Psi-\pi$$

where π is calculated for the *RWC* measured corresponding to the water potential measurement. The turgor loss point, defined by *RWC* and water potential, was taken as the measured point where the calculated turgor potential was less than 0.05 MPa.

The relative amount of apoplastic and symplastic water can be calculated as the intercept of the osmotic potential line with the *RWC* axis. As this intercept must be extrapolated beyond the measured data points, it is susceptible to large shifts with small outlying data points. Thus, the value of this significant point is often unreliable, and for this reason was excluded from the analysis.

RESULTS

Soil and shoot water status

The soil below stable-dune species had significantly lower soil water content to soils below mobile-dune species, or from un-vegetated stable-dune areas (Figure 2.4.1b). This difference was not evident in the shoot water potentials of the two mobile- and stable-dune species (Figure 2.4.1a).

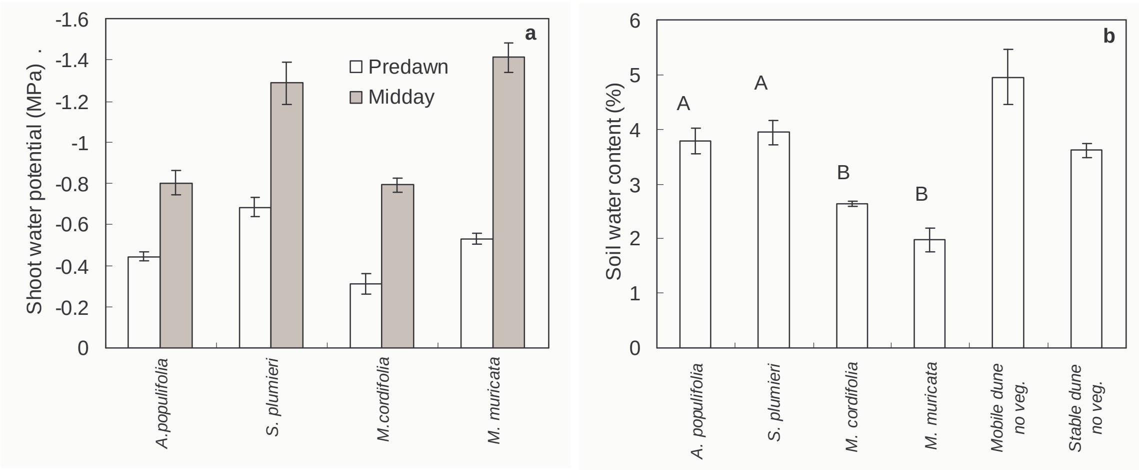


Figure 2.4.1: Predawn and midday water potentials (a), and soil water contents (b)**,** measured on a summer day for four species of dune plants. Bars are standard error's of the mean (n = 5 for all samples). Letters indicate a significant difference measured using Tukey's HSD *post hoc* test, for a one way ANOVA including only species (areas of no vegetation had n = 2).

Pressure volume curves

S. plumieri had the steepest (most elastic) pressure volume curves of the four species (Figure 2.4.2). The other three species displayed similar curves (Figure 2.4.2). The mobile-dune species displayed turgor loss at high RWC's in comparison with the stable-dune species (Figure 2.4.3c). At the turgor loss point, *A. populifolia* had the greatest water potential, while *S. plumieri* the least water potential (Figure 2.4.3b). The two species with the lowest osmotic potentials (2.4.3a), *S. plumieri* and *M. muricata*, also had the lowest midday water potentials (Figure 2.4.1a). None of the species studied had predawn, midday, or osmotic potentials as low as those measured for partly drought stressed coastal dune plants, or desert plants (Table 2.4.1). These results are confirmed by the two year period of measurements undertaken by Ripley (2002), for two of the study species at OWR (Table 2.4.1). The midday water potentials of the four study species were between 20 and 50% higher than the osmotic potentials at the turgor loss point (Table 2.4.1). There were significant differences between species only for the osmotic potentials at full turgor (one-way ANOVA, $P <$ 0.03).

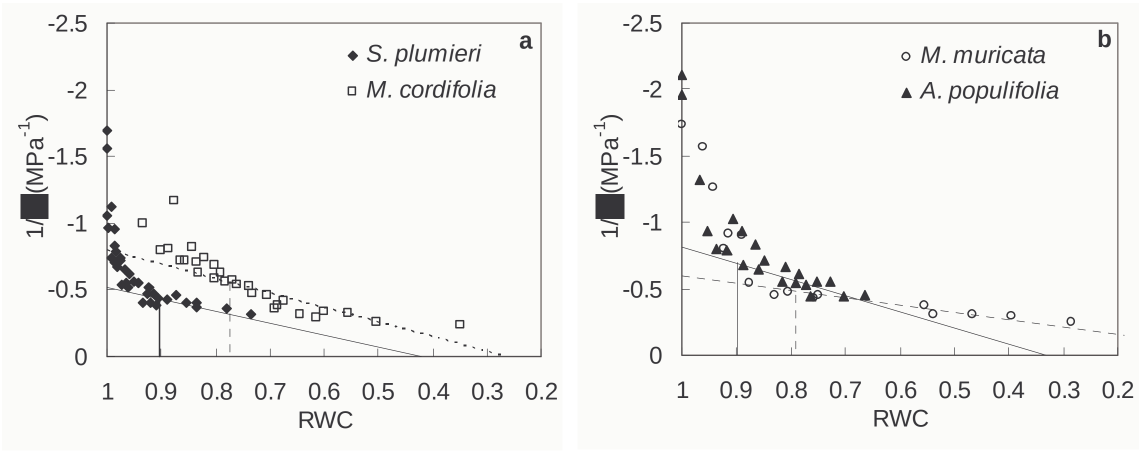


Figure 2.4.2: Pressure volume curves for four species of mobile (closed symbols, solid lines) and stable-dune plants (open symbols, dashed lines), measured as the reciprocal of water potential and relative water content. Diagonal lines represent the osmotic component of the PV curve, and are extrapolated back to the RWC axis to find the relative apoplastic water content, and the pressure axis to find the osmotic potential at full turgor. The vertical lines indicate the turgor loss point.

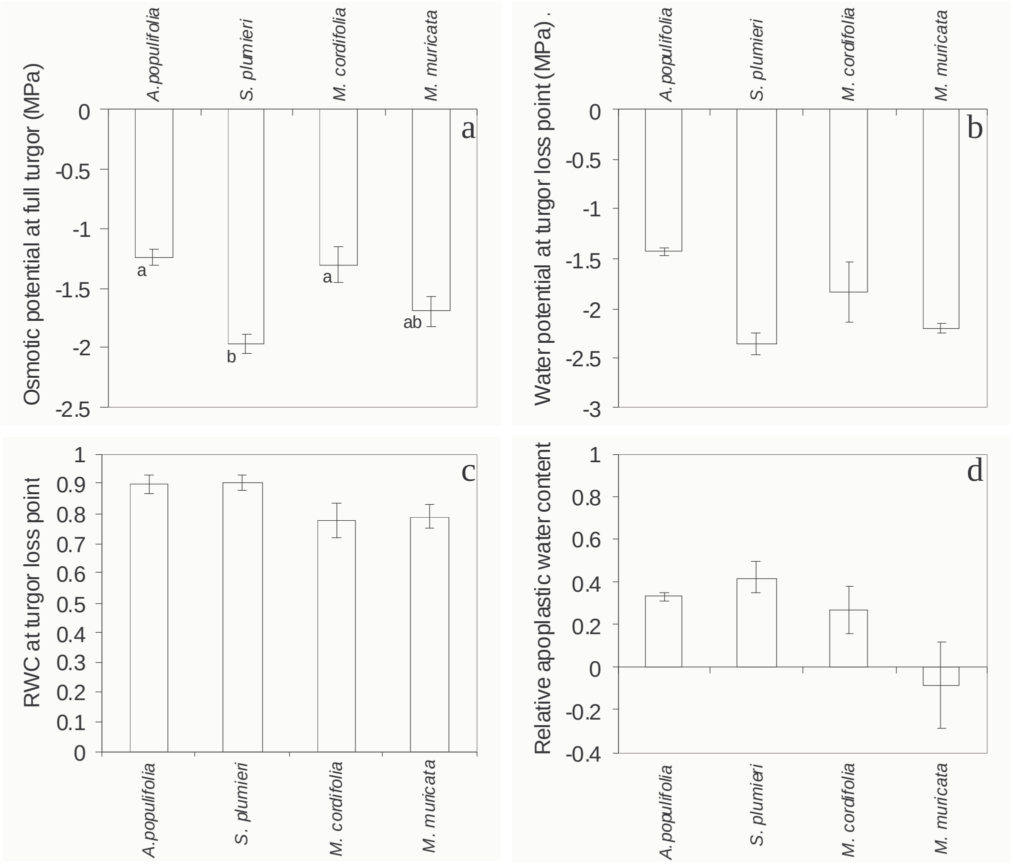


Figure 2.4.3: The important hydraulic parameters calculated from the pressure-volume curves presented in Figure 2.4.2. The parameters are: the osmotic potential at full turgor (a), the water potential at the turgor loss point (b), the relative water content at the turgor loss point (c), and the relative apoplastic water content (d). Error bars represent standard errors of the mean (n = 3). Apart from the osmotic potential all other parameters were not significantly different between species.

Table 2.4.1: Pressure volume and other hydraulic components of plants from coastal dune, desert, and mesic habitats.

Habitat	Rainfall (mm)	Species	Predawn water potential MPa	Minimum daily water potential MPa	Osmotic potential at full turgor MPa	Osmotic potential at turgor loss point MPa	Apoplastic water content Prop.	W_f/W_d Ratio
Saltmarsh,	in pots	*Suaeda calceoliformis* [a]			-1.3 to -1.6	-1.9 to -2.0	0.86 to 0.89	8.2 to 8.6
Sonoran desert	~200	*Atriplex polycarpa* [b]	-1.8 to -3.1		-2.5 to -3.0	-3.0 to -3.9		
	~200	*Olneya testa* [b]	-1.6 to -2.4		-2.2 to -2.9	-2.9 to -3.7		
	~200	*Larrea tridentate* [b]	-1.7 to -4.5		-2 to -2.7	-3.0 to -3.7		
	~200	*Baccharis sarothrioides* [b]	-1 to -1.6		-1.5 to -2.0	-2.0 to -2.7		
	~200	*Encelia farinoa* [b]	-1 to -2.9		-1.7 to -2.3	-2.2 to -3.2		
Temperate forests	mesic	6 Angiosperm tree species [c]	-0.05 to -0.2					
		6 Conifer tree species [c]	-0.04 to -0.6					
Coastal dunes	1796	*Ammophila arenaria* [d]	-0.1 to -0.2	-0.9 to -1.5	-1.25 to -1.5	-1.6 to -2.0	0.45 to 0.58	
	1796	*Elymus mollis* [d]	-0.1 to -0.2	-1.1 to -1.65	-1.4 to -2.0	-1.7 to -2.4	0.51 to 0.55	
	1325	*Ipomoea pes-caprae* [e]			-0.26 to -0.30		0.16 to 0.22	
	1325	*Coccoloba uvifera* [e]			-1.2 to -1.3		0.63 to 0.80	
	820	*Atriplex leucophylla* [f,g]	-0.2 to -1.4	-0.2 to -2.3				
	390	*Atriplex leucophylla* [f, g]	-0.3 to -2.1	-0.4 to -3.2				
	820	*Cakile maritima* [g]	-0.25 to -0.8	-0.65 to -1.6				
	390	*Cakile maritima* [g]	-0.4 to -2.0	-0.6 to -2.8				
	390	*Abronia maritima* [g]	-0.7 to -1.6	-1.1 to -2.3				
	390	*Ambrosia chamissonis* [g]	-0.2 to -0.6	-0.3 to -1.9				
	618	*Arctotheca populifolia* [h]		-0.81 to -1.31	-1.24	-1.31		
	618	*Ipomoea pes-caprae* [h]		-0.75 to -1.17	-1.48	-1.65		
	618	*Scaevola plumieri* [h]		-1.47 to -1.60	-1.46	-1.61		
	618	*Arctotheca populifolia* [i]	-0.4 to -0.5	-0.6 to -0.9	-0.81	-1.4	0.33	19.6
	618	*Scaevola plumieri* [i]	-0.6 to -0.8	-1.0 to -1.4	-0.51	-2.4	0.42	7.5
	618	*Myrica cordifolia* [i]	-0.2 to -0.5	-0.7 to -0.9	-0.79	-1.8	0.27	4.0
	618	*Metalasia muricata* [i]	-0.4 to -0.6	-1.3 to -1.7	-0.59	-2.2	na	3.7

[a] Youngman and Heckathorn, 1992; [b] Monson and Smith, 1982; [c] Becker *et al.*, 1999; [d] Pavlik, 1985; [e] Greaver and Herbert, 2004; [f] DeJong and Barbour, 1979; [g] DeJong, 1979; [h] Ripley, 2002; [i] This study

DISCUSSION

Is coastal foredune vegetation water limited?

Two forms of evidence indicate that vegetation growing on non-arid coastal foredunes is not water limited. Firstly, almost all of the soil water is available to plants, and is rarely reduced to drought levels. Secondly, dune plants from non-arid areas do not usually show signs of water stress, and possess few of the characteristics distinctive of drought tolerance (Table 2.4.1). These statements are supported by a broad range of literature as follows: Soil water potential is reduced to drought levels at extremely low soil water contents (<1%), resulting in virtually all soil water being freely available to plants (Rundel and Jarrell, 1989). A number of authors find that soil water is rarely drawn down to drought levels (e.g. van der Valk, 1974). The soil water content of Lake Huron dunes was rarely less than 3.5% during the growing season (Baldwin and Maun, 1983). The minimum soil water potential, at depth, of mesic and arid Californian dunes, was above -1.5 MPa through out the growing season (de Jong and Barbour, 1979).

During four years of monitoring dune plants at OWR none were observed to be wilting, although wilting is particularly obvious in these species when a shoot is excised. This observation is confirmed by the two year monitoring experiment of Ripley (2002), at this site. Three foredune species where shown to never reach the turgor loss point during this period (Ripley, 2002). Similar data remains to be collected for stable-dune species. The water potential and pressure-volume curve characteristics measured in this study were similar to a range of mesic, but not xeric species (Table 2.4.1). This indicates that these dune species have hydraulic characteristics which would be unable to cope with water stress conditions. A previous study by the author at OWR (Gilbert, 1999) demonstrated that the two stable-dune species *Chrysanthemoides monilifera* and *Mimusops caffra* had a greater range of daily water potentials (less than -0.3 to greater than -1.6 MPa) than *S. plumieri* (from -1.3 to -1.5 MPa). Furthermore individuals of *C. monilifera* growing at different points along the dune zonation (at 3 and 10 metres above sea level) had similar leaf water potentials through out the day. Such preliminary results indicate that mobile- and stable-dune plants may not have considerably different water availabilities.

Three obvious exceptions must be mentioned, the dry surface layer of sand has been found to limit the growth of seedlings (Maun, 1994), arid areas have species with characteristic drought-tolerant physiologies (C_4 dicot's, and CAM plants, Johnson, 1977 and 1982), and the distribution of species may relate to the seasonality and quantity of rainfall (Peter *et al.*, 2003). The latter example, of the South African distribution of *S. plumieri*, is particularly interesting, as the distribution of this species appears to be limited by low amounts of summer rainfall (Peter *et al.*, 2003).

Is there a gradient of water availability across a dune zonation?

A number of studies have measured soil water content in different areas of the dune zonation. The zones with the highest cover often have the highest soil water contents. For instance, the thicket transition zone had 92% cover, and the highest soil water contents (Baldwin and Maun, 1983). Other studies confirm this trend: for higher biomass areas to have greater soil moisture contents (Sykes and Wilson, 1991; Stevenson and Day, 1996; Lichter, 1998; Wilson and Sykes, 1999). However, this trend is paralleled by increasing organic matter content (Sykes and Wilson, 1991; Lichter, 1998), and thus higher capacity to hold water. This trend is not representative of the soils studied here, which had little trends in the amounts of non-particulate organic matter (Table 2.2.2).

Pressure volume curves of stable-dune species were remarkably similar to the mobile-dune species (Figure 2.4.2), particularly when it is considered that the species range from herbaceous to woody plants. The reduced water content of soil from under stands of stable-dune species, was above drought levels, and did not result in lower water potentials for stable-dune plants (Figure 2.4.1). The pressure volume curve results indicate that stable-dune species are no more tolerant of desiccation than mobile-dune species. However, the data presented here are not sufficient to conclusively state that higher biomass stable-dune plants are not water limited. An explanation for the availability of water in the high biomass stable-dune zone may be that these species have low transpiration rates, relative to mobile-dune species (Chapter 8). Thus, these two areas may have similar rates of water loss per ground surface area. This reduction in transpiration may have important consequences, such as lowered photosynthetic rates, and therefore, a lack of ability to grow fast in response to burial. The vertical distribution of roots, total rooting volumes, and rooting density will all affect the amount of water available to plants of different species. Therefore, there are a number of unmeasured factors that would greatly influence the water available to perennial stable-dune plants.

Are the traits of foredune plants xero-, meso-, or halophytic?

Classically xeromorphic leaf characteristics, such as succulence, C_4 and CAM metabolism, and thick cuticles are contrasted by dune plants also possessing mesophytic characteristics, like high transpiration rates and large leaves (Barbour *et al.*, 1985). Dune soils are generally low in moisture content, but the majority of this water is freely available, and has rarely been shown to reduce to drought levels (e.g. Ripley and Pammenter, 2004a). Thus, these results support the hypothesis that adult dune plants, from mesic climates are not often water stressed. Therefore, many of the leaf characteristics otherwise associated with xeromorphy are more likely to be halophytic adaptations. Leaf succulence for instance, correlates well with species tolerance of seawater inundation (Table 2.3.1). A second reason that many foredune species have thick semi-succulent leaves may be related to light, and high photosynthetic rates per unit leaf area. Leaves with a thick double palisade layer are called heliophytic by Barbour *et al.* (1985). It has been shown that this leaf anatomy allows *I. pes-caprae* to maximise light interception, by absorbing the considerable amounts of light reflected by the sand (Greaver and Herbert, 2004). Species with similar anatomy are: *S. plumieri* (this study Chapter 8), *Abronia maritima*, *Convolvulus soldanella* and *Franseria bipinnatifida* (Purer, 1936). A consequence of this anatomy, may be a low internal diffusion of CO_2, requiring the leaves to be amphistomatous, contributing to these leaves having high transpiration rates. The unusually high stomatal conductances measured for South African coastal dune species (Chapter 8 and Ripley, 2002), also appear at odds with the interpretation that these species have adaptations to conserve water.

Few dune species have hydraulic characteristics of xerophytic plants. These traits include, mesic leaf water potentials (Table 2.4.1; Figure 2.4.1a; de Jong and Barbour, 1979; Pavlik, 1985; Ripley, 2002), and typical mesophytic pressure volume curves (Figure 2.4.2; Pavlik, 1985; Ripley, 2002). In conclusion, foredune plants do not have xerophytic traits, but do have some halophytic traits.

SECTION 3: ZONATION OF COASTAL DUNE SPECIES

Ecophysiological explanations of dune zonation: Testing whether differences in species carbon and nitrogen economies can result in zonation on coastal dunes in the Eastern Cape

Chapter 3

Introduction to ecophysiological explanations of zonation

Zonation and succession — Review of factors causing dune plant zonation — Overview of the three chapters within this section

INTRODUCTION

Plant zonation and succession on Eastern Cape coastal dunes

The spatial change in the dominance of species on partially vegetated coastal dunes in the Eastern Cape is striking (Figure 1.1, and Plate 3.1), but the general position of species and the appearance of the dunes are thought to remain the same in the long-term. Sequential changes in species dominance over space, rather than time, distinguish zonation from succession. Thus, the coastal dunes in the Eastern Cape are examples of zonation. Dune succession occurs along prograding coastlines, such as parts of northern Kwa-Zulu Natal (Weisser and Backer, 1983), and results in similar spatial species sequences to those found along the Eastern Cape coast (see comparison in Chapter 1). In other ecosystems zonation and succession have been found to occur by similar mechanisms (Smith and Huston, 1989). Thus, these two processes may be comparable.

Sand burial (Chapter 2.1), high storm water levels, and salinity or salt spray, which form gradients of decreasing severity from the sea towards the land, are likely to play a role in maintaining the zonation sequence. As a result of such factors, coastal dune zonation has been referred to as “arrested” succession (Carter, 1995). The degree of stasis is dependent on the process by which the zonation is maintained. For instance, the observed zonation at OWR could be formed by the daily prevention of stable-dune species from colonising the mobile-dunes by the harsh environmental gradients. Salinity and salt spray may be examples of such influences. Alternatively, periodic disturbance events in the mobile-dune zone may kill any individuals of typical stable-dune species that may have become established in between disturbance events. Storm overwash events or unusually large burial or erosion may result in such dynamics. The former may be called an “arrested” succession, while the latter case clearly has some successional dynamics in which the successional state is periodically returned to an earlier state by an extreme disturbance. In the context of dune succession, Carter (1995) refers to this as regressive succession (also see Kent *et al.*, 2001).

Without long-term studies of vegetational change, the nature of zonation and successional dynamics is difficult to establish. Indeed, many important studies of dune succession which measure spatial changes make the assumption that observed zonation (often assumed to be a chronosequence) is representative of a temporal successional process, (e.g. Avis and Lubke, 1996; Franks, 2003; Feagin *et al.*, 2005; Lichter, 1998, 2000; van der Putten *et al.*, 1993), a number of studies do measure temporal changes (e.g. Allen and Allen, 1988; Martinez *et al.*, 2001; Morris *et al.*, 1974). As this assumption is prevalent in the large body of evidence for dune successional mechanisms, it is surprising that it is not acknowledged more often.

Inverse trait correlations

The resources a plant can access are limited. The quantity and quality of traits to which resources can be allocated is also limited (Bloom *et al.*, 1985). This results in plants allocating resources to different sets of traits, and consequently reacting to environmental factors in different ways. A series of traits to which resource allocation is limited is referred to as an inverse trait correlation (McCook, 1994), or a trait trade off. An example is the worldwide leaf economics spectrum of Wright *et al.* (2004), in which all plants invest limited carbon and nitrogen in a range of leaf properties. At the extremes, leaves are short lived, highly productive and poorly defended, ranging to long lived, slowly productive and well defended. Inverse trait correlations that differentially affect the ability of plants to respond to an environmental factor may result in species’ zonation, or succession (Huston and Smith, 1987). In addition, such trait correlations often provide a mechanism for the exclusion of “early” successional species from “later” areas.

Plate 3.1: Photograph of the zonation of dune species at Old Woman's River mouth. The dominant plant in the lower biomass zone (the mobile zone) is *Scaevola plumieri*, with *Ipomoea pes-caprae* (the vine), and *Arctotheca populifolia* (short light grey herb) present. The zone of higher biomass (stable zone) is characterised by *Myrica cordifolia* (the short dominant shrub), *Metalasia muricata* and *Stoebe plumosa* (taller lighter-coloured shrubs).

A number of traits have been suggested to affect the ability of a species to respond to burial (see Chapter 7 for discussion of many of these traits), salinity and salt spray, competition, or herbivory. Less often have these traits been shown to have an associated inverse trait correlation.

Differential responses of species to environmental factors result from a physiologically and phylogenetically limited array of plant traits. The inverse trait correlations that cause dune succession are likely to result in differential responses of species to the environmental gradients that cause zonation. Thus, the same physiological mechanisms are likely to underpin dune succession and zonation (Smith and Huston, 1989). The zonations found along the Eastern Cape coast are likely to be maintained via similar environmental factors, and mechanisms, to those that form dune successions at Mtunzini. Hence the spatial similarity of the species sequences at the two sites (Chapter 1).

A review of both the succession and zonation literature is presented here to outline possible causes of the observed zonation on coastal dunes in the Eastern Cape. Three reasons are given for the inclusion of successional theory in this introduction: 1) authors in the coastal dune literature use the terms zonation and succession interchangeably, 2) A discussion of dune zonation would be poorer without reference to the larger body of literature on dune succession, and 3) the zonation of plants on coastal dunes in the Eastern Cape appears to be similar to the zonation produced by successional processes on prograding coastlines, suggesting that similar processes may be affecting the plant distributions.

A distinction should be made between the different ecological processes present on coastal dunes and those in dune slacks. Dune slacks are characterised by variable water levels, and distinct vegetation relative to dunes (Avis and Lubke, 1996). At the main study sites, OWR and Cintsa West, and the example of succession at Mtunzini, dune slacks are largely absent, and for this reason the dynamics of this system were not included in this thesis. Nor are any of the zonation and succession explanations, included here, intended to explain dune slack succession (see Grootjans *et al.*, 1998 for a review of dune slack ecology).

Succession and zonation on coastal dunes

Coastal dunes are used as textbook examples of succession (Smith, 1996; also see Begon *et al.*, 1996, and McNaughton and Wolf, 1973), and were the first ecosystems that were extensively studied with relation to succession (Cowles, 1899; Oosting and Billings, 1942; Olson, 1958). However, the mechanisms whereby dune succession or zonation occur remain controversial (Dech and Maun, 2006). In particular, interactions between the co-varying environmental factors that determine species distributions remain to be studied. At least five major factors have been suggested as primary determinants of zonation or succession (reviewed below): burial, salt spray or salinity, facilitation and competition, nutrient dynamics, and soil pathogens and/or mycorrhizae. This section looks at three of these environmental factors and the interactions between the factors: burial responses and competition for height amongst all species found along the dune zonation (Chapter 4), the interaction between nutrient availability and the burial responses of four dune species (Chapter 5), and the nutrient dynamics of two species, and the influence of nitrogen-fixation by *Myrica cordifolia* (Chapter 6).

OVERVIEW OF FACTORS THAT MAY CAUSE SUCCESSION AND ZONATION

Plant succession can occur via many different mechanisms (McCook, 1994), however, three general succession models have been proposed to describe the basic characteristics of the many examples of succession (Connell and Slatyer, 1977). The facilitation, tolerance and inhibition models of succession refer to the interaction that the coloniser species have with species that subsequently arrive at the site. These interactions are considered as net responses, not in a mechanistic manner (McCook, 1994). Thus, facilitation may occur via any of the many possible inverse trait correlations, or relate to any of the many resources that may limit growth. As a result, McCook (1994) stresses the need to determine causal mechanisms of succession in addition to assigning a general model to a particular example. The facilitation model of succession has most frequently been applied to dune successions (Connell and Slatyer, 1977). This model suggests that coloniser species are able to grow at a new site, facilitating the growth of other species unable to colonise the site. Once the environment has been modified by the coloniser species, the secondary species are able to out-compete the initial coloniser species.

Sand burial

Filtering of species unable to respond to burial seems the most likely general explanation of the distributions of species on dunes (van der Valk, 1974). Maun and Perumal (1999) found a strong correlation between the burial responses of 13 species and the position of these species on a dune succession. Similarly, a study of 11 moss species demonstrated that the position of a species on the dune zonation was related to the tolerance of the species to burial (Martinez and Maun, 1999). A strong relationship between sand movement and species succession or zonation has been found in both temperate and subtropical dune ecosystems, suggesting that sand movement is an important factor in dune succession (Dech and Maun, 2006; Martinez *et al.*, 2001).

Connell and Slatyer (1977) suggest that the vegetation dynamics of coastal dunes may be good examples of their facilitation model of succession, but went further to suggest that there is little experimental evidence for this suggestion. Subsequent studies have found support for a number of the criteria necessary for the facilitation model to hold: 1) that only burial tolerant coloniser species are capable of growing in “early” successional disturbed areas (Maun and Perumal, 1999), and 2) that these species reduce the amount of sand burial thereby making this environment more suitable for burial sensitive “late” succession species (Moreno-Casasola, 1986). Martinez *et al.* (2001) found that colonisers must gain a significant cover before other species are able to live in mobile-dune areas, suggesting some moderation of the environment must occur before species succession takes place. The final criterion of the facilitation model; that of a negative interaction between “late” and coloniser species, remains to be integrated into this model of dune succession or zonation. Little evidence is available to show that the “late” succession species have negative effects on colonisers resulting in competitive exclusion. As a minimum we need an explanation of why the coloniser species are excluded from later areas of the dune successions or landward areas of dune zonations.

A number of traits could allow particular species to respond to burial: e.g. plastic biomass allocation responses (Maun, 1998), and fast growth rates associated with a short leaf longevity (Ripley and Pammenter, 2004b). Such traits may limit burial responsive species from occupying other areas of the dune zonation e.g. the low soil nutrients of stable-dune areas may preclude species which rapidly loose their leaves, and nutrients, requiring species with leaf longevity. Thus, the missing negative interaction between “late” and coloniser species could result from unequal competitive interactions due to unknown inverse trait correlations (McCook, 1994). The inhibition of *Ammophila arenaria* growth, by a soil pathogen complex, is a possible candidate for the missing negative interaction between “late” and coloniser species (van der Putten *et al.*, 1993), although limited to one or a few species.

Nitrogen and nitrogen-fixing plants

Nitrogen is the most limiting nutrient in dune ecosystems (Willis, 1965; and literature cited in Chapter 2.2), since potassium, and micronutrients are abundant in seawater and salt spray (Pakeman and Lee, 1991b, Rozema *et al.*, 1985). The distribution of nitrogen is heterogeneous in nitrogen-form, in time and space, but generally increases in quantity and decreases in variability with distance from the sea (Cain *et al.*, 1999). Corresponding with zonation sequence from sea to inland, different areas have varying amounts of available nitrogen. Strandline accumulations of washed up macroalgae are large sources of nitrogen for plants growing in seaward positions (Pakeman and Lee, 1991a and b). Large foredune species may also benefit from this nitrogen-supply by extending roots seaward (pers. obs.). However, in general, foredune species are nitrogen-limited (literature cited in Chapter 2.2). Landward areas may have nitrogen-fixers, or increasing standing biomass, these represent greater quantities of nitrogen, and due to increased soil organic matter, greater nitrogen retention (Kellman and Roulet, 1990).

Nitrogen-fixing plants are often large or dominant species in many coastal dune areas, world-wide (Doing, 1985; e.g. *Acacia* spp., *Canavalia* spp., *Casuarina* spp., *Chamaecrista chamaecristoides, Myrica* spp., and *Strophostyles umbellata*). The salinity inhibition of the legume and actinorhizal nitrogen-fixing symbiosis (Cordovilla *et al.*, 1994; Sande and Young, 1992) may limit these species distributions to areas landward of the influence of increased soil salinity (Erickson and Young, 1995), but they may be tolerant of salt spray (Griffiths and Orians, 2003). Exceptions do exist, such as *Casuarina equisetifolia* which had increased biomass production and nodulation at low to moderate soil salinities (Ng, 1987). As dune plant growth is generally nitrogen limited, and nitrogen-fixing species can enhance local soil nitrogen levels (Vitousek and Walker, 1989), nitrogen-fixers have the potential to enhance the growth of neighbouring plants. Such facilitation has been shown for *Myrica pensylvanica* which enhanced the growth of *Ammophila breviligulata* and *Solidago sempervirens* (Shumway, 2000). Thus, landward areas of dune zonation may be locally altered by the presence of nitrogen-fixing species (Morris *et al.*, 1974).

Thus, a secondary influence on species distributions may be nutrient dynamics, in particular, nitrogen-fixing species. Nutrient limitation may have interactive effects on the burial responses of species. As dune plants are nutrient limited, adaptations that reduce the amount of nutrients needed to elongate stem or replace leaf area would be beneficial when responding to burial. The interaction between burial and nutrient limitation has been studied for greenhouse grown *Cakile edentula* (Zhang, 1996), for which nutrients had no effect on this species burial response.

Recently, many grass species have been shown to have endophytic associations with nitrogen-fixing bacteria (Dalton *et al.*, 2004). A number of common dune species like

Ammophila arenaria and *Elymus mollis* are included in this group, potentially explaining the high rates of growth of these species on the nutrient limited dunes (Dalton *et al.*, 2004). Older literature, including acetylene reduction analyses, both confirm and disagree with this finding for *Ammophila arenaria* (e.g. Fay and Jeffrey, 1992; Abdel Wahab and Wareing, 1980), possibly due to the reported heterogeneity of nitrogen reduction activity (Dalton *et al.*, 2004).

Salt spray and salinity

Adaptations allowing survival of high osmotic stress play a role in the zonation of dune species (see Chapter 2.3), but a number of reasons suggest that this factor may not be the primary determinant of zonation on the dunes of the Eastern Cape coast (see further discussion in Chapter 2.3). In general, most coastal dune species appear to have considerable tolerance of salt spray (of 12 dune species measured by Barbour and De Jong, 1977, 10 had considerable tolerance of salt spray) relative to a non-coastal species. Thus, tolerance of salt spray may be considered as a general adaptation to living on coastal dunes as a whole, and not necessarily determining zonation patterns. A number of important questions about salt spray and salinity remain to be answered:

1) the leaf hypertrophy of many dune plants could be salt spray or salinity related adaptations, or in arid areas a drought adaptation, or a combination of all three. What proportions of the salt content of a succulent leaf derives from the soil-water, and salt spray?
2) Are plants with long leaf longevity more susceptible to salt accumulation than plants with short lived leaves?

Soil pathogens and mycorrhizae

Van der Putten *et al*. (1993) found that the build up of soil-borne pathogens that were specific to two dominant dune stabiliser species, result in the degeneration of these species with time. Similarly, Van der Stoel *et al*. (2002) found that a short-term build-up of pathogens in newly colonised soil was correlated with a decrease in *Ammophila arenaria* growth rate. It has further been suggested that this phenomenon allows *Ammophila arenaria* to increase growth after burial, as the newly deposited sand layer contains no pathogens (de Rooji-van der Goes *et al.*, 1998). Alternatively, Little and Maun (1996) found that the growth of *Ammophila breviligulata* was not affected by nematodes in the presence of mycorrhizae, or after burial. This study contradicts the previous studies, but the authors maintain that nematodes may not explain the burial response of *Ammophila*, but in conjunction with soil fungal pathogens, and beneficial mycorrhizae these may play an important role in the dune ecosystem (Little and Maun, 1996). A subsequent study showed that the effect of soil fungi, and nematodes on the growth of *Leymus arenarius* was not removed in the presence of mycorrhizae (Greipsson and El-Mayas, 2002).

While these studies are highly relevant to the dynamics of dune succession, none have been conducted *in situ*. This is an important distinction, as pot experiments, especially on plants from such extreme conditions as coastal dunes, are microcosms of a much more complex dune environment. As Little and Maun (1996) point out these experiments are also contingent upon soil sterilisation and pot growth not having an undue effect on the growth of plants and soil organisms.

Knevel (2001) conducted pot transplant experiments on three dune species from the area of this study (including the non-invasive *Ammophila arenaria*). These species had specific nematode fauna, but lacked a consistent increase in growth when grown in soil taken from under other species, or in sterile soil (Knevel, 2001). For the two native species soil sterilisation had no influence on growth, while for *Ammophila arenaria* soil sterilisation resulted in a decrease in growth. These results suggest that soil pathogens, or mycorrhizae, play a small or even positive role in dune species growth. Thus, it remains unclear what role soil-pathogens play in determining dune zonation in the Eastern Cape.

Nucleation, facilitation and competition

Franks (2003) suggested that increased seedling germination and accumulation of seeds of other species under established plants results in facilitation allowing new species to colonise stressed and disturbed areas. Martinez (2003) found that *Chamaecrista chamaecristoides* allowed two late coloniser species to germinate and survive in more extreme conditions due to increased shade and protection. Franks and Peterson (2003) tested 8 species of dune plants, in Florida, for an overall presence of competition and facilitation. The presence of other plants, of any species, resulted in an increase in growth in buried plants, but had no effect on unburied plants (Franks and Peterson, 2003). Thus, no competition was detected, even for high planting densities, while facilitation occurred when plants where buried. Silander and

Antonovics (1982) tested dune species in North Carolina, for interspecific interactions between a number of dune species, using a species perturbation/removal approach. They found that removal of two dominant dune species had both positive and negative effects on the remaining species, and this was site specific. That study, however, made no suggestion about what the nature of these interactions were. Thus, the nature of interspecific plant interactions is poorly understood, with a general lack of tests of specific mechanisms, apart from those related to nucleation. While nucleation *per se*, is unlikely to determine dune zonation in the Eastern Cape, the competitive and facilitative interactions of plants, must play an important role in determining species presence or absence.

Herbivory

Even herbivores have been cited as possible causes of coastal dune succession. Herbivory caused by wallabies on Australian dunes affected species differentially, reducing the establishment of some foredune species (Ramsey and Wilson, 1997). Tortoise beetles (*Aspidomorpha deusta*) and caterpillars (*Aedia leucomelas*) or burial were shown to affect the growth of the dune coloniser, *I. pes-caprae* (Bach, 1998). However, when both factors occurred burial reduced the effects of the herbivory (Bach, 1998). The differential grazing of rabbits could result in changes in zonation on dunes in the United Kingdom (Boorman and Fuller, 1982). Personal observations (2003-2007) at OWR found that herbivores browsed extensively, in a highly species-specific manner, on the dune zonation (bushbuck, *Tragelaphus scriptus*, on *Metalasia muricata*; porcupine, *Hysterix africaeaustralis*, and tortoise beetles, *Aspidomorpha deusta*, on *I. pes-caprae*; tortoises, *Chersina angulata*, on tide-mark *Arctotheca populifolia*; and *Imbrasia cytherea* caterpillars on *M. cordifolia*). Such herbivory may be catastrophic for the browsed plant as it has reduced resources to deal with other disturbances, such as sand burial. As herbivores are often species specific, dune plants are nitrogen-limited, and many chemical defences against herbivory are nitrogen-based (Craine *et al.*, 2003), the role of herbivory in coastal dune ecology deserves more attention. Dune species' zonation does occur in close proximity to developed areas, or areas where less herbivory may occur. Therefore, it is likely that the effect of herbivory on dune zonation is more species specific than general. Herbivory modulates established dune zonations.

Other factors

Wind, light competition, and water may all affect the zonation of dune species.

Gale force winds (>17.2 m s^{-1}) are common along the coastline of the Eastern Cape, while calm days are rare (1 in 600 days, Chapter 2.1). In addition to causing sand movement and salt spray, strong winds result in increased transpiration and physical damage. Although coloniser species are short, and may gain some boundary layer protection, the strongest winds are found seaward of the foredunes, where these species occur. Lortie and Cushman (2007) found that wind is a major cause of species performance on coastal dunes. They, surprisingly, related the effect of wind to its physical effects and size sorting of the sand which affects soil moisture and nutrient capacity. While this is a potentially interesting and new view on dune zonation, the study did not account for wind causing both salt spray and burial. Thus these results could equally be accounted for by differential species responses to either salt spray or burial.

Dune zonation is typified by increasing vegetation height, leaf area index (*LAI*), and biomass with distance from the sea (Figure 1.1). Competition for light, nutrients, and water are likely to increase along this gradient. It is unclear what role these gradients have in determining dune zonation.

De Jong (1979), Pavlik (1985), Ripley (2002), found little evidence for water stress in dune coloniser species. Indeed, Hester and Mendelssohn (1989) found that the foredune dominant grass, *Uniola paniculata*, was more severely affected by water logging than drought. In part, the older literature cites water stress as an important factor in dune ecology (Salisbury, 1952; Ranwell, 1972). This may be due to conflation of xeromorphic adaptations with those of halophytic adaptations, such as leaf succulence/hypertrophy (Kearney, 1904), and CAM metabolism. Few studies investigate whether high biomass stable-dune species show signs of water stress. Rainfall quantity, in conjunction with the season in which rainfall occurs, appears to limit the distribution of *S. plumieri* along the South African coast (Peter *et al.,* 2003; Robertson *et al.,* 2003). Rainfall may be important were strong seasonal or low rainfall occurs (Peter *et al.,* 2003), or high biomass develops (Ripley and Pammenter, 2004a). Water is not a likely factor in coloniser species physiology at OWR (Chapter 2.4; and Ripley, 2002), but may become more important at higher biomasses in the stable-dunes. If this is the case, the higher stable-dune biomass may drawdown soil water content to lower soil water potentials and to drought levels more frequently. Thus, it is possible that stable-dune species require traits that allow more negative xylem water potentials and less vulnerability to cavitations, in comparison to dune colonisers.

Finally, even fire has been attributed to influencing dune zonation: “Healthy cottonwood potentiate marram’s inhibition through fertilization by easily decomposed leaves whereas senescent cottonwood indirectly facilitate succession by acting as lightning rods that start fires which remove the strong inhibition effect of thick marram litter” (Poulson, 1995).

What causes dune zonation?

The succession of dune systems in Virginia can be successfully modelled using a facilitation model, with unspecified mechanisms or environmental gradients (Feagin *et al.*, 2005). Suggestions have been made that succession occurs in response to salinity or salt spray (Barbour and De Jong, 1977), burial (Maun and Perumal, 1999; Moreno-Casasola, 1986), nitrogen-fixing plants (Shumway, 2000; Morris *et al.*, 1974), and soil pathogens (van der Putten *et al.*, 1993; van der Stoel *et al.*, 2002). Most authors conclude that a single factor is insufficient to explain zonation as a whole (e.g. Barbour and De Jong, 1977; Wilson and Sykes, 1999). In the special case of lacustrine dunes, burial in conjunction with differing species traits were sufficient to explain zonation, as salinity is absent from this system (Lichter, 2000, and Dech and Maun, 2006).

These numerous factors are all likely to affect dune zonation. Beyond conducting important correlative studies (Sykes and Wilson, 1990a), experimental investigations of dune zonation are limited to investigating a few factors at a time. Interactions between factors are of great interest, and not often studied experimentally. Thus, the interactions between light competition and burial, burial and nutrient limitation, and nutrient limitation and nitrogen-fixing plants were investigated using correlative or manipulative experiments.

The following hypotheses were tested in the three chapters of this section:

1) Could the inverse correlation between plant burial responses and the ability to grow tall result in the zonation of dune vegetation?

The high rates of burial and the steep decrease in burial with distance from the beach suggest that burial is potentially a major factor determining zonation at OWR and Cintsa West (**Chapter 2**). Thus, the traits that correspond to burial tolerance across all species found in the zonations were studied (**Chapter 4**). An inverse trait correlation was hypothesised between burial and competitive traits, potentially resulting in differential responses to burial and competition, and thus species' zonation.

2) Contrasting growth, allocation and photosynthetic responses of four coastal dune plant species are related to the rates of natural sand burial and nutrient limitations

The interaction of the high burial and low nitrogen levels was investigated to determine whether species, at different positions along the zonation, had varying burial responses based upon nutrient limitation (**Chapter 5**).

3) On the effects of nitrogen-fixation on the growth of two species of dune plants along the dune zonation

The presence of two nitrogen-fixing species, and extremely low levels of soil nitrogen, could result in nutrient-patch dynamics. Differing growth due to higher nitrogen levels near stands of nitrogen-fixers may modulate the burial responses of otherwise nutrient limited species. Thus, these two species may secondarily modify the proposed zonation patterns caused by burial and light competition. The effect of nitrogen-fixation on the growth of non-fixing plants was determined by comparing plants growing within and distant to stands of the nitrogen-fixer, *Myrica cordifolia* (**Chapter 6**).

Chapter 4

Could the inverse correlation between plant burial responses and the ability to grow tall result in the zonation of dune vegetation?

Facilitation model of dune plant succession: Is this adequately described? — Proposal of the stem tissue density model explaining mechanism of facilitation and reciprocal abilities of plants in different areas of the dune succession — Experimental evidence for stem tissue density determining: burial elongation rate of stem, maximum height of growth of a species, and stimulation of growth of species in response to burial — An inverse trait correlation — A discussion of the mechanism of dune species succession

"[T]he steep lee slopes of the slowly advancing dune-complex are first captured by social perennial herbs like *Ammophila* and *Asclepias*. Then shrubs like *Cornus*, *Salix*, and *Prunus* grow up and gradually drive out the herbs which grew there first, because they are ill-adapted to the shade." Cowles (1899)

INTRODUCTION

In Chapter 1 comparable species sequences were observed at OWR, Cintsa West (Figure 1.1), and Mtunzini. These sequences were thought to have resulted from spatial changes in environmental conditions (zonation at OWR and Cintsa West), or temporal changes in the environmental conditions (succession at Mtunzini; Weisser and Backer, 1983). This zonation or succession of plant species from shore landwards remains poorly explained for dunes on the eastern coastline of southern Africa. However, the observed zonation correlates with a number of environmental factors, such as sand burial and the light environment measured as competitor cover and height (Chapter 1, Figure 1.1). Thus, the following question is asked: Can a simple (parsimonious) model of dune zonation be formulated, that explains a large amount of the variation of species distributions? If such a model is possible, then what other factors explain the residual variation of species distributions?

This chapter attempts to provide a zonation model, incorporating two environmental factors and a number of plant traits, that allow species to specialise in different areas of the dune environmental gradient.

Facilitation model

Coloniser species are able to survive in an extreme environment, these species modify the conditions so that other species may grow there, thus facilitating the change of species with time. The ‘later’ species may have greater competitive ability eventually excluding the colonisers. This general scheme was suggested as the facilitation model of succession (Connell and Slatyer, 1977). This model has been used to explain coastal dune succession, but Connell and Slatyer (1977) suggested that little evidence was available to test this hypothesis. Since this suggestion, much effort has been made to answer this question, and undoubtedly dune zonation or succession is much better understood. However, two areas lack clarity: many different mechanisms and environmental factors have been suggested to result dune zonation or succession, and which combination of these is most important remains unclear. In this section a plausible mechanism of succession, an inverse trait correlation, and two environmental factors explaining a large degree of variation in the observed zonation were investigated. Other chapters of this thesis demonstrate that other factors such as salinity (Chapter 2.3) and nutrient dynamics (Chapter 5 and 6) were also important in determining growth and species distribution in this ecosystem.

Inverse trait correlations

Species specialise; one species is generally unable to have suitable traits to occupy all positions in an ecosystem. As a result of limited resources, species are restricted to allocating resources to a few traits. This limitation causes the formation of inverse trait correlations between species, which allocate resources to different combinations of traits (McCook, 1994). Species’ zonation is then formed by such inverse correlations in response to severe environmental gradients. A similar example to the one discussed in this section are the dynamics of trees in tropical forests. Gap pioneer species grow fast, and approach biomechanically critical heights due to low wood densities and wood mechanical strength. Shade tolerant species, however, have high wood densities, resulting in slow growth, but high persistence and stem strength (see van Gelder *et al.*, 2006 about tree mechanics, and Muller-Landau, 2004 concerning growth rates). Inverse trait correlations are important as these often provide a mechanism whereby zonation or succession can occur.

Proposal of a dune zonation model: the stem tissue density hypothesis

A major factor in determining the zonation of species on coastal dunes is the differential responses of mobile- and stable-dune species to burial (Maun and Perumal, 1999). The following mechanism is proposed to answer the questions: How are mobile-dune species able to respond to burial? Why are these species excluded from more stable areas of the dunes?

The reduced carbon costs associated with lower stem densities, given similar stem diameters, may allow mobile-dune species to have high stem elongation rates, and survive burial. Alternatively, stem tissue densities may be reduced in response to burial, allowing higher elongation rates. Lower stem tissue densities result from greater proportions of stem tissue being made up of less lignified cell types such as parenchyma (Niklas, 1995). Lignification increases the material strength (and decreases the elasticity) of stems (Niklas, 1995), thus low density stems are associated with lower material strength (and greater elasticity), and consequently can grow to lower potential heights, for a given stem diameter. Therefore, an inverse trait correlation is proposed between stem elongation rates and plant stature, based upon the relationships of these traits with stem tissue density. This correlation may result in differential abilities of species with low and high stem tissue density to respond to burial or compete with tall vegetation.

The theoretical determinants of stem elongation rate and plant height need more elaboration. A detailed introduction to the concepts used to define the stem tissue density hypothesis is given below.

Stem tissue density

An important concept of stem mechanics is the difference between the bulk or fresh stem tissue density (ρ_{wet}), and dry mass stem tissue density (ρ_{dry}). Dry mass stem tissue density gives a good indication of the carbon cost of stem production, an important burial-related production characteristic, while bulk stem tissue density relates to the amount of weight a stem supports, a mechanical characteristic. For this reason, a link must be made between these two measures of stem tissue density. Fortunately, these two parameters are strongly correlated ($R^2 = 0.68$; Van Gelder *et al.*, 2006).

Stem elongation rate

Increased stem elongation rates are an important response of partially buried plants (Maun and Lapierre, 1984; Maun, 1998). Factors that enhance stem elongation would result in shoots maintaining a greater photosynthetic leaf area, fitness, and survival, under burial conditions. Enhanced elongation rate can be constitutive or inducible, depending on the phenotypic plasticity of the species (e.g. Gartner, 1991). The majority of stem mass is likely to be made up of carbon (roughly 45% of leaf dry weight is carbon, and stems have considerably more carbon than leaves, Whittaker and Marks, 1975), thus, dry stem tissue density largely represents the carbon and energy cost of producing a set volume of stem. Therefore, a reduction in stem density represents a reduction in the cost of energy, carbon, and other elements or compounds, for a given amount of stem elongation.

Theoretically, the potential rate of stem elongation for a shoot (l_{elong}, cm 30 days^{-1}), is dependent on the rate of biomass allocation to stem growth (B, g 30 days^{-1}), the dry mass density of the stem (ρ_{dry}, g cm^{-3}), and the diameter of the stem at the level of elongation (e, cm). Thus, stem elongation rate for a given allocation of stem biomass can be estimated as:

$$l_{elong} = \frac{\mathrm{B}}{\rho_{dry} * csarea} = \frac{\mathrm{B}}{\rho_{dry} * \pi * (\frac{e}{2})^2} \qquad \text{eqn 4.1}$$

An important feature of this equation is that lower stem tissue densities result in disproportionately larger stem elongation rates. As stem mechanical elasticity relates to stem diameter and tissue density, these two parameters are highly related.

A trade-off between woody density and shoot growth rate has been observed in the forestry literature (Downes *et al.*, 2002), tropical forest ecology (Muller-Landau, 2004), and for liana’s or climbing plants (Darwin, 1867; den Dubbelden and Verburg, 1996). Thus, it is well established that stem density relates to production, but this has not been recognised in the coastal dune literature. A number of other correlations have been observed between stem tissue density and *RGR* (negative, Huante *et al.*, 1995; Castro-Diez *et al.*, 1998), the weight of roots relative to total biomass (negative, Huante *et al.*, 1995), herbivory (Loehle, 1996), hydraulics (Hacke *et al.*, 2001; Taneda and Tateno, 2004), strength and height (positive, Van Gelder *et al.*, 2006; weakly negative, Preston *et al.*, 2006).

What determines plant height?

Three models of stem mechanical function have been suggested to determine the height to which a plant can grow, depending on the characteristics of the supportive stem (Niklas, 1993a). The elastic similarity or stress similarity, and the geometric similarity models are thought to determine the maximum heights of woody and non-woody plants, respectively (Niklas, 1993a), and the allometry of animal bones (McMahon, 1973; Biewener, 2005). However, Niklas (1993a) found a large amount of variation in the scaling of plant height, leading Niklas to suggest: “Accordingly, subscription to the empirical relevancy of any one of the three scaling models, or to hypotheses derived from first principles which purport to explain why plant height scales according to one of these models, are viewed as highly suspect.” In addition, Niklas (1993a) suggested that this variation was due to the false assumption that all plants are made of the same tissue.

An alternative explanation of height scaling relationships is that of Niklas and Spatz (2004) who suggest that growth and hydraulics limit plant height. The current study attempted to avoid these pitfalls, by: 1) correlating stem tissue density directly to stem and height parameters rather than fitting a specific model, 2) searching for hydraulic constraints related to stem characteristics, and 3) comparing a broad range of species for which generalisations were possible. Niklas (1993a) found that the relationship of height to diameter for extant vascular plant species was scaled linearly, on average, allowing broad generalisations regardless of mechanical model that best describes individual species. For the data mentioned in Niklas (1993a; Figure 2) the differences in the woody and non-woody species were apparent for non-woody plants greater than 4 metres in height. As no non-woody dune species reach such heights, this reduces the effect of not adopting a particular model. Alterative models of what determines plant height to diameter scaling relationships are given by Niklas and Spatz (2004).

Geometric similarity model

The geometric similarity and elastic similarity models are described below, to allow an insight into possible determinants of plant height. The elastic similarity model is then used to demonstrate the implications of changes in stem density on a possible inverse correlation between stem elongation and plant height. These models are not used to evaluate the stem tissue density hypothesis in the discussion, but serve as demonstrations of how an inverse correlation between stem tissue density and elongation rate could occur.

The geometric similarity model of plant height suggests that mechanical considerations are not necessary for short structures, the reason that this model is often applied to non-woody plants and small animals (Norberg, 1988). By maintaining a geometric similarity (proportional relation between height and diameter), a simple construction algorithm is used for growth, and implies various interactions between competing plants (Norberg, 1988). As far as this author is aware, this growth model does not depend on stem tissue density or stem mechanical strength, beyond the obvious cost of growth.

The elastic similarity model

The elastic similarity model is the most widely used mechanical model of plant height, and is applied to the majority of tall plants in most environments (woody trees). This principle suggests that the structure of a plant is maintained in such a manner that all components are mechanically stable, with some degree of safety (McMahon, 1973). The height to which a beam-like plant can grow, depends upon the strength, shape, and cross-sectional area of the stem, and the amount of weight the stem supports. This weight is proportional to the bulk (fresh) tissue density of a stem (ρ_{wet}), a large component of which is water (44-90% for measurements done on a range of species studied here, similar to the data of Van Gelder *et al.*, 2006).

The formula for the critical length of a vertical column at which the column buckles under its own weight (l_{crit}) is (McMahon, 1973):

$$l_{crit} = C\left(\frac{E}{\rho_{wet}}\right)^{\frac{1}{3}} d^{\frac{2}{3}} \qquad \text{eqn 4.2}$$

Where E is the modulus of elasticity, ρ_{wet} is the fresh density of stem, d the diameter of the column, and C a constant that depends on how weight is distributed along the column (e.g. whether the column tapers or is cylindrical). E may be considered a function of ρ_{wet}, a measure of the quantity of water and structural tissue per volume of stem. The nature of this function is important in determining whether changes in ρ_{wet} affect plant height, as a strictly proportional relationship would result in no change in the term E/ρ_{wet}. Thus, in order for increases in ρ_{wet} to have an effect on plant height, E must relate allometrically, or linearly (with an intercept), to ρ_{wet} (see Appendix 2 for an explanation). McMahon (1973) assumed that E/ρ_{wet} remains constant, however, this ratio was shown to vary in herbaceous species (Niklas, 1995), but the changes in this ratio were unclear in some woody species (van Gelder *et al.*, 2006), and approximately constant in others (Niklas, 1993a). Niklas (1993b) shows that different tissue types have different E/ρ_{wet} ratios, explaining much of the variation in fits of equation 4.2 and the variation in the other study by Niklas (1993a). Further, variation may be caused by variation in the safety factor by which species prevent buckling (Rich *et al.*, 1986), as no species should grow to l_{crit} as the smallest wind would cause buckling. Variation in this safety factor has been shown to vary with the successional status of species (Van Gelder *et al.*, 2006; Rich *et al.*, 1986), with early successional species growing fast at the expense of little safety from mechanical failure.

The stress similarity model is less widely used, but has a similar form to the elastic similarity model (breaking strain replaces E, and both exponents become square-roots, equation 4.2; Niklas, 1993b). This model implies that structural organs are maintained under similar strains, so that a particular organ is not unduly stressed to breaking point.

Stem diameter

The diameter of a stem responding to burial, or supporting a plant, has variable effects on the rate of elongation, or maximum height of the plant (equations 4.1 and 4.2). Thus, it is important to consider diameter as a trait affecting both elongation and height. An important distinction is the difference between the diameter supporting a stem (named d) and that responding to burial (named e). For short plants, d and e may be similar, while for tall plants these are invariably different. What are the relative costs of reducing stem diameter or tissue density? Halving the diameter of a stem responding to burial would result in a four times increase in elongation, while halving density would result in a two times increase in elongation, for the same mass of stem allocated to growth. A reduction in diameter would be associated with a reduction in supported leaf area, a severe penalty. Therefore, the costs of reducing diameter must be studied before further suggestions are made.

Halving the diameter of supportive stem would result in a consistent decrease in potential height for all stem tissue densities (by parameterising equation 4.2 a value of 37% decrease in height was estimated). Alternatively, halving stem tissue density results in a variable (0 to 40%) decrease in height depending on the densities selected (high to low).

Unless, there are penalties (lower supported leaf area) for reducing diameter under burial conditions, it would be advantageous for the plant to respond in this manner, along with a reduction in stem tissue density. Under stable conditions a reduction in stem tissue density is of greater benefit as a reduction in stem diameter would result in considerable reductions in potential height in comparison to a reduction in density.

Summary of the stem density model

A basic outline of this model is given in Figure 4.1. The proposed zonation model is comparable to the general facilitation model applied to species successions, differing in space rather than time. This model answers two questions about the facilitation model, 1) that mobile-dune species are not competitive in the stable-dune, as they can not grow tall, and 2) this model provides a mechanism for the zonation, by proposing an inverse trait correlation between plant height and growth after burial. Mobile-dune species have low stem tissue densities allowing them to grow fast when buried, but preventing them from being competitive as they can not grow tall. Stable-dune species are competitive, growing tall with high stem tissue densities, but have low production rates under burial conditions (Figure 4.1).

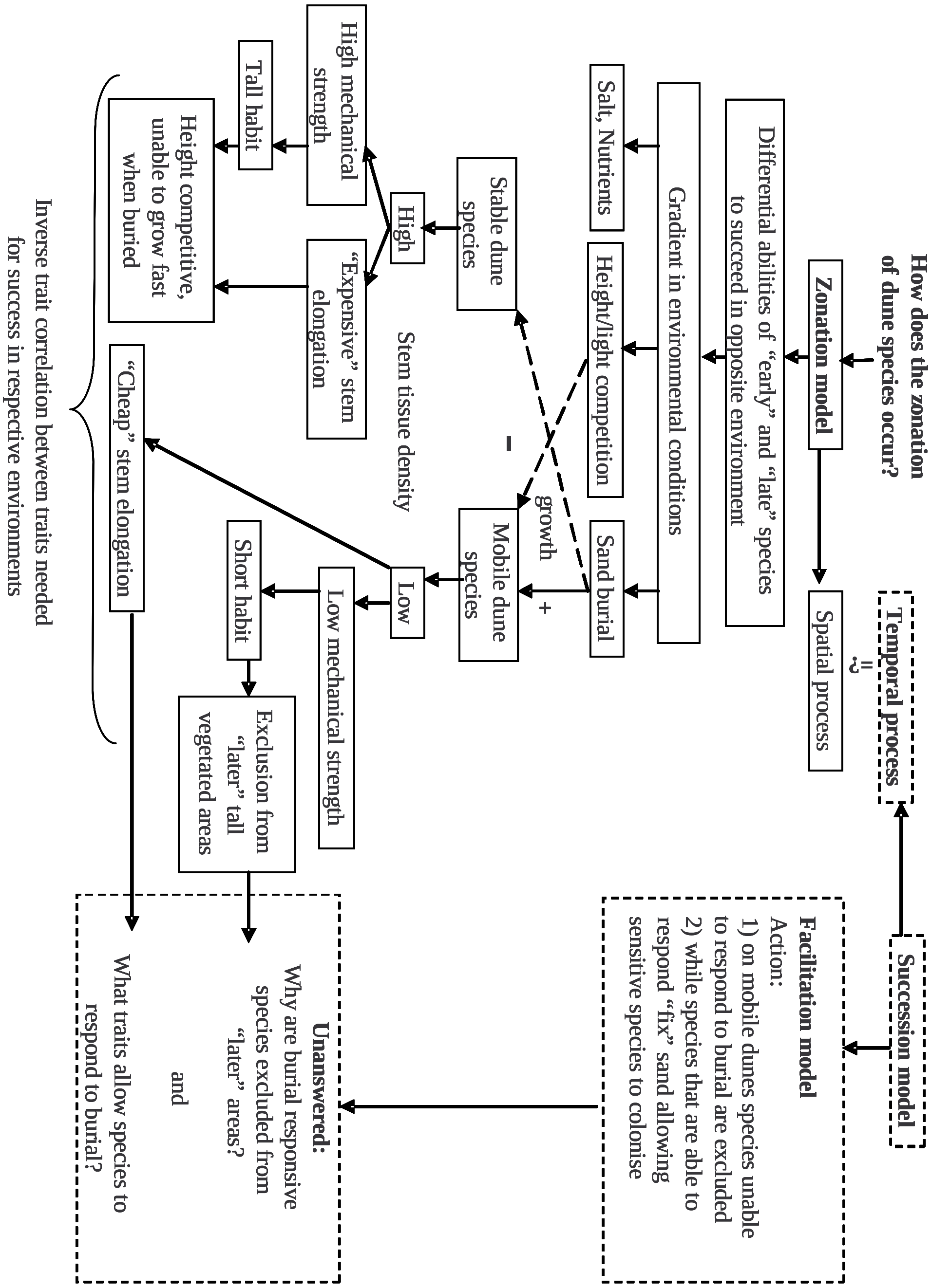


Figure 4.1: Proposed model of dune plant zonation due to an inverse trait correlation between the ability to compete with tall vegetation and respond to burial. The dashed boxes represent the facilitation model of succession, while the solid boxes the proposed zonation model. Common questions and features are indicated. Dashed arrows represent negative influences of factors, while solid arrows represent the positive influences of factors on others.

The literature cited in the previous paragraphs demonstrated that a number of relationships exist between: stem tissue density, growth rate, stem mechanical strength, stem proportions, and maximum height. In the stem tissue density model these parameters interrelate suggesting that an inverse correlation between plant height and growth rate, is theoretically possible (Figure 4.2). For this mathematical model the elastic similarity model was used, one of a number of competing models describing maximum plant height to which a plant can grow. This model was chosen as it is the most widely used for predicting the biomechanical maximum height of plants (Niklas, 1995). Non-woody plants may not be best described using this model, however a similar inverse correlation between plant height and growth rate is thought to exist for the geometric similarity or stress similarity models.

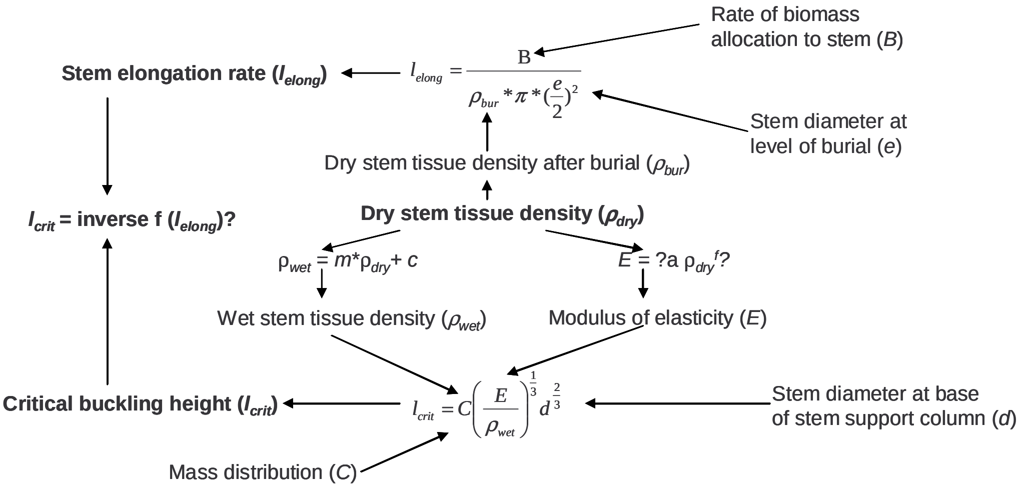


Figure 4.2: Theoretical equations and factors relating dry stem tissue density to critical buckling height, and stem elongation rate (bold). Dry stem tissue density, along with other variables, determines both the critical buckling height of a plant stem (the maximum possible height to which a stem can grow), and the rate of stem elongation for a buried plant. The use of the Greenhill equation for l_{crit} is dependent on the assumption that the elastic similarity model holds for determining plant height (Niklas, 1993a). The relationship between dry stem tissue density and the modulus of elasticity was determined experimentally not theoretically for a range of coastal dune species, hence the question marks.

AIMS

In the above sections it has been demonstrated that, theoretically, an inverse trait correlation could exist, between stem elongation and maximum height, and underpinned by dry stem tissue density. Each theoretical relationship in this scheme (Figure 4.2) was experimentally verified to test the validity of the stem tissue density hypothesis.

The aim of this section was to test:

1) If an inverse correlation exists between stem elongation rate and the height to which a species can grow.
2) If this correlation is determined through the relationships of stem tissue density with mechanical strength, stem elongation rate, and maximum plant height.

3) Whether such an inverse correlation could result in the observed zonation of dune species, thus explaining how:
    a. mobile-dune species were able to survive burial, and
    b. why these species lack the ability to compete in tall areas of the stable-dune zone.

The following specific questions were asked:

- Are mobile-dune species limited to areas of high burial and low vegetation?
- Do mobile-dune species have lower costs of stem production relative to stable-dune species?
- Does the low cost of stem production result in a lack of height-competitive ability, in both absolute height and stem mechanical terms?

METHODS AND MATERIALS

Study sites

Transects and the stem tissue densities of species were sampled at Old Woman's River (OWR) mouth and near Cintsa West on the Eastern Cape coastline of South Africa. Both sites have similar subtropical dune flora, mean maximum temperatures greater than 20ºC all year round, and the majority of rain falls in summer, with some winter rainfall. These sites have typical shore-to-inland vegetation gradients with a large vegetated incipient mobile foredune and more stable relict dunes leading to well developed thicket or forest.

Transects

Five belt transects, at each study site, were used to assess the position of species within the dune zonation. Each transect consisted of a linear series of adjacent 2 by 2 m quadrats starting at the first vegetation landward of the high tide mark and ending two metres into the dune thicket or forest. The procedure used for these transects is explained in greater detail in Chapter 1. At OWR, burial rates were measured at 2 metre intervals along the transects; these measurements are described in Chapter 2.1.

Species were considered typical of either the mobile-dune or stable-dune zone based upon their abundance in areas with high rates of burial.

The initial transects used quadrats of 2 by 2 metres and within these large quadrats were areas of tall and short vegetation. Thus, this quadrat size grouped species that were not necessarily competing for light. For this reason a second series of five transects were surveyed at OWR, to assess the height of species along the original five transects, at a smaller scale to the previous measurements. The original 2 by 2 m quadrats were reduced to four consecutive 50 by 50 cm quadrats, in which species presence and height were measured. A total of 296 quadrats were measured along the five previously established burial transects.

Two grass species (*Sporobolus virginicus* and *Ehrharta villosa*) were present in the transects, but were found in fewer than three quadrats. As this was insufficient to conclusively assign a zonation rank to these two species, they were excluded (and a couple of rare dicot species). Thus, this study does not form a test of the influence of grasses on dune zonation.

Species occurrence relative to competitor height and burial rate

The distribution of each species was plotted as the range of competitor heights, and burial rates for the quadrats in which the species occurred, at OWR. A minimum convex polygon, including all points where a species occurred, was fit to the data.

Stem tissue density and species occurrence

In order to correlate the characteristic stem tissue density of a species to the environment, the occupied environmental space of each species was summarised as the average burial rate and competitor height for all quadrats in which a species occurred. Thus, correlations of the stem tissue density of 13 species with the two environmental factors could be made for OWR. To expand the number of species and the statistical power of this analysis the measured burial data for OWR was extrapolated to all quadrats measured at both sites, based upon a strong relationship found in Chapter 2.1 (burial rate = max(-0.306 * distance from beach + 3.63,0.1833), $R^2 = 0.50$, $P < 0.001$, n = 82, data summarised in Figure 1.1). In this way, similar correlations could be plotted for 23 species, representing both sites. The estimated burial rates coincided with notes made on the burial state of plants in each quadrat.

Table 4.1: Definitions of variables and constants used in this section.

Parameter	Value and unit	Description
Variables:		
θ	degrees	angle of deflection of stem under point load P
ρ_{bur}	g cm^{-3}	dry weight density of stem under burial conditions
ρ_{dry}	g cm^{-3}	dry weight density of stem supporting leaf area
ρ_{wet}	g cm^{-3}	fresh weight density of stem supporting leaf area
d	cm	diameter of stem supporting leaf area
dx	m	deflection at distance x from point load where θ was measured
e	cm	diameter of stem where elongation occurs
E	GN m^{-2}	modulus of elasticity
F	N	flexural stiffness of stem
I	m^{-2}	second moment of cross sectional area
L	cm	length of stem from point load to fixed end of cantilever
l_{crit}	cm	critical length a stem can grow, vertically, before buckling under its own weight
l_{elong}	cm	rate of stem elongation under burial conditions
M	N	weight of point load P * 9.8
P	N	point load on stem used in calculation of E
R	cm	average radius of a stem used in calculation of E
r	cm	average radius of the pith of a stem used in calculation of E
Constants:		
a	14.0 [a]	constant scaling ρ_{dry} to E
B	1g 30days^{-1} [b]	rate of biomass allocation to stem growth during burial
m	0.39 [a]	rate of change of ρ_{wet} with changes in ρ_{dry} (slope)
c	0.97 g cm^{-3} [a]	value for ρ_{wet} at ρ_{dry} is 0 (intercept)
f	2.26 [a]	exponent of power function relating ρ_{dry} to E
C	0.792 [c]	relates to the distribution of weight along a stem

[a] experimentally determined for a range of species in this study,

[b] assumed value,

[c] a value of 0.792 has been widely used in the literature, and derives from Greenhill's formula for l_{crit} when weight is distributed along the entire stem column (McMahon, 1973).

Stem tissue density

The bulk stem tissue density, used in elasticity calculations (ρ_{wet}), is poorly related to stem strength (ρ_{wet} α modulus of elasticity; R^2=0.20; n = 30; Van Gelder *et al.*, 2006). The dry stem tissue density (ρ_{dry}) predicts stem strength better (R^2=0.54; same data set). For this reason it was decided to use ρ_{dry} as a functional proxy for both production and mechanical parameters.

Dry mass stem tissue density (ρ_{dry}) was measured on all abundant species at both sites, for five, or more, stems per species (average n = 12). To increase the power of this analysis the stem tissue density samples included six species which were not present in the ten transects, but were common dune species at both sites. Stem tissue density was calculated as the dry weight of the stem divided by fresh volume. Stem volume was measured using Archimedes' buoyancy principle as follows: a short section (1-3 cm) of stem from below the lowest leaf was fixed to the tip of a pin which was used to submerge the stem in a vial filled with distilled water, on a 4-place balance (Model 200G, Mettler-Toledo Inc. Columbus, Ohio). The weight of the water displaced by the stem multiplied by the density of water gives the volume of the stem. As the total dry weight cost of stem extension is of interest for buried plants, stem tissue density was measured including bark and pith tissue, where present. In order to standardise the measurement of stem tissue density across all species, measurements were made of the stem below the lowest leaf. Thus, the measured stem density was representative of the stem needed to support leaf area, or elongate in response to burial.

Mechanical measurements

The mechanical properties of stems of straight-stemmed species were measured using a cantilever apparatus similar to that used by Wagner *et al.*, (1998). As the mechanical properties of herbaceous and woody stems relate to the water content of the stems (Niklas, 1992), stems were hydrated in water overnight, prior to making measurements. Selected straight stems of 3 to 20mm diameter were placed in foam wrapping between two horizontally clamped pieces of wood (8 cm width). The edge of the lower piece of wood was rounded to prevent unnecessary pressure on the stem segment. A piece of wire was attached to the stem 8, 10, 15 or 20 cm from the clamp depending on the diameter of the stem, however a number of species had only short sections of straight stem often necessitating a compromise length to diameter ratio of ca. 10 (see Niklas, 1992, pp. 174). A series of increasing weights of water were added to a container suspended from the wire in the form of a single cantilever point load. The angle of deflection for each weight was measured by marking the stems position on a rigid piece of paper adjacent to the stem. Young's modulus of elasticity (E), a measure of the applied force required to bend a stem, normalised for cross sectional area and shape, was calculated similarly to Wagner *et al.*, (1998), but see Niklas

(1992) for further calculations. The *E* was calculated for the largest deflection angle measured that was less than 5^{o} (Niklas, 1992). The second moment of cross sectional area (*I*), a parameter taking into account stem shape and cross sectional area, was calculated using the hollow stem formula of Niklas (1992), assigning pith parenchyma (were present) as the hollow component. The hollow stem formula resulted in better within-species correlations between flexural stiffness and *I*, than a circular stem formula. Thus,

$$I = \frac{\pi * (R^4 - r^4)}{4} \qquad \text{eqn 4.3}$$

where *R* is the average radius of the stem, and *r* the average radius of the pith. Where pith is absent, *r* = 0, and the formula becomes that for a circular stem. Flexural stiffness (*F*) relates to the material strength of the stem rather than strength due to shape, and is given by the formula,

$$F = \frac{P * (2 * L^3)}{6 * dx} \qquad \text{eqn 4.4}$$

where *P* = point load, *L* = length of the stem from the point load to the fixed end of the cantilever, *dx* = deflection in metres at distance *x* from *P* where angle was measured.

$$P = M * \cos(\theta * \frac{L}{100}) \qquad \text{eqn 4.5}$$

and

$$dx = \frac{L - L * \cos(\theta)}{\tan(\theta)} \qquad \text{eqn 4.6}$$

where θ is the angle of deflection of the stem under the point load and *M* the weight of the point load, in Newton's. From flexural stiffness and the second moment of area the modulus of elasticity can be calculated:

$$E = \frac{F}{I} \qquad \text{eqn 4.7}$$

Stem elongation: measurement and estimation

The allocation of biomass to stem elongation is dependent on the burial state of a shoot. For this reason, rates of stem elongation were measured after a single burial treatment for seven species of dune plants, from 11 March 2007 to 6 May 2007. Species representing a wide range of growth forms were selected: *Ipomoea pes-*

caprae, *Scaevola plumieri*, *Hebenstretia cordata*, *Carpobrotus deliciosus*, *Passerina rigida*, *Metalasia muricata*, and *Myrica cordifolia*. Two adjacent shoots of each species were selected and assigned to a burial or unburied treatment. Shoots were buried to the level of the apical bud with homogenous beach sand in 12 cm diameter PVC tubes. Shoots were marked with flexible wire a set length down the stem, allowing the calculation of stem elongation rate with subsequent length measurements. After 56 days the stem distal to the growth marker was harvested for each shoot. The reproductive, leaf, stem, and total dry biomass, distal to the marker was measured for each shoot after seven days of drying in a 60^{o}C oven. Prior to drying *C. deliciosus* shoots were frozen to speed up the drying process. The stem elongation of *A. populifolia*, was measured in the course of a similar burial event for the experiments described in the following chapter. The data for this species were added to those of the seven other species.

As the stem elongation data was limited to 8 species, stem elongation rate (l_{elong}) was estimated for other 24 species for which stem tissue density was measured, using equation 4.1. As the stem diameter (e) and ρ_{dry} were measured for the stem segment supporting the shoot leaf area, the magnitude of l_{elong} is largely dependent on the characteristic stem diameter which supports leaf area for a species. For instance a 1 mm diameter stem of *M. muricata* will grow 3 m, while a 10 mm wide stem of *S. plumieri* will grow 5 cm, with 1 g of biomass allocated to stem growth. However, the leaf area supported by these stems was not comparable. For this reason, all species could be evaluated using a standard 1 g of biomass allocated to stem production, 1 cm^2 of stem cross sectional area, and the stem tissue densities of unburied stems. In this case, equation 4.1 becomes the reciprocal of stem tissue density. This estimated elongation rate was not intended as an actual value for stem elongation, rather as a relative indicator of the elongation rate a species could achieve for a standard sized stem. There was strong significant correlation between estimated and measured elongation rates (Figure 4.11, 8 species, $R^2 = 0.81$, $P<0.007$), validating the use of this measure for a general comparison of stem elongation rates between species.

Comparison of the density of buried and unburied stems

A survey of a number of sites along the Eastern Cape coastline found areas where 10 species were naturally buried, even though some of these species were typical of dune thicket. From each site stems of buried and unburied individuals were harvested and stem tissue density was measured, as detailed above. Equation 4.1 was used to estimate the stem elongation rate of a standard sized stem, as outlined in the previous paragraph.

Leaf to stem area ratios

Leaf to stem area ratios (leaf area supported by a cross sectional area of stem) were measured for 15 species, for on average 8 shoots per species. A shoot was cut below the lowest leaf, where stem tissue density and cross sectional area were measured, and the leaf area distal to this point was measured using image analysis software (WinDIAS, Delta-T Devices, Cambridge, U.K.). Leaf to stem area ratios may be biased by an allometric relationship between leaf area and cross sectional area. For these measurements an allometric relationship was avoided as all shoots were measured from a standard point (below the lowest leaf), minimising a large range in shoot sizes within a species. In addition, for species which had a range of shoot sizes there was little indication of deviation from an isometric relationship between leaf area and cross sectional area (data not shown).

Analysis

The presence of three climber species in the stable-dune zone (*Rhyncosia caribae*, *Cynanchium obtusifolium* and *Rhoicissus digitata*) could potentially complicate the analysis of the relationship between stem tissue density and maximum height. These species achieve tall growth by using other species as support with little investment in stem mechanical strength (Darwin, 1867; den Dubbelden and Verburg, 1996). For this reason, these species were excluded from the analysis of the relationship between stem tissue density and maximum height, or *E*. Standardised major axis (SMA) regression (Warton *et al.*, 2006, and Appendix 1 for an explanation of this procedure) was used to determine if the relationship between stem tissue density and maximum height was consistent between mobile- and stable-dune species (implemented in the program SMATR, Falster *et al.*, 2006). SMA regression calculates lines which best approximate the underlying relationship between the variables, and is a better fitting procedure, for model parameterisation, than ordinary least square fits (Warton *et al.*, 2006, also Niklas, 1993a). To test whether *S. plumieri* and *I. pes-caprae* where limited to areas of shorter vegetation, a Monte Carlo randomisation procedure was used. The average height of competing vegetation in quadrats where the species was present or absent was compared to the distribution of average heights for 1000 random shuffles of the entire data set (Manly, 1997). This procedure was repeated for two of the most common stable-dune species, *C. obtusifolium*, and *P. rigida*. Spearman rank order correlations were used to test for associations between burial, or competitor height, and stem tissue density for 13 species from OWR, or 24 species from both sites.

RESULTS

Zonation of plants in relation to burial and competition

The annual net sand burial rate and the maximum height of plants along shore to forest transects showed opposite and mutually exclusive patterns (Figure 1.1). Namely, the areas of net increase in sand level (the zone extending from the high tide mark to half way to the thicket/forest) had low vegetation, in all cases less than 100 cm in height (Figure 1.1). The area inland to this displayed no net sand accumulation, but had increasing maximum vegetation heights up to 450 cm (Figure 1.1). The occurrence of species along these transects corresponds roughly to these zones, with 7 species found in the mobile-dune zone, and 17 species found mainly in the stable-dune zone. The presence of a number of the mobile-dune species such as *I. pes-caprae* and *S. plumieri* in the stable-dune zone was noted.

A minimum convex polygon was adequate to summarise the occupied environmental space of each species. However, this technique did result in an overestimate of the occupied space of *S. plumieri* as the polygon for this species included a large number of environments in which this species was not present (Figure 4.3e and n). The occupied environmental spaces of 13 abundant species measured in transects at OWR, show a clear distinction between the mobile- and stable-dune species (Figure 4.3). The mobile-dune species were present in areas of shorter competitors, and varying burial rates (Figure 4.3a-f). The stable-dune species where restricted to areas of low burial and varying competitor heights, with a few exceptional quadrats (Figure 4.3g-m). It should be noted that the transects at OWR did not include the type of taller vegetation measured at Cintsa West, which was included in Figure 1.1.

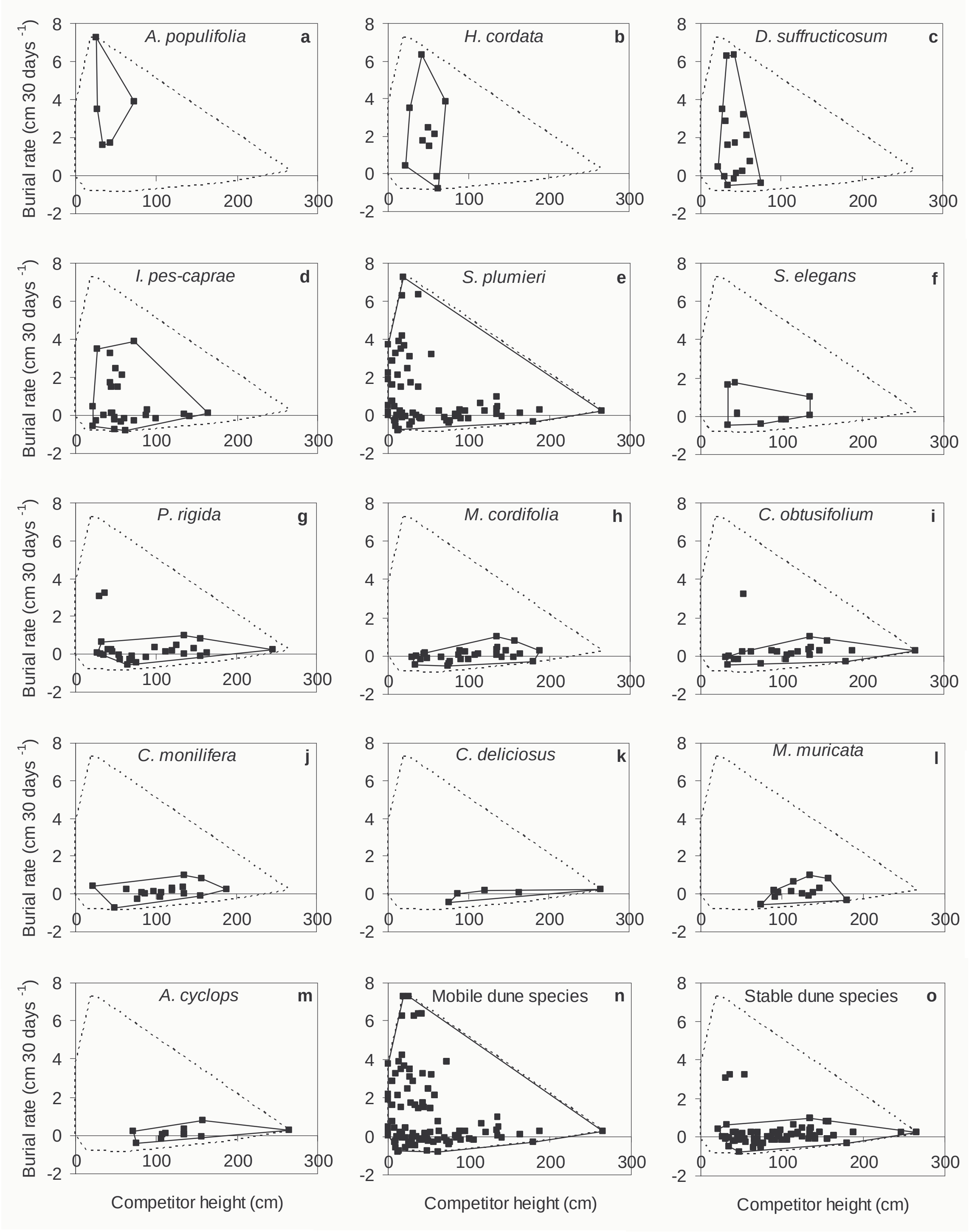


Figure 4.3: Occupied environmental space of abundant mobile-dune species (a-f) and stable-dune species (g-m), and environmental space occupied by all mobile- (n) or stable-dune (o) species at OWR. Points represent the burial rate and maximum height of competitors for all quadrats where the species was present. The occupied environmental space was represented by a minimum convex polygon (solid line) for the plotted quadrats, for *P. rigida* and *C. obtusifolium* three quadrats were excluded. The large polygon (dotted) represents the total occupied space of all 13 species.

The inclusion of data from Cintsa West would result in a number of stable-dune species having larger occupied spaces, including taller areas, but not areas of higher burial (pers. obs.). A number of less common thicket and forest species, if included, would be restricted to stable areas of vegetation taller than those presented in Figure 4.3, e.g. *A. natalensis*, *B. discolor*, and *M. caffra* (pers. obs. and see Figure 1.1).

The distribution of *S. plumieri* extends to all the space occupied by the mobile- and stable-dune species (Figure 4.3e and n or o). The distribution of *I. pes-caprae* also includes areas of tall vegetation. As the stem tissue density hypothesis predicts that species with low stem tissue density will be unable to compete in the stable-dune area due to taller competitors, the presence of these two species in the stable-dune zone was further investigated.

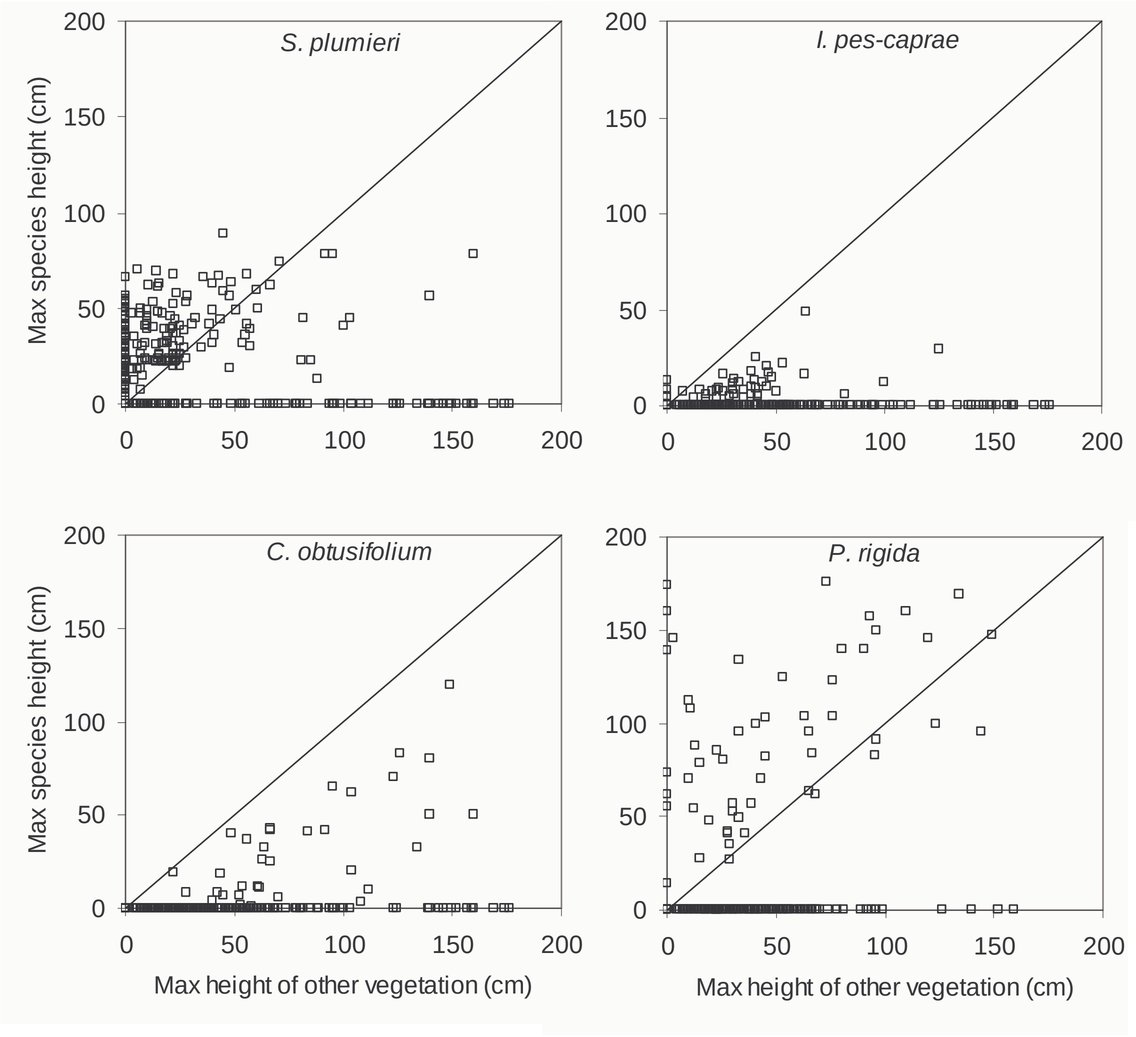


Figure 4.4: Distribution of the maximum height of a species in a 0.25 m^2 quadrat in comparison with the maximum height of all other species in the quadrat (n = 296). The diagonal line represents the 1:1 line under which species were shorter than competitors, within a quadrat.

When typical mobile-dune species are present in areas of low burial, are they limited to areas of low vegetation?

The occupied environmental spaces (Figure 4.3) were measured with large 2 by 2 m quadrats, possibly grouping adjacent areas of different vegetation heights. For this reason further investigations of the heights of competitors used a reduced quadrat size. Figure 4.4 shows the range of heights for four of the most abundant species compared to competitor height, sampled in 296 0.25 m^2 quadrats. *S. plumieri* and *I. pes-caprae* occupy areas where competing vegetation was significantly lower than expected if each species was distributed randomly ($P = 0.006$ and $P = 0.0504$, with 1000 and 10000 randomisations, respectively, see Methods for details). *S. plumieri* shoots were taller than competitors, in the majority of quadrats. *I. pes-caprae* was shorter than the other vegetation in the majority of quadrats, where this species was present. *C. obtusifolium* and *P. rigida* show the opposite trends, with both species growing in areas where competing vegetation was taller than expected if each species was distributed randomly ($P < 0.001$ with 1000 randomisations, for both species). The climbing species, *C. obtusifolium*, was restricted to growing to less than the heights attained by surrounding vegetation, while *P. rigida* consistently grew taller than competing vegetation (Figure 4.4).

Does stem tissue density relate to the distributions of species relative to burial and competition?

Ten sampled mobile-dune species had a significantly lower stem tissue density than 19 stable-dune species ($P<0.001$, one tailed t-test with unequal variances; Figure 4.5). This result was not dependent on the inclusion of the three climber species in the stable-dunes, or the five species which were not present in the transects.

According to the stem tissue density hypothesis, stem tissue density should relate to the occupied environmental space of a species, in terms of burial and height competition. For this reason, the correlations of stem tissue density with the average burial and competitor heights for quadrats where species were present were investigated (Figure 4.6a). In Figure 4.6a there was an indication that low stem tissue density may be associated with species which occupy quadrats with higher burial. But, a limited number of species (13) were present along the transects for which burial was measured. For this reason, burial rates were estimated for all ten

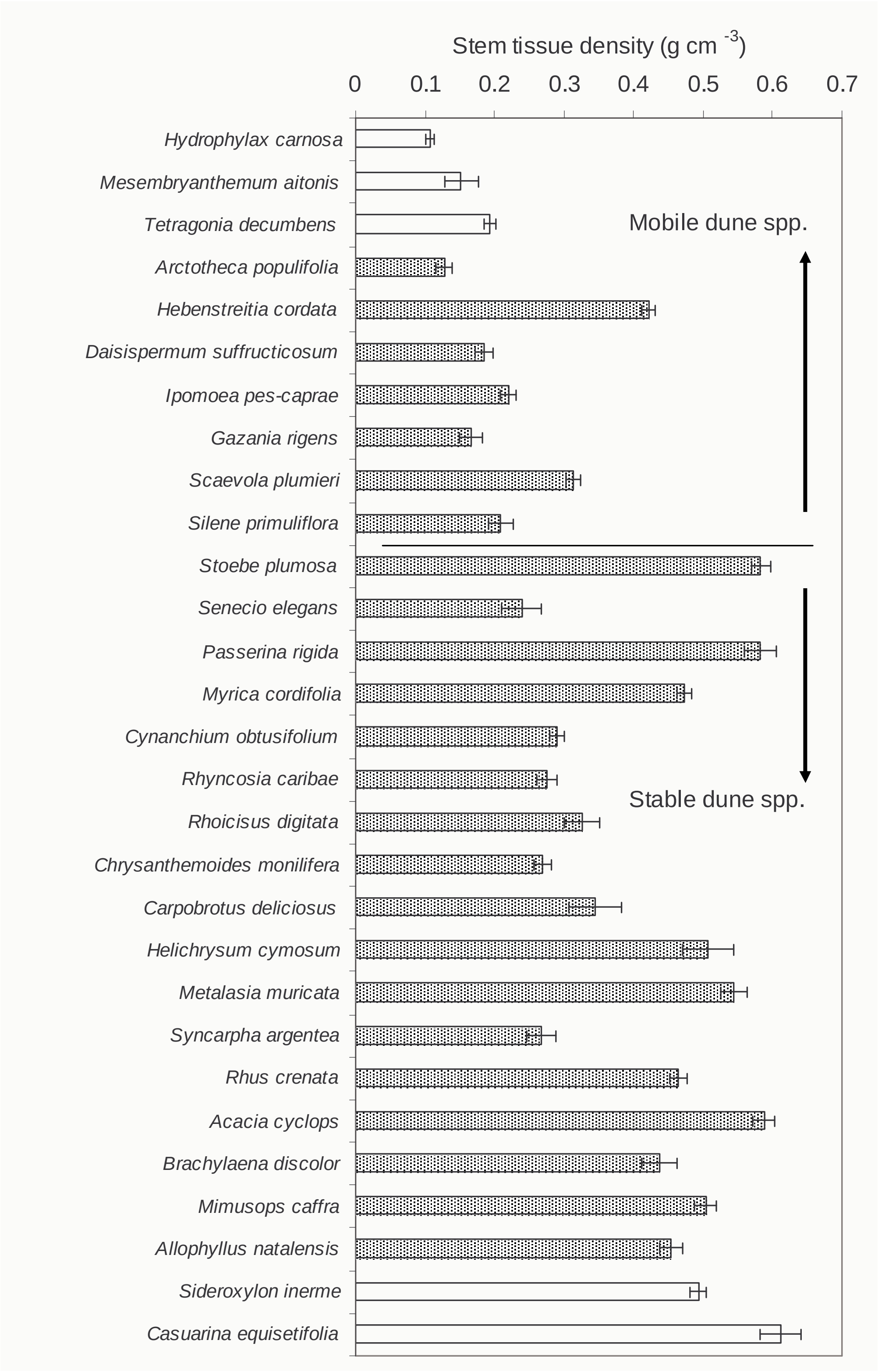


Figure 4.5: Stem tissue density of 24 coastal dune species present in the transects (closed bars) and 5 species not present in the transects (open bars). Bars are means ±one standard error, n = 5 (mean). Mobile-dune species have significantly lower stem tissue densities than stable-dune species (*P*<0.001, one tailed t-test with unequal variances).

transects, at both OWR and Cintsa West, increasing the number of species to 24 (see Methods for more details). Estimated burial rates corresponded well to notes on the burial state of each quadrat. The position of each species in the figure produced from measurements at OWR (Figure 4.6a), and that produced by estimation of burial at both OWR and Cintsa West (Figure 4.6b), was similar. This similarity suggests that this method was sufficiently robust to make the following comparisons.

There was a very strong hyperbolic relationship ($R^2 = 0.84$, n = 24, $P<0.001$) between the average competitor height and estimated burial rate for all species (Figure 4.6b). There was a low, but significant, correlation between the estimated burial rates and stem tissue density of species (Spearman’s rank order correlation; $R_{Spearman}^2 = 0.26$, n = 24, $P < 0.02$), which became more significant when the three climber species were excluded ($R_{Spearman}^2 = 0.32$, n = 21, $P <0.008$). A significant negative correlation was found between the height of competitor plants and stem tissue density ($R_{Spearman}^2 = 0.22$, n = 24, $P < 0.025$), with similar results when climbers were excluded ($R_{Spearman}^2 = 0.28$, n = 21, $P < 0.02$). The mobile-dune species, *I. pes-caprae* and *S. plumieri*, although present in the stable-dune areas (Figure 1.1) where found, on average, in low vegetation (ca. 50 cm; Figure 4.6b).

Does stem tissue density relate to stem elongation after burial?

The stem elongation rates measured for seven species during a burial experiment, and that of *A. populifolia* measured separately, correspond inversely to the stem tissue density of unburied plants (Figure 4.7). It is important that the stem tissue density of an *unburied* shoot relates to elongation rate after burial, as this parameter is then common to determining the height to which a species can grow, as the stem tissue density of a *buried* shoot may not relate to mechanical constraints.

Does stem tissue density relate to the strength of a stem, and the maximum height to which a plant can grow?

There was a highly significant positive relationship between stem tissue density and modulus of elasticity (E), for herbaceous and woody species combined (Figure 4.8). The power function fit had a smaller Akaike’s information criterion (AIC) value than a single linear fit or fits of separate lines for herbaceous and woody species (AIC values indicate the information lost by a model in comparison to the actual data, and

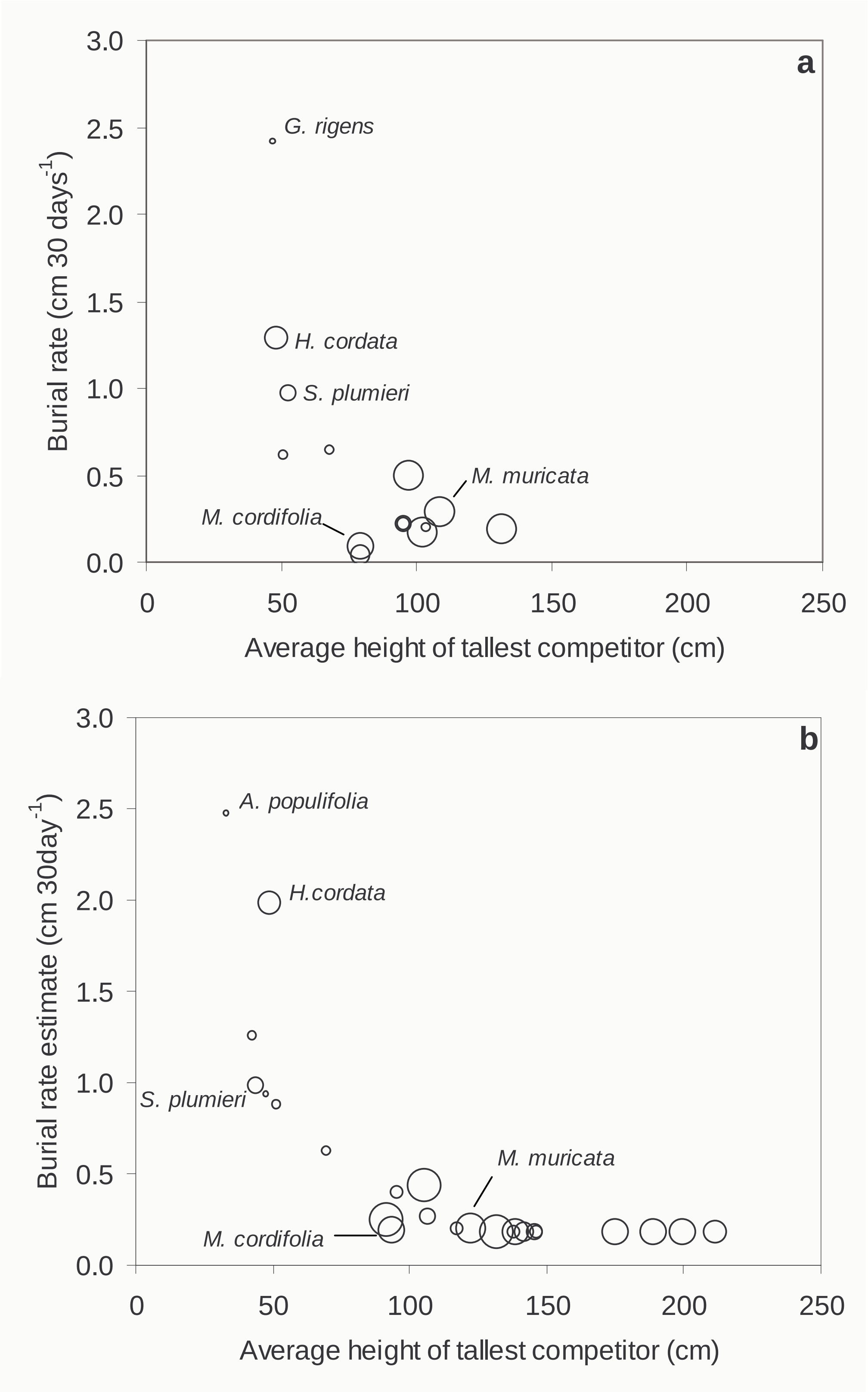


Figure 4.6: The relationship between stem tissue density and the average burial rate and competitor height of species along transects where burial was measured (a), or where burial was estimated (b) as a function of distance to open beach (derived from Figure 1.1.; see Methods for details). Bubble size was scaled proportional to the average stem tissue density for a species. Only the four main study species and two extreme species were labelled.

penalises complex models; Johnson and Omland, 2004). Thus, a power function appears to best explain the data, suggesting that while the herbaceous and woody species may appear to have different linear functions, this was an artefact of a power relationship and separation of the two tissue types along the density axis.

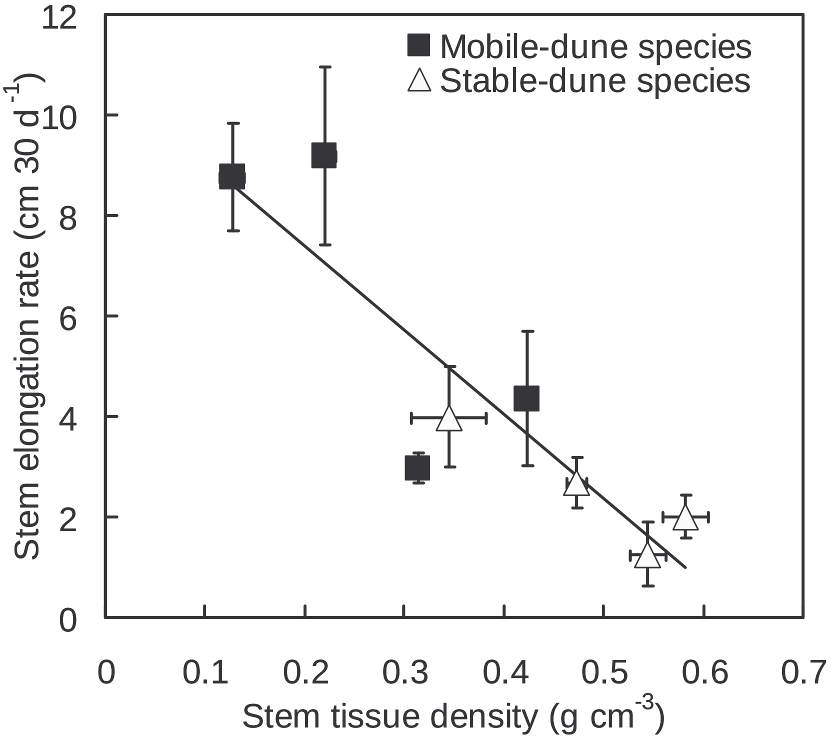


Figure 4.7: Relationship between stem tissue density and stem elongation for 8 species of dune plants, measured under burial conditions. Error bars are standard errors of the mean. The regression line: y = -16.8*x+10.8, $R^2 = 0.78$, $P < 0.004$.

The different stem tissue densities of the two stem tissue types, and a non-linear relationship, results in herbaceous species having a lower *E*, in general, to woody species. The power function relationship between *E* and ρ_{dry}, results in an increase in height with increases in stem tissue density (equation 4.2). The measurement of *E* takes into account stem size and shape, but the maximum heights of each species were achieved at very different stem diameters. Thus, a comparison of *E* to maximum height may be inappropriate. This relationship was not significant ($R^2 = 0.06$, n = 17, $P > 0.33$).

The maximum height which a species attained was highly correlated to stem tissue density (Figure 4.9). Woody and herbaceous stemmed species shared the same relationship, with the exception of the three species of climber. Standardised major axis regression (SMA) analysis procedures (Warton *et al.*, 2006) found that herbaceous species shared a common slope with woody species (Test statistic = 2.83,

$P > 0.09$), with no difference in elevation (Wald test statistic = 0.167, $P > 0.68$). There was a significant shift along the common axis between the two tissue types (Wald test statistic = 33.9, $P < 0.001$).

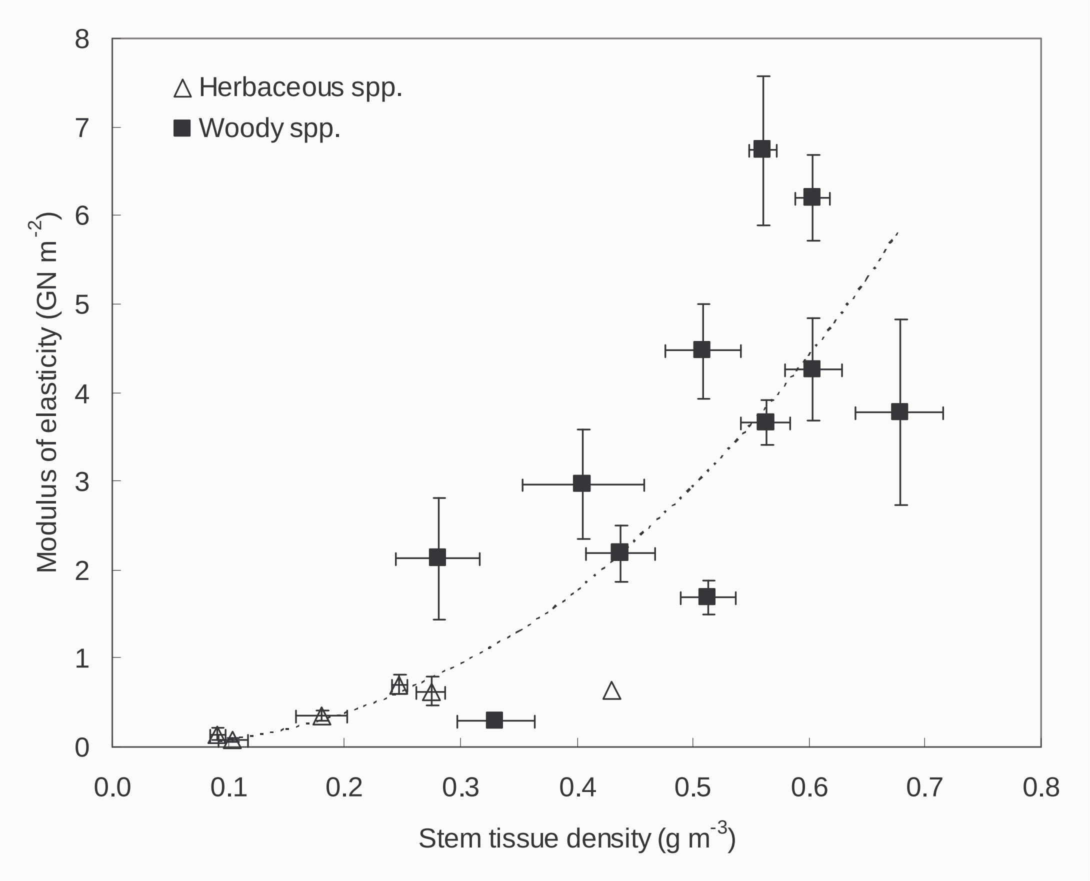


Figure 4.8: Modulus of elasticity (*E*) related to stem tissue density of herbaceous and woody species. The regression line was a power function ($y = 14.0*x^{2.26}$, n = 17, $R^2 = 0.80$, $P < 0.001$).

Is there an inverse correlation between elongation rate after burial and the maximum height to which a plant can grow?

A significant relationship exists between stem tissue density and stem elongation after burial, and with maximum height, but does this result in an inverse correlation between the latter two variables? The eight species for which stem elongation was measured showed a strong relationship between stem elongation and maximum height (Figure 4.10). In order to extrapolate the measured stem elongation rates to as many species as possible an estimate of stem elongation was used (equation 4.1, Methods). This estimate was highly correlated to actual measured stem elongation rates (Figure 4.11).

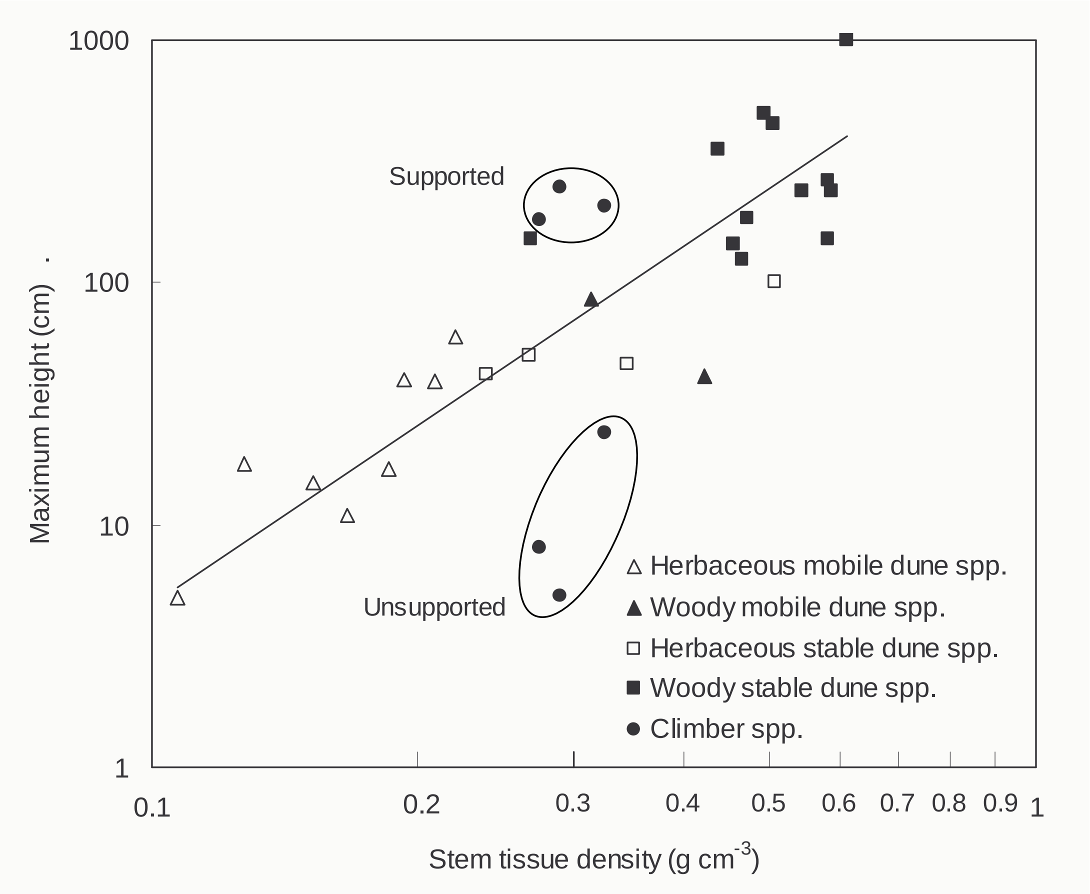


Figure 4.9: Stem tissue density and maximum height for groups of mobile or stable-dune species, and herbaceous or woody species, and supported or unsupported climbing plants. The maximum heights of three species of climber were shown for plants either supported or unsupported by other vegetation (circled), these species were not used for allometric analysis. The power function line was $y = 1327*x^{2.45}$ ($R^2 = 0.79$, n = 26 species, $P < 0.001$).

A strong inverse correlation was found between the maximum height and estimated stem elongation rates for the majority of species sampled (Figure 4.12, $R^2 = 0.71$, n = 27, $P < 0.001$). This correlation was similar to that found for the eight species for which stem elongation was measured, not estimated (Figure 4.10). A clear separation exists between the mobile- and stable-dune species for either or both axes (SMA analysis of log log data found a significant shift along a common slope, between mobile- and stable-dune species, Wald statistic = 28.7, $P < 0.001$). The majority of mobile-dune species were herbaceous, but two were woody, and attained lower maximum heights than woody stable-dune species. Conversely, the generally woody stable-dune species, included four herbaceous species, which had significantly lower stem elongation rates than herbaceous mobile-dune species (t-test, $P < 0.005$). The maximum heights which the three species of climbers attained when supported by other vegetation were exceptions to this trend.

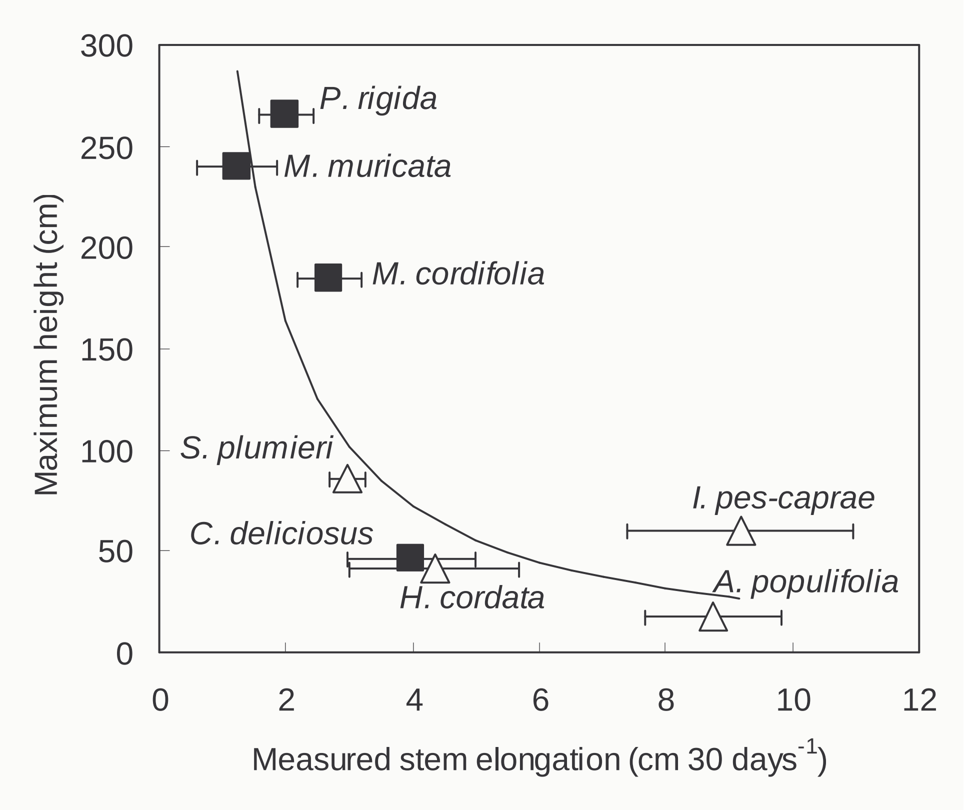


Figure 4.10: Relationship between the maximum height and measured stem elongation after burial for 8 species of dune plants. Error bars are standard errors of the mean. The regression lines ($y = 370.7*x^{-1.18}$, $R^2 = 0.72$, $P < 0.007$).

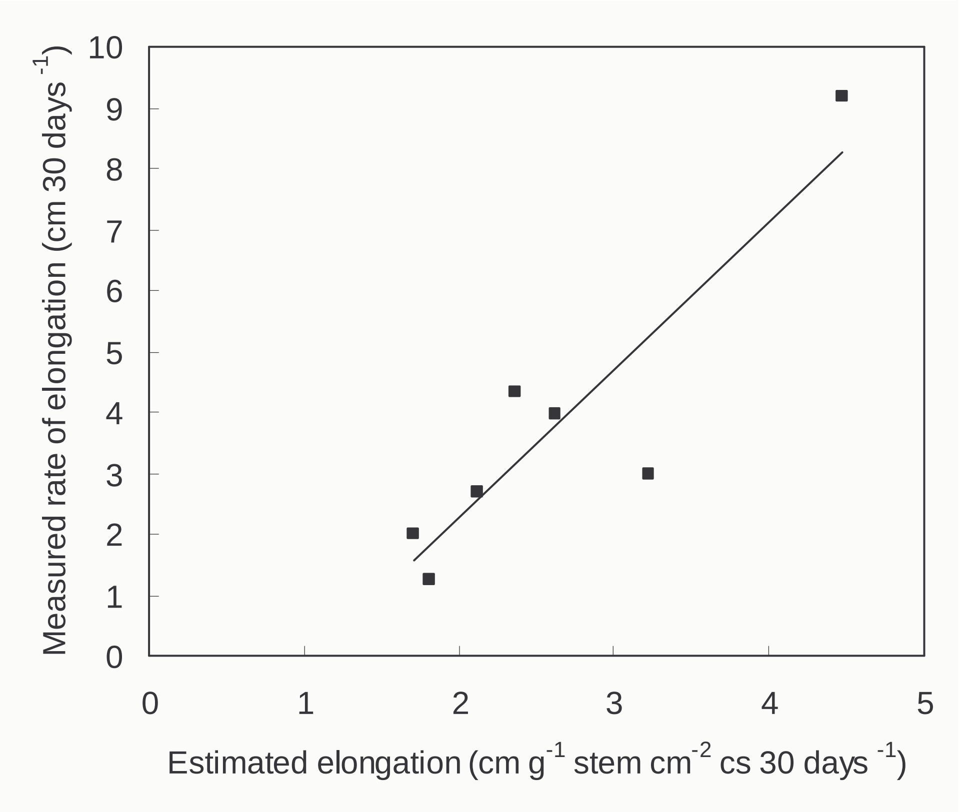


Figure 4.11: Validation of the correlation between estimated and measured stem elongation. $R^2 = 0.81$, 7 species, $P < 0.007$. cs = cross-sectional area.

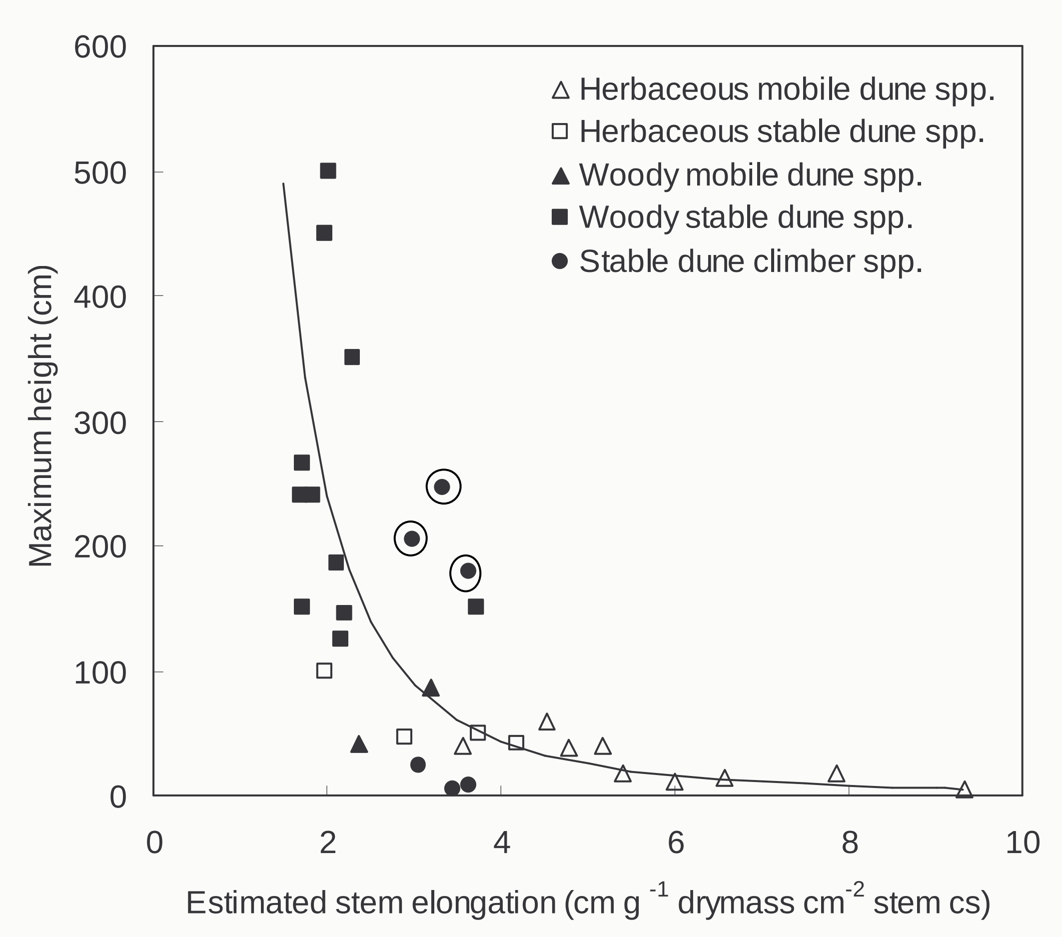


Figure 4.12: Inverse correlation between the maximum measured height of a species and the estimated rate of stem elongation. Circled points represent measured heights of three species of climber, unsupported heights are also shown. The point for *C. equisetifolia* was omitted from the plot, but not the regression (>>600cm tall). The regression line is a negative power function, as expected from the relationship between height and density, and density and stem elongation ($y = 1327*x^{-2.46}$, n = 27, $R^2 = 0.79$, $P < 0.001$).

Could other stem tissue density related traits be important for dune plant ecophysiology?

Stem tissue density was reduced in buried stems of some species, but not others (Figure 4.13b; a significant effect of species, burial, and interaction; $F_{9,193} = 114.5$, $P < 0.001$, $F_{1,193} = 86.0$, $P < 0.001$, $F_{9,193} = 7.4$, $P < 0.001$, respectively). This trend corresponded with the position of the species along a dune transect, with species found nearer the mobile area having greater reductions in stem density upon burial. Stem elongation rates were estimated as the inverse of stem tissue density (see Methods). As a consequence, lower stem densities would result in disproportionately increasing amounts of estimated stem elongation (Figure 4.13a). Thus, the small decrease in stem density of *A. populifolia* in buried shoots results in a disproportionately large increase in estimated stem elongation rate, in comparison to species with higher stem densities (Figure 4.13a).

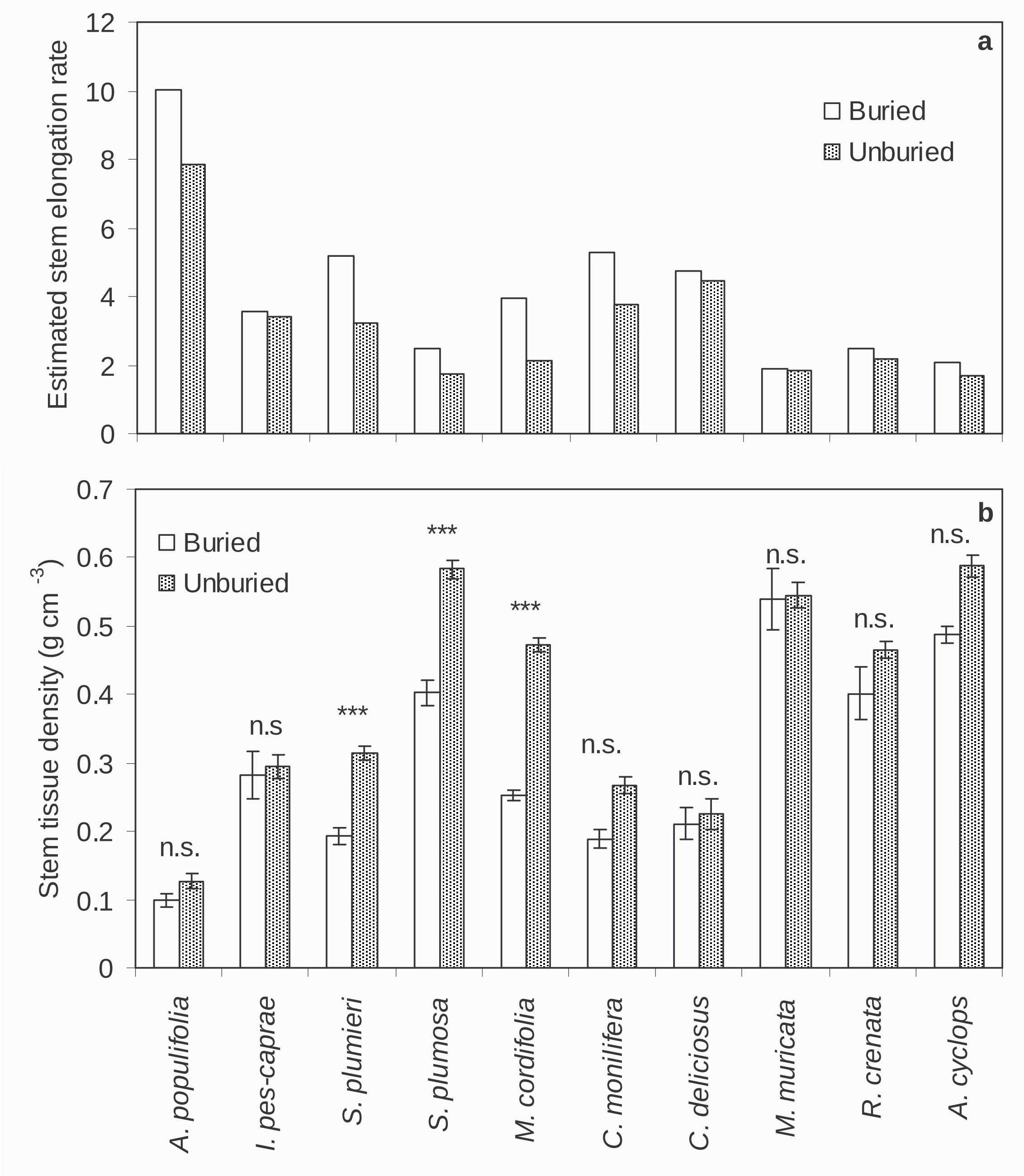


Figure 4.13: Estimated elongation rates (a) and tissue densities (b) of stems from naturally buried or unburied plants of ten species. Estimated stem elongation rates were calculated as the inverse of stem tissue density (see Methods for explanation). Species were ordered by their position along transects from the start of vegetation landward (left to right).Tukey HSD *post hoc* comparisons were calculated for the densities of buried and unburied stems of each species. n.s., not significant; ***, $P <$ 0.001.

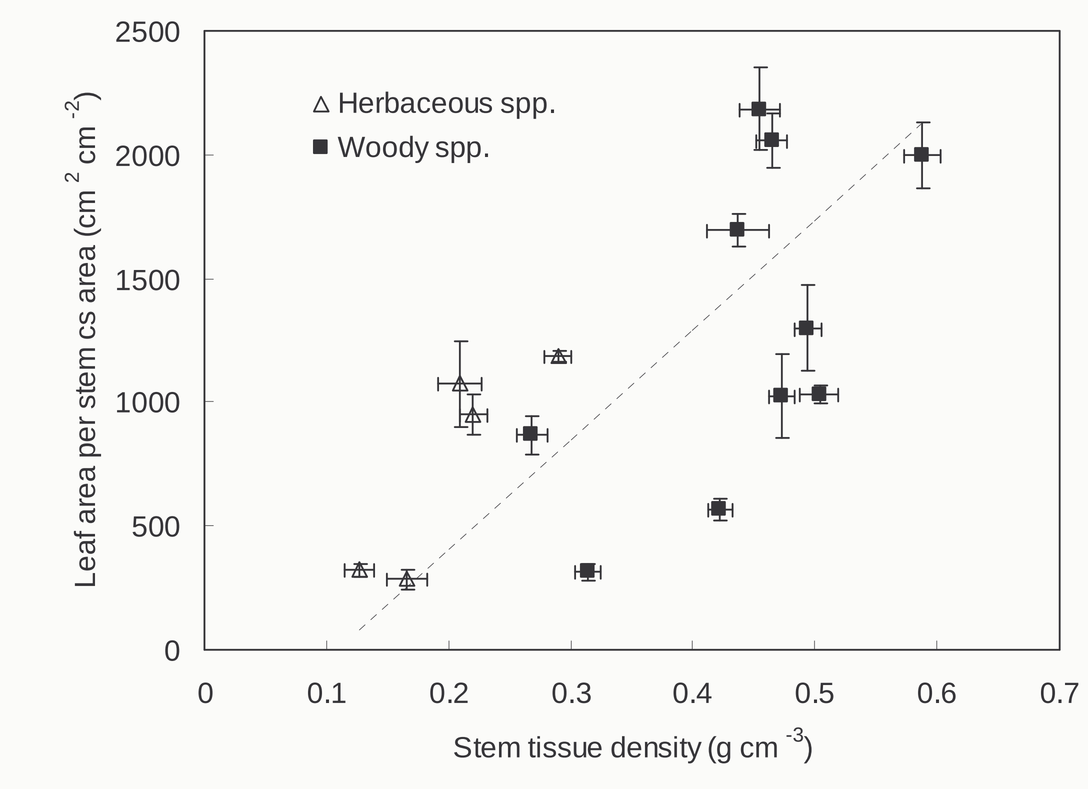


Figure 4.14: Leaf area (cm^2) supported by 1 cm^2 stem cross sectional area related to stem density for 14 species. Points represent means with standard errors for a species. The line represents a SMA regression line y = 4434*x-481 ($R^2 = 0.45$, $P < 0.007$).

The leaf area supported by 1 cm^2 of stem varies considerably between species (Figure 4.14). There was a moderate significant relationship between the leaf to stem area ratio and stem tissue density for a species. Low stem tissue density species were thus associated with low supported leaf areas, a second inverse trait correlation with stem elongation rate. As estimated stem elongation rate was calculated as the inverse of stem tissue density, the inverse trait correlation between elongation and supported leaf area has the same statistics as the relationship in Figure 4.14.

DISCUSSION

Zonation of dune plants in relation to burial and competition

The dune environmental gradient was divided into two distinct zones, in each there were different important environment factors and species (Figure 1.1). The mobile-dune zone was characterised by high rates of burial, low vegetation, and fast growing, generally herbaceous, species. The stable-dune zone was characterised by little burial, tall, and generally woody vegetation, a number of the mobile-dune species were also present.

An analysis of the environmental spaces that 13 species occupied (Figure 4.3), found that, in general, stable-dune species were not present in areas where there were high rates of burial, and most mobile-dune species were limited to areas of short vegetation. The realised niche space is the range of environmental variation that a species can occupy indefinitely (Hutchinson, 1957). Measuring the realised niche of a species, is dependent on the effects of dispersal limitation and the lack of long-term measurements being unimportant, factors that limit the interpretation of these results (Figure 4.3). In addition, the burial and competitor height axes were correlated, further limiting this interpretation. At the time of sampling there were few seedlings, apart from those of *D. suffructicosum*. Thus, the measured distributions of the species may be representative of the areas where a species can survive in the long term, rough estimates of Hutchinson's realised niche (Pulliam, 2000). Despite these limitations, mobile- and stable-dune species have largely non-overlapping distributions (e.g. Figure 4.3a, b, and c compared to k, l, and m). The two mobile-dune species that were found in stable-dune vegetation were limited to areas of short vegetation (Figure 4.4). Mobile-dune species generally had a decreasing proportional abundance further into the stable-dune zone (see *I. pes-caprae* and S. *plumieri*, Figure 1.1), further supporting the hypothesis that the burial tolerant mobile-dune species were limited to areas of low vegetation.

Does stem tissue density relate to the distributions of species relative to burial and competition? Both transect and environmental space data suggest that zonation, or species distributions, were well correlated with burial and competitor height (Figure 1.1 and 4.3). Stem tissue density was significantly lower in mobile-dune species, relative to stable-dune species (Figure 4.5). In addition, stem tissue density correlates significantly to the average burial and competitor height for quadrats where a species occurred (Figure 4.6).

These results suggest that burial and competitor height may play an important role in the zonation of dune species. Stem tissue density was lower in mobile-dune species, consistent with the stem tissue density hypothesis.

Inverse trait correlation

A strong non-linear inverse trait correlation exists between stem elongation and plant maximum height (Figures 4.10 and 4.12), regardless of whether stem elongation was measured for 8 species, or estimated for 27 species. This strong relationship was also present, regardless of the large species differences in shoot sizes, stem diameters, and possibly variable allocation to stem growth in response to burial. The non-linear relationship was such that species were either able to grow tall or have high rates of stem elongation. This dichotomy was evident for the species from the mobile- and stable-dune zones, with the position of a species on this inverse correlation corresponding to the zone in which the species occurs (Figure 4.12). It is difficult to compare this data set of herbaceous and woody species to the literature, as many studies with comparable data are limited to measurements on forest trees (Muller-Landau, 2004; Van Gelder *et al.*, 2006) or woody species (Preston *et al.* 2006). However, Muller-Landau (2004) demonstrates that the relative growth rate of juvenile trees is negatively correlated to wood density similar to this study. The growth rates and height of adult trees do not relate strongly to wood density (Muller-Landau, 2004; Van Gelder *et al.*, 2006). Preston *et al.* (2006) found a weak negative correlation between wood density and maximum height that was not significant when phylogenetic contrasts were taken into account. It is possible that when herbaceous species are included in these relationships the broadened range of variation in wood density results in stronger relationships.

The species typical of the mobile-dunes had 50% lower stem tissue densities than stable-dune species (Figure 4.5). This result was consistent with the hypothesis that low stem tissue density allows mobile-dune species to survive burial through cheaper stem production costs. These results are in accordance with those of Chapter 5 in which it was found that stem tissue density of four species of dune plants: 1) related inversely to stem elongation rates, 2) predicted the ability of a species to respond to burial, and for two species 3) stem tissue density changed with burial state in a similar manner to the inducible responses of stem elongation to burial. These findings were supported by the differing abilities of species from different areas of the dune zonation to reduce stem tissue density (Figure 4.13b). The six-fold range of stem tissue densities found in this study (0.11 to 0.62 g cm^{-3}) correspond to a similar six-fold range in the length of potential stem elongation, assuming that all stems have a similar diameter.

Stem tissue density was positively correlated with stem strength and the maximum height to which a species' grows, and negatively with the stem elongation after burial (Figure 4.7 and 4.9). Therefore, the relationships between these traits and stem tissue density could underlie this inverse trait correlation. Whether a species was herbaceous or woody largely determined stem tissue density and related characteristics (Table 4.2). In general, this may be the reason that herbaceous and woody plants occur in different areas of the dune zonation. There were exceptions, the woody-stemmed *S. plumieri* was dominant in mobile-dune areas and some stable-dune areas of short vegetation. This species combines an inducible reduction in stem tissue density (Figure 4.13) with a lower stem tissue density than other woody species (Figure 4.5), and thus can survive burial. A number of herbaceous species were abundant in stable-dune areas, but the most abundant were climbers, a clear exception to short stature herbaceous species.

Table 4.2: Summary of traits related to stem tissue density for herbaceous and woody species. Means ± one standard error of the mean. Sample sizes per grouping, range from 5 to 15 species, depending on the trait. All comparisons are significantly different at the 95% level of confidence.

Species group	Stem tissue density g cm^{-3}	Stem elongation rate cm 30 days^{-1}	Stem strength (*E*) GN	Height cm	Supported leaf area cm^2 LA cm^{-2} CS [a]	Dune position [b]
Herbaceous	0.21±0.02	9.26±3.37	0.54±0.19	33±7	569±179	39±9
Woody	0.49±0.03	1.71±0.43	3.48±0.58	194±31	1391±230	77±6
Herb/Woody %	42%	540%	15%	17%	41%	na

[a] cm^2 leaf area per cm^2 stem cross sectional area,

[b] Dune positional rank, 0 = start of vegetation, 100 = thicket or forest.

Stem mechanical strength and maximum height

Mobile-dune, and herbaceous, species had considerably lower maximum heights than stable-dune species (Figure 4.9). The reason for this difference may be related to two aspects of the relationship of stem tissue density with maximum height: 1) a strong power relationship between stem tissue density and maximum height (height = 1327*density$^{2.45}$), and 2) the separation of the stem tissue densities of the herbaceous and woody species (Figure 4.9). In contrast to this study, a weak negative relationship was found between stem tissue density and maximum height for woody plants in California (Preston *et al.*, 2006).

The mechanical properties of herbaceous and woody species differ due to a shift from hydrostatic to other structural mechanisms (Niklas, 1992). The mechanical properties and stem density of woody species varies with the quantities of parenchymatous, sclerenchymatous, primary, and secondary vascular tissue (Niklas, 1992), and the ratio of tracheids to fibres (Baas, 1986). Herbaceous stems lack structurally strong secondary thickening, and therefore, have a lower mechanical strength. For this reason, a fundamentally different stem density to mechanical strength relationship was expected for stems made up of these different tissue types (Niklas, 1993a). This appeared to not be the case for the species surveyed in this study. A possible reason may be that stems are composed of varying combinations of these tissue types, thus a continuum exists for the tissue composition of stems. However, as the relationship between ρ_{dry} and E was allometric and stem tissue densities differed between species, the herbaceous species had lower E's than woody species (Figure 4.8).

The stem tissue density hypothesis mirrors recent work done on wood density in tropical forests. There succession was partially explained by an inverse correlation between fast growing, low wood density, low mechanical strength pioneer trees and slow growing, high wood density, high mechanical strength shade tolerant trees (Van Gelder *et al.*, 2006).

A mechanical or hydraulic tradeoff?

The mechanical structure of stems, metabolic limitations on growth rate, and hydraulic limitations on height, diameter, and leaf area are some of the factors limiting plant height (Niklas and Spatz, 2004). For instance, wood density relates to hydraulic parameters and drought tolerance (Hacke *et al.*, 2001). More dense wood is associated with smaller vessels, and less vulnerability to cavitation. Thus, the gradient of increasing stem tissue density from the mobile-dunes to the stable-dunes is indicative of increasing mechanical strength, maximum heights, and decreasing vulnerability to drought. A strong linear relationship also exists between stem tissue density and the leaf area supported per stem cross-sectional area (Figure 4.14). This finding suggests that hydraulic limitations were a second implication for plants with low stem tissue densities. Taneda and Tateno (2004) found a negative correlation between the mass specific conductivity and stem tissue density of a broad range of species. This negative trend, may suggest that low stem density plants should have larger leaf areas. But, the positive relationship between stem tissue density and leaf area stem area ratio (Figure 4.14) suggests that the net effect of hydraulic limitations is to have lower leaf areas with lower stem tissue densities. This is not an exception, as a positive correlation was found between tree species stem specific conductivity and leaf area to stem area ratio (measured as the Huber value, van der Willigen *et al.*, 2000). In summary, low stem tissue density stems have high stem elongation rates, with little mechanical strength, and can support less leaf area.

The findings of Preston *et al.* (2006) suggest that there are limits to the comparison of stem tissue density with hydraulic parameters. Those authors found that within a small range of wood densities, wood characteristics varied to a large degree, suggesting that these two characteristics cannot be considered to be the same evolutionary axis.

Herbaceous low stem density plants (with low drought tolerance) may avoid drought stress by living on low biomass mobile-dunes were soil water is rarely limiting (de Jong, 1979; Pavlik, 1985; Ripley and Pammenter, 2004a). Alternatively, the higher biomass stable-dunes may cause greater dry down of the soil water levels, necessitating stable-dune species to have greater drought tolerance, and thus higher stem tissue densities. These considerations do not take into account differences in growth form and physiology, which are also important in determining species drought sensitivity (e.g. the inducible CAM species *Carpobrotus deliciosus*, or the constitutive CAM species *Tetragonia decumbens*; Sayed, 2001).

The mechanical derivations of MacMahon and Niklas are fundamental to the height or scaling to which a plant can potentially grow. However, Niklas and Spatz (2004) found that hydraulic and growth scaling laws described species height to diameter relationships better than theoretical mechanical limits. Thus, l_{crit}, the maximum height a plant can grow to in mechanical terms, does not reliably predict the actual plant height, as hydraulics and growth scaling laws limit this relationship to a greater degree. For this reason, l_{crit} was not calculated in this study, while the stem strength parameter modulus of elasticity was used as an estimate of mechanical function. This discussion does not imply that mechanical strength has no role to play in determining the ability of a shoot to withstand gale force winds, or maintain a competitive leaf area with a minimum of support costs. Rather, this debate supports a more holistic view of what determines plant height, including mechanical, growth, and hydraulic limitations (Taneda and Tateno, 2004).

Inducible reductions in stem density

Post burial stem elongation rate was estimated from the stem tissue density of stem segments below the lowest leaf of unburied shoots. The use of the stem density of unburied shoots represents a necessary simplification, as the measured stem tissue density relates to considerations of both growth and plant height. However, the density of the buried stem physiologically determines growth under burial conditions. Thus, species may maintain high-density strong stems under stable conditions, and reduce stem density under burial conditions, when stem strength was not a consideration. This strategy appears to be used by a number of species with intermediate unburied stem tissue densities (Figure 4.13b). Species at the extremes of the measured zonation showed less ability to reduce stem tissue density, although the small reduction in stem tissue density of *A. populifolia* would result in a large increase in elongation rate (Figure 4.13a).

A vital aspect of the inverse relationship of stem density and elongation rate was that increases in stem elongation in buried stems of lower density are a function of both, the relative reduction in density, and the actual density. In evolutionary terms, the rewards for reducing stem tissue density, in terms of elongation and survival of burial, increase hugely with lower stem tissue density. For this reason, species that encounter burial in their natural environment may be selected, in a run-away fashion, towards a minimum possible stem tissue density. Alternatively, for species that are newly arrived in a dune environment, burial will act as a filter disproportionately discriminating against species with higher stem densities. Indeed, two species typical of areas of high burial, *A. populifolia* and *H. carnosa*, have extremely low stem tissue densities (minimum's of 0.06 and 0.07 g cm^{-3}), in comparison to other dune species (Figure 4.5). The recorded minimum stem tissue densities for plant stems ranges from: 0.04 g cm^{-3} for greenhouse-grown seedlings of 62 semi-woody or woody plants (Castro-Diez *et al.*, 1998), to 0.1 g cm^{-3} for a large range of tropical trees (Williamson, 1984, and Van Gelder *et al.*, 2006). To emphasise how low these values are, *Typha* or bulrush fronds, including the volume added by aerenchyma had densities of 0.14 g cm^{-3}, and a minimum density of 0.092 g cm^{-3} was measured for *leaves* of 597 woody species (Niinemets, 1999). Therefore, a stem dry mass density of less than 0.1 g cm^{-3} may be a functional minimum density for most species, below which little reduction may be possible. Secondary functions of herbaceous stems may prevent further reductions in stem tissue density, such as, salt dilution in the form of hypertrophy, carbohydrate storage, and photosynthesis. These functions would be beneficial to plants growing in the extreme burial environment of the coastal foredunes.

A further advantage of low stem tissue densities for buried plants, may be the low investment in stem tissue, allowing these species to successively senesce buried stems in favour of the growth of unburied stems (Ripley, 2007, pers. com.). The dry weight density determines the longevity and rate of growth of leaves and roots (Ryser, 1996), and most likely stems too.

Dune plant strategies

Five dune plant strategies are proposed to relate to burial and height competition (Table 4.3):

1) **Fast growing species:** *A. populifolia* had extremely low stem tissue densities, and was unable to reduce stem tissue density in response to burial (Figure 4.13b), as a result this species achieved very low maximum heights. *A. populifolia* is characteristic of the seaward edge of dune vegetation and was consequently subject to the highest rates of burial of any species. These traits may represent the extreme strategy of constitutive fast growth, and short stature.

2) **Inducers:** *S. plumieri* had the highest stem tissue densities of the mobile-dune species, however, the species had the ability to induce lower stem tissue densities in stems responding to burial (Figure 4.13). This mechanism may allow these species to achieve taller stature, under stable conditions, while still allowing a considerable response to burial. A number of other woody species surveyed show inducible changes in stem tissue density in response to burial (Figure 4.13b), however, none have distributions in the mobile-dune area, and all reduce stem tissue density to higher absolute levels than *S. plumieri*.

3) **Climbing plants:** Climbers clearly represent a different strategy to those of other stable-dune species (Figure 4.12). These species have low stem tissue density, but were able to achieve tall heights through using other species as support (Figure 4.9). Richards (cited in Putz, 1990) found that low wood density liana's had high rates of stem extension, as these species did not need to allocate carbon to support structures. This suggestion stems back to Darwin (1867), and these observations support this idea. The fast growth of climbers has been suggested as a potential adaptation

allowing these species to survive in sand dune habitats (Isnard *et al.*, 2003). In the Eastern Cape, climbing species do not appear to have colonised the mobile-dunes, as the three stable-dune climbing species were limited to areas of little burial.

4) **Low stable-dune species**: The three low stature herbaceous stable-dune species were rare, and generally were associated with the less vegetated areas of the stable-dunes.

5) **Woody species**: The woody species were generally restricted to stable-dune areas, where they are competitive with other species for height.

Other work on the functional types of beach plants has differed based upon the type of environment or plant characteristic studied. For instance the three plant functional types of Garcia-Mora *et al*. (1999) illustrate that dune plants may fall into a winter annual group or perennial groups either capable or incapable of withstanding burial. When the response to burial is measured plants form a number of loosely defined species groups: those negatively affected by shallow burial, those stimulated by burial at shallow depths, and species that show stimulated growth at deeper depths (Maun and Perumal, 1999). The criteria for species grouping used in this study, focuses on shifts in plant characteristics with burial, and species zonation position in relation to burial and light competition. Thus, different functional groupings are suggested here, while past groupings are relevant to different research questions.

Table 4.3: Possible strategies of dune plants relating to burial and height competition.

Strategy	Environment	Stem density	Plant Stature	Examples
Fast-growing spp.	Mobile-dunes	Low	Short	*I. pes-caprae*, *A. populifolia*
Inducers	Mobile-dunes	Inducible in response to burial	Moderate	*S. plumieri*, *H. cordata*
Creepers	Stable-dunes	Low	Tall – supported by other vegetation	*C. obtusifolium*, *R. caribae*, *R. digitata*
Low stable-dune species	Stable-dunes	Low-Moderate	Low-Moderate restricted to areas of low vegetation	*C. deliciosus*, *S. argentea*, *H. anomalum*
Woody spp.	Stable-dunes	High	Tall	*M. muricata*, *R. crenata*, *A. cyclops*

An evaluation of the stem tissue density hypothesis

Desired qualities for zonation models, are that: 1) the model should explain, in a mechanistic manner, the observed zonation, possibly through an inverse trait correlation, 2) the model should explain why reciprocal movements of species do not occur, 3) for the sake of generality, the model should explain a large amount of the variation in the distribution of as many species as possible, not just one species.

This chapter tests the stem tissue density hypothesis, by generating a theoretical framework that is then validated by the strong correlations between the measured parameters. These correlations outline a probable mechanistic basis for the observed inverse trait correlation. The position of species on measured dune zonations are then compared to those predicted for the species based upon their position on an inverse trait correlation. The observed zonation positions follow the predicted positions closely, suggesting that the stem tissue density hypothesis is a likely explanation of dune zonation in the Eastern Cape. A number of *ad hoc* modifications to the hypothesis were needed to allow a more close approximation of natural processes, for instance, species were able to alter stem tissue density based upon burial state.

Ultimately, the hypothesis remains to be further tested by an experimental procedure, such as, reciprocal transplants, or further *in situ* manipulations of burial state. To some degree the following chapter tests this with four typical dune species (Chapter 5).

Is the stem tissue density hypothesis a global zonation or successional model?

The stem tissue density hypothesis of dune zonation and succession may be limited to mobile-dune systems that have an increasing height of vegetation with distance from the sea. Examples could be mobile-dune systems along the south-eastern coasts of Africa, and the Great Lakes of North America (Lichter, 1998). Mobile-dune systems that have no land-ward increase in *LAI* and height must, necessarily, be maintained by other processes then the one suggested here. Examples are: arid coastal areas (which often have little inland vegetation) or places where dune vegetation is replaced by low grasslands. However, the effects of stem density on growth rates and burial survival may remain important, and thus it would be interesting to look for inverse correlations between stem tissue density and potentially important ecosystem processes in these environments e.g. vunerablity to herbivory, water stress, or plant longevity.

Coastal dune ecology: What is required to determine the mechanisms of zonation and succession?

A growing synthesis of coastal dune ecology is appearing in the literature, in which a number of factors are considered important in driving dune ecology. Screening experiments with large numbers of species (Maun and Perumal, 1999; Martinez and Maun, 1999) have found that the ability of a species to respond to burial has the potential to determine which species are filtered out of zones of high burial. It is important to find the general traits, such as stem tissue density, that allow the species to respond to burial. In addition, the concept of differential filtering of species based upon traits is more useful when inverse trait correlations are considered, as this is necessary to explain zonation or succession (McCook, 1994). The findings in this chapter suggest that stem tissue density fulfils both of these criteria; it explains how species have different responses to burial, and provides an inverse correlation between burial response and the ability to grow tall.

Chapter 5

Contrasting growth, allocation and photosynthetic responses of four coastal dune plant species are related to the rates of natural sand burial and nutrient limitations

Importance of studying the *in situ* interactions of environmental factors on plant growth — Experimental evidence of plant response to factorial burial and nutrient addition treatments — Shifts in species ability to respond to burial and nutrient limitation explain position of each species on dune zonation gradient — Stem tissue density corresponds with species response to burial

INTRODUCTION

Many studies have found sand burial to be an important environmental factor that promotes the growth of dune species (Maun & Lapierre, 1984; Yuan *et al.*, 1993), influences the distribution of species (Maun, 1998; Wilson & Sykes, 1999), and filters out species or seedlings incapable of responding to burial (Van Der Valk, 1974; Moreno-Casasola, 1986; Maun, 1998; Maun & Perumal, 1999). Other studies have shown that coastal dune sand is nutrient poor and contains little organic matter, which limits plant growth (Willis, 1965; Maun, 1994). In particular, low soil nitrogen limits growth on many dune systems (Kachi & Hirose, 1983; Willis, 1965; Shumway, 2000). The high growth rates required for a shoot to survive burial are potentially limited by the low nutrient availability of dune sands. Given the importance of burial and nutrients in limiting dune plant growth, the greenhouse experiments of Zhang (1996) on *Cakile edentula* are exceptional in considering the interaction between burial and nutrient limitation. Thus, it seems important to study the interaction between burial and nutrient limitation as a factor influencing production, survival and the distribution of plants on coastal dunes.

Factors such as nematodes, and salinity or salt spray may also affect coastal dune species differentially (e.g. van der Putten *et al.*, 1993; Sykes and Wilson, 1990a). Knevel (2001) conducted transplant experiments on three dune species from the area of this study, between soils of different origins (including the non-invasive *Ammophila arenaria* and *Arctotheca populifolia* which was included in the current study). These species had specific nematode fauna, but lacked a consistent increase in growth for plants growing in soil from other species, or sterile soil (Knevel, 2001). These results suggest that soil pathogens, or mycorrhizae, play a small or even positive role in dune species growth. Thus, it remains unclear what role soil-pathogens play in determining dune zonation in the Eastern Cape. An alternative explanation of zonation: salt tolerance, may be influenced by changes in nitrogen metabolism (e.g. osmotic metabolites, Pakeman and Lee, 1991a), thus analysis of nutrient limitation experiments may interact with salt tolerance, but for the sake of practicality salinity treatments were excluded from this study.

The proximity of a source of mobile sand, the ability of a species to trap sand, wind, soil moisture, and other factors determine the *in situ* rates of burial experienced by a species, and the distribution of species on the dunes (Hesp, 2002). High rates of sand burial in conjunction with nutrient limitation have the potential to filter out species lacking in growth characteristics enabling them to survive burial. Characteristics that allow these plants to have reduced carbon- and nitrogen-costs of compensatory growth to burial would be of great advantage. Thus, species found at different points along natural dune burial gradients are expected to display contrasting carbon- and nitrogen-related growth characteristics and show different responses to burial relating to their *in situ* burial regime. A survey of the literature finds; that coastal dune species do show variable responses to moderate burial (Sykes & Wilson, 1990b; Maun & Perumal, 1999), from tolerance (Bach, 1998), decreased growth (Sykes & Wilson, 1990b; Franks & Peterson, 2003) to increased growth and biomass accumulation (Disraeli, 1984; Maun & LaPierre, 1984; Maun, 1994). However, it remains unclear why some species are able to respond to burial, what mechanisms are responsible for the increased growth responses (Maun, 1998), and how these responses relate to nutrient availability.

In order to survive high rates of burial, plants must increase the production of stem (Maun & Lapierre, 1984), while also replacing their buried photosynthetic leaf area (Kent *et al.*, 2005). As growth of coastal dune plants is often limited by nutrients (e.g. Willis, 1965), a positive burial response necessitates the acquisition of nutrients, efficient use of nutrients that the plant already has, and efficient remobilisation of nutrients sequestered in senescing tissue. Thus, characteristics such as photosynthetic-nitrogen-use efficiency, nitrogen resorption from senescing leaves and stem tissue production costs are likely to be relevant in explaining burial related growth responses. During periods of substrate stability, plants benefit more from producing leaf area and associated short internodes than allocating resources to elongating stems. Thus, within a species, stem and leaf area production are expected to show different responses between buried and unburied conditions.

This study investigated the interaction between burial and nutrient limitations of four species of dune plants by using a factorial burial by nutrient addition experiment. The four species were selected based upon their dominance in different areas of a burial gradient. The selection includes species that naturally experience partial burial daily, to species that rarely experience burial. Plant growth responses were measured in terms of the maintenance of photosynthetic leaf area and stem elongation. Characteristics that allowed plants to respond to burial were measured on unburied shoots and included: stem tissue density, photosynthetic nitrogen-use-efficiency, leaf longevity, the mean time that nitrogen is retained in a shoot and measures of leaf nitrogen remobilisation (these data are given in Chapter 8: The nitrogen cost of carbon fixation). Specifically, we investigated whether: (1) Growth and the nature of the burial response recorded for each species relates to the *in situ* burial rates experienced by that species. (2) Species that are able to respond to burial possess characteristics allowing reduced carbon- and nitrogen-costs of stem elongation and photosynthetic leaf area production. (3) Allocation of carbon photoassimilates to stem elongation and photosynthetic leaf area production differ between unburied and burial conditions. (4) Soil nutrients limit growth and the magnitude of the burial response of a particular species.

MATERIALS AND METHODS

Study site

This study was conducted on the vegetation zonation to the west of Old Woman's River mouth, Eastern Cape Province, South Africa (See Chapter 1 for more details).

Plant species

Four species were selected based upon their importance in the dune ecosystem (high abundance and cover relative to other species) and their position along a burial/zonation gradient: *Arctotheca populifolia, Scaevola plumieri, Myrica cordifolia* and *Metalasia muricata*.

The four species are abundant in different regions of the dune succession with: *A. populifolia* found in areas of mobile sand from the high-tide mark to the top of the seaward-side of the first mobile-dune, *S. plumieri* found along most of the dune succession, but with highest biomass on both sea- and land-ward faces of the first mobile-dune where considerable sand movement occurs. *M. cordifolia* is found inland from the first mobile-dune, often occurring on the edge of the mobile sand region and *M. muricata* found in the rear on stable-dune sand (Lubke, 1983).

Measurement of in situ sand burial regime

The method of measuring sand burial regime for the four species was reported in Chapter 2.1 pp. 22.

Experimental design

A factorial burial by fertiliser addition experiment was initiated on 19 November 2004 (early summer) on all four species. Plots of homogenous vegetation for each species were selected and randomly assigned to unburied, burial, fertiliser addition and combined burial and fertiliser treatments. Unburied and fertiliser addition plots consisted of 50 by 50 cm staked areas. Pine frames, 50 by 50 cm were placed over burial plots. The heights of the frames (10, 20, 30 or 40 cm) depended on the height of the vegetation. The frames were filled to five cm below the tips of the highest leaves with freshly blown sand from four similar sites. This resulted in the total burial of most of the shoots in the frame and burial of at least 75% of the leaf area of marked shoots. The level of sand was monitored regularly and adjusted to the original height of burial. A full-nutrient long release fertiliser (15g of ‘Horticote 13:6:11 (30) plus’, 130g N kg^{-1}, 60g P kg^{-1}, 110g K kg^{-1}, 12g Mg kg^{-1}, 200 mg B kg^{-1}, 500mg Cu kg^{-1}, 2000mg Fe kg^{-1}, 600mg Mn kg^{-1}, 200mg Mo kg^{-1}, 150mg Zn kg^{-1}, Efecto, South Africa) and a short release fertiliser (15g of Wonder 3:2:1 28 SR*, 140g N kg^{-1}, 93.3g P kg^{-1}, 46.7g K kg^{-1}, Agroserve Pty., South Africa) were placed at 10-20 cm depth at four points per fertiliser addition plot (i.e. 60g Horticote and 60 g Wonder per plot), in addition to a surface application of 15g of each fertiliser. As the Horticote fertiliser took more than a year for all nutrients to be released, and due to high rates of leaching this amount of fertiliser was considered to be appropriate. A fourth treatment of combined burial and fertiliser addition was also installed in a similar manner to the burial and fertiliser addition plots. Fertiliser was reapplied to *M. cordifolia* and *M. muricata* plots on 6 February 2005. The number of plots for each treatment was *A. populifolia* (6), *S. plumieri* (6), *M. cordifolia* (5) and *M. muricata* (5). *A. populifolia* plots were positioned on the seaward face of the foredunes, while plots for all the other species were placed in homogenous stands of vegetation to the landward side of the foredune. To avoid within-individual nutrient dilution effects, individuals of *S. plumieri* and *M. cordifolia* that extended out of the 0.25 m^2 fertiliser addition plots were treated with similar fertiliser additions for up to 2 metres beyond the experimental plots.

Measurements on the factorial experiment

Prior to administering treatments, a single representative shoot in each replicate plot was marked and monitored throughout the course of the experiment. As species had different response times to burial, the experiment was terminated and shoots were harvested on different dates for each species: *A. populifolia* (16 December 2004, 27 days), *S. plumieri* (6 February 2005, 77 days), *M. cordifolia* and *M. muricata* (15 July 2005, 236 days). The following parameters were measured on the marked shoots at intervals during the duration of the experiment: the number of leaves produced and lost, lengths and widths of the third, fifth, eighth, tenth, fifteenth and twentieth leaves (if present) and the shoot height above the reference level of the original sand burial. The area of individual leaves was calculated from relationships between leaf length multiplied by width and leaf area for each species. The total leaf area of each shoot was estimated by summing the measured leaf areas and interpolated leaf areas between the third, fifth, eight, tenth, fifteenth and twentieth measured leaves. The total leaf area estimates were highly correlated with the total leaf areas measured on harvested shoots using image analysis software (WinDIAS, Delta-T Devices, Cambridge, U.K.). As *M. muricata* has very small ericoid leaves, a set of ten 5 cm long shoots were marked at the start of the experiment and a random sample of two marked shoots per plot was harvested at regular intervals. The average change per plot in mass or length of the marked stems of *M. muricata* was taken to represent leaf and stem production rates.

Gas exchange

Gas exchange measurements were made on young fully illuminated leaves of *A. populifolia* and *S. plumieri* on the 16 December 2004 (summer), using a LCA2 broad leaf chamber IRGA (Analytical Development Company, Hoddeston, England). Young fully illuminated shoots of the small leaved species, *M. cordifolia* and *M. muricata*, were measured on both the 16 December 2004 (summer) and 19 July 2005 (winter) with a transparent conifer chamber attached to a LI-COR 6400 (LI-COR Biosciences, Lincoln, USA). Measurements of rates of photosynthesis were taken under natural conditions between 11:00 and 15:00. During this period PAR incident on the leaf surface was (mean ±std. dev.) 2116 ±334 μmol m^{-2} s^{-1} (16 December 2004) and 1493 ±233 μmol m^{-2} s^{-1} (19 July 2005). At the two measurement dates cuvette CO_2 concentrations were 376.0 ±8.7 and 378.3 ±9.1 μmol mol^{-1}, respectively; VPD was 2.3 ±0.4 and 2.0 ±0.2 kPa and leaf temperature 29.4 ±1.5 and 28.3 ±1.1^{o}C. The LCA2 was calibrated prior to use with standard CO_2 cylinders and a dew-point apparatus.

Measurements of plant characteristics

Stem tissue density measurements were made on stems harvested from the burial by nutrient addition experiment, and supplemented with stems from naturally buried plants. Stem segments below the lowest leaf were used to measure the density of stem needed to support leaves. Stem tissue density was calculated as stem dry mass divided by the fresh volume of stem. Fresh volume was measured as the weight change of a water container after immersion of the stem in the water, as according to Archimedes' buoyancy principle (described in detail in Chapter 4).

Rooting experiment

Root ingrowth bags are often used to determine root activity, but these have the disadvantages of disturbing a volume of soil, and possibly changing the nutrient dynamics of the soil, depending on what medium the bag is filled with (Caldwell and Virginia, 1989). An alternative is root ingrowth sheets, which add no nutrients, and disturb a minimal plane of soil (Caldwell and Virginia, 1989). On 4 December 2005 40 sheets of nylon mesh (0.0625 m^2) were placed vertically in the soil, below the dry sand layer (from 20 to 45 cm depth). A flat spade was used to install these root ingrowth sheets with minimal damage or compaction to the surrounding sand. The ingrowth sheets were placed under homogenous stands of each of the species, apart from *A. populifolia*, for which only small individuals were available. The ingrowth sheets were excavated and removed on the 21 January, 48 days later. The day after removal, the ingrowth sheets were viewed under a bifocal dissecting microscope, and the root number and identity of the roots determined. A type collection of roots from the common species present at the site was made, and used as a comparison to identify the ingrowth sheet roots. The roots of *S. plumieri* and *M. muricata* were particularly distinctive.

Analysis

Repeated-measures ANOVA was used to determine the effects of two-way combinations of burial and fertiliser addition treatments on shoot height and total leaf area per shoot of *A. populifolia*, *S. plumieri* and *M. cordifolia* over time. Mauchly's test statistic was not significant for any of the analyses, suggesting that uncorrected F-ratios were appropriate. Two-way ANOVA was used to investigate the effects of combinations of burial and fertiliser addition treatments on the photosynthetic rate and leaf area of all species, and rates of shoot production, and total leaf area production per shoot of *M. muricata*. Logarithmic transformations were used where necessary to increase normality or homogeneity of variances. Interspecies comparisons of growth characteristics under unburied conditions were made using one-way ANOVA, or alternatively Kruskal-Wallis non-parametric ANOVA by ranks was done when transformation did not result in homogeneity of variance. Significant interspecies differences were found using the Tukey HSD *post hoc* test, and a multiple comparison Kruskal-Wallis ANOVA by ranks for parametric and non-parametric analyses, respectively.

RESULTS

In situ sand burial regime

The two mobile-dune species, *A. populifolia* and *S. plumieri*, were exposed to higher net rates of sand burial and higher maximum rates of burial than the two species on the stable-dunes, *M. cordifolia* and *M. muricata* (Table 5.1). The net rate of sand burial was highly variable for *A. populifolia*, and it was exposed to both high rates of erosion and deposition. The net rate of burial of *S. plumieri* shoots was less variable, but showed a significant net increase in sand level over time ($P < 0.03$, Table 5.1). The net rates of sand burial adjacent to *M. cordifolia* and *M. muricata* shoots (Table 1) were not significantly different from a zero change in sand level ($P > 0.31$ and 0.39, respectively). Sand burial rates correlate inversely with species stature (Table 5.1). Mobile-dune species that encountered high amounts of burial were short, and vulnerable to burial, while stable-dune species were tall and least vulnerable to burial (Table 5.1).

Table 5.1: Maximum and average rates of burial of each species in comparison to the rates of shoot growth, the height of leaves and total plant height above the sand surface.

	Maximum rate of burial [a]	Net rate of burial over period of measurement	Growth rate of unburied shoots	Height of lowest leaf above sand	Total plant height
	cm 30 day^{-1}	cm 30 day^{-1}	cm 30 day^{-1}	cm	cm
A.populifolia	17.5	2.4 ± 4.0 b	11.0 ± 4.7 a	5 ± 5 c	10 ± 5 c
S. plumieri	27	2.7 ± 1.4 a	0.1 ± 2.1 b	11 ± 14 ab	26 ± 13 a
M. cordifolia	2.3	0.2 ± 0.5 c	0.8 ± 0.6 b	16 ± 15 bc	30 ± 14 b
M. muricata	5	0.0 ± 0.1 c	1.9 ± 0.9 b	71 ± 33 a	90 ± 35 a

[a] maximum rate of burial for any 30 day period during the period of measurement
Different letters in each column represent significant differences at $P < 0.05$, for Tukey HSD tests of a one-way ANOVA or multiple comparisons calculated for a Kruskal-Wallis Ranks test. Means ± one standard deviation.

Factorial burial by fertiliser addition experiment

In all species, buried plants survived burial of between 70 and 95% of the total photosynthetic leaf area of a shoot.

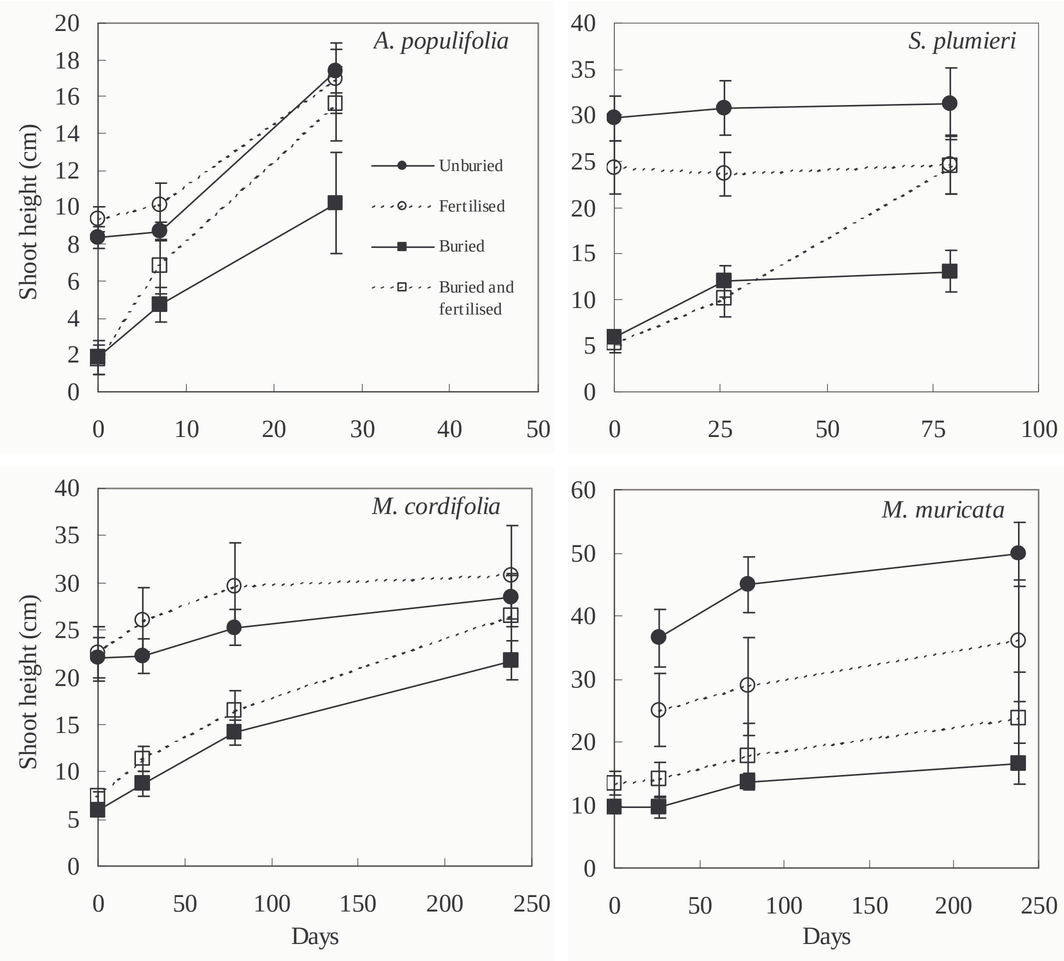


Figure 5.1: Shoot height measured above sand level of four species under unburied (closed circle), fertiliser addition (open circle), burial (closed square) and burial by fertiliser addition (open square) treatments imposed on day zero. Error bars are 1 standard error on either side of the mean, n = 3-6 depending on species and treatment. Refer to Table 2 for within-species statistics.

Burial elicited contrasting responses in vertical stem elongation among species, burial, time-by-fertiliser addition, and the time-by-combined burial and fertiliser addition treatment effects (Figure 5.1, Table 5.2). *A. populifolia* shoots had similar high rates of stem elongation under unburied and fertiliser addition conditions, but showed significant time-by-burial, time-by-fertiliser addition and time-by-burial and fertiliser addition interactions and thus greater rates of stem elongation under the burial treatments. *S. plumieri* had small rates of stem elongation under unburied and fertiliser addition conditions, but displayed a large inducible stem elongation response to burial. The addition of fertiliser to buried shoots of both species allowed stems to maintain elongation rates for a longer period such that shoot heights were similar to the unburied plants by the end of the experiment (Figure 5.1, Table 5.2). *M. cordifolia* stem height production had significant burial and time-by-burial effects suggesting an inducible response to burial. While *M. muricata* had a uniform rate of production irrespective of treatment (Figure 5.1, Table 5.2). The maximal rates of stem elongation differed widely between species, with mobile-dune species having significantly higher rates.

Table 5.2: Effects of burial, fertiliser addition and a combined burial and fertiliser addition on shoot height and total leaf area per shoot of four species of coastal dune plants.

		A. populifolia		*S. plumieri*		*M. cordifolia*		*M. muricata*[a]	
Variable	Effect	DF	F	DF	F	DF	F	DF	F
Shoot height	Burial (B)	1,13	39.81***	1,17	82.85***	1,16	41.07***	1,13	0.81ns
	Fertiliser(F)	1,13	1.73ns	1,17	0.18ns	1,16	0.60ns	1,13	0.01ns
	B*F	1,13	0.01ns	1,17	3.68ns	1,16	1.93ns	1,13	0.39ns
	Time (T) [b]	2,26	86.84***	2,34	25.56***	3,48	70.24***	na	
	T*B	2,26	13.84***	2,34	26.13***	3,48	28.67***	na	
	T*F	2,26	3.63*	2,34	3.70*	3,48	0.22ns	na	
	T*B*F	2,26	3.35ns[c]	2,34	3.34*	3,48	0.25ns	na	
Total leaf area per shoot	Burial (B)	1,13	10.59**	1,17	27.53***	1,16	7.71*	1,12	1.08ns
	Fertiliser(F)	1,13	11.02**	1,17	1.09ns	1,16	0.68ns	1,12	0.62ns
	B*F	1,13	0.70ns	1,17	0.25ns	1,16	2.11ns	1,12	0.07ns
	Time (T) [b]	1,13	97.64***	2,34	77.73***	3,48	7.29***	na	
	T*B	1,13	4.45ns	2,34	58.84***	3,48	6.20**	na	
	T*F	1,13	33.28***	2,34	7.62**	3,48	1.58ns	na	
	T*B*F	1,13	0.02ns	2,34	1.08ns	3,48	0.94ns	na	

[a] As data for *M. muricata* were not collected in a repeated manner a two-way ANOVA was done for the rates of shoot growth and leaf area production.

[b] Time is the repeated factor in the repeated-measures ANOVA done for *A. populifolia*, *S. plumieri* and *M. cordifolia.*

[c] $P < 0.051$

Significance levels: ***. $P < 0.001$; **, $P < 0.01$; *, $P < 0.05$; ns, $P \geq 0.05$

Burial significantly increased the production of leaf area in the two mobile-dune species and *M. cordifolia* in the short-term, but had no effect on *M. muricata* (Figure 5.2, Table 5.2). In *A. populifolia* and *S. plumieri* the increased leaf area production under burial conditions resulted in a full or partial replacement of lost photosynthetic leaf area, respectively. The mobile-dune species had significant time-by-fertiliser addition and time-by-combined burial and fertiliser addition interactions, thus fertiliser addition enabled buried shoots of the mobile-dune species to maintain high growth rates for longer periods, while the stable-dune species showed no response (Figure 5.2, Table 5.2).

Photosynthetic rate was significantly affected by time in *M. cordifolia* and *M. muricata* (Figure 5.3) and *M. cordifolia* showed a small (25%) significant increase in photosynthetic rate with burial, but the treatments had no significant effect on the photosynthetic rates of the mobile-dune species. As the photosynthetic rate per unit leaf area remained constant, the changes in total leaf area per shoot of the mobile-dune species represented the replacement of buried photosynthetic leaf area or an increase in the total photosynthetic leaf area, under burial or fertiliser addition conditions, respectively (Figure 5.2). The stable-dune species took longer to replace leaf area lost to burial than the mobile-dune species, and showed no effects of fertiliser addition (Figure 5.2). Average leaf size was significantly affected by fertiliser addition with no interactions for *A. populifolia* and *S. plumieri* (Figure 5.4).

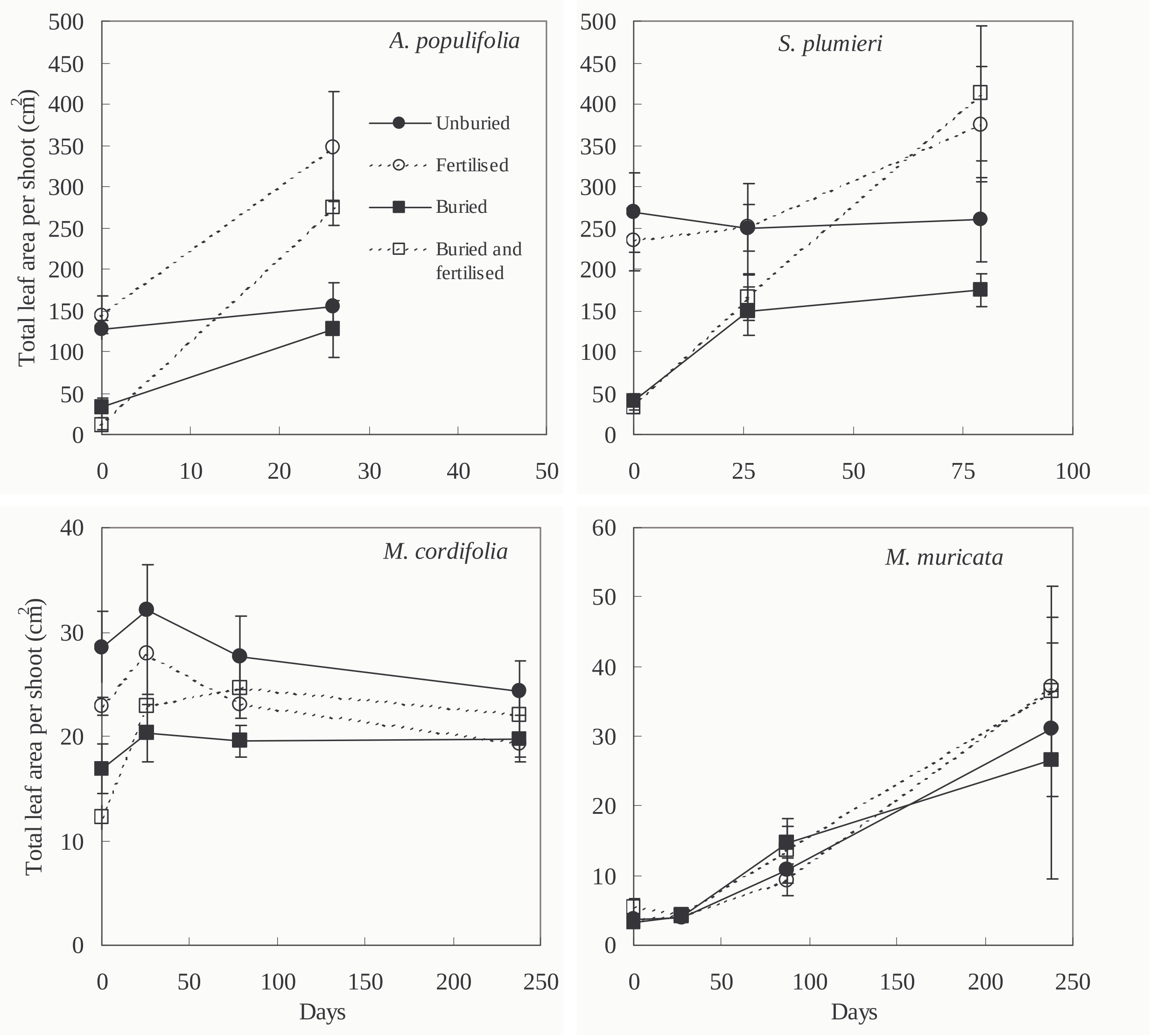


Figure 5.2: Total leaf area per shoot of four species under unburied (closed circle), fertiliser addition (open circle), burial (closed square) and burial by fertiliser addition (open square) treatments imposed on day zero. Error bars are 1 standard error on either side of the mean, n = 3-6 depending on species and treatment. Refer to Table 2 for within-species statistics.

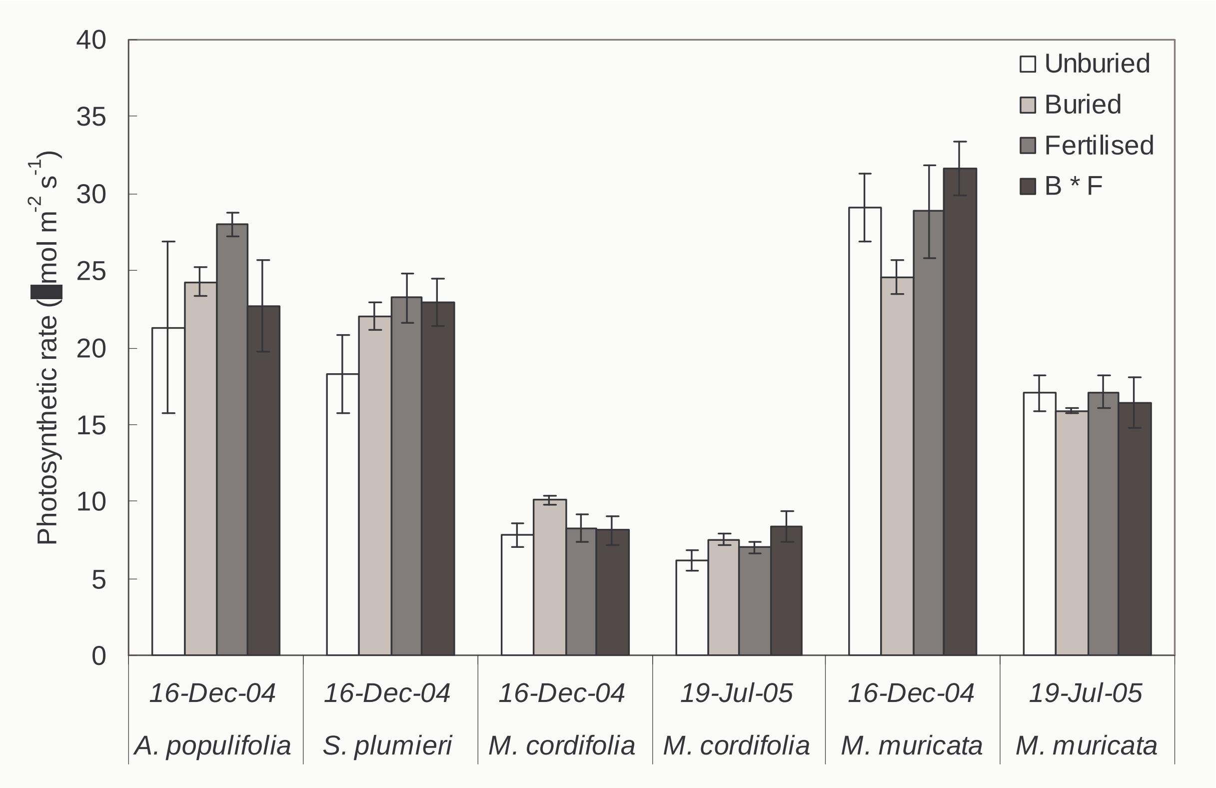


Figure 5.3: The photosynthetic rate of four species of dune plants. Error bars are 1 standard error.

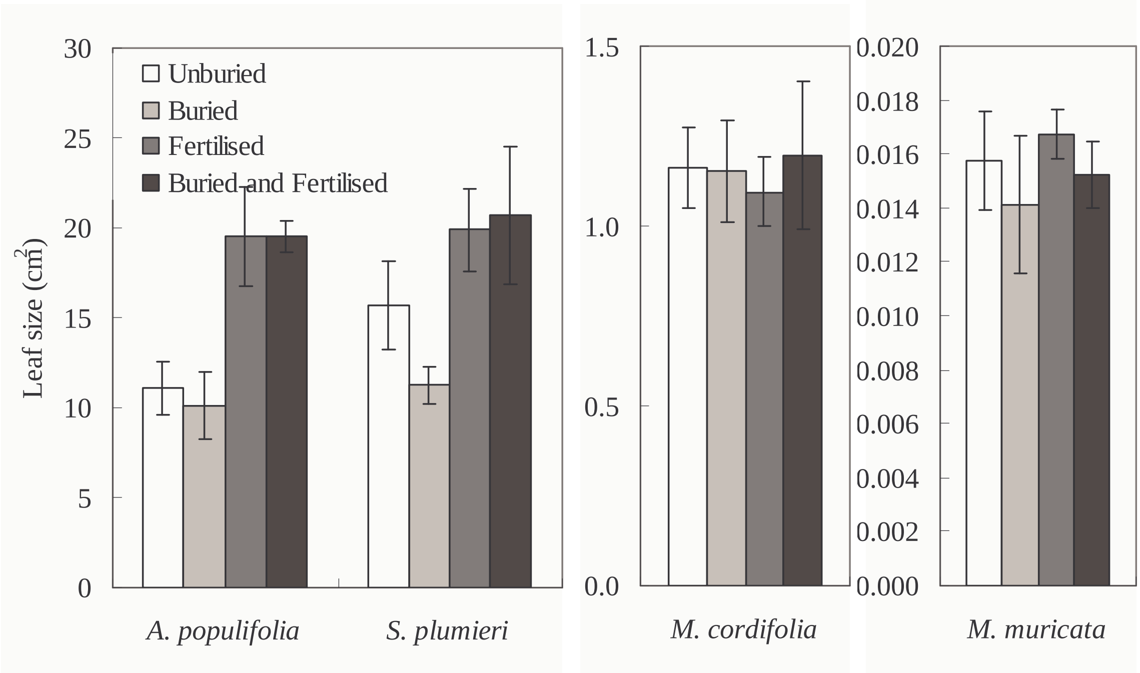


Figure 5.4: The average leaf size of four species of dune plants**.** Error bars are 1 standard error.

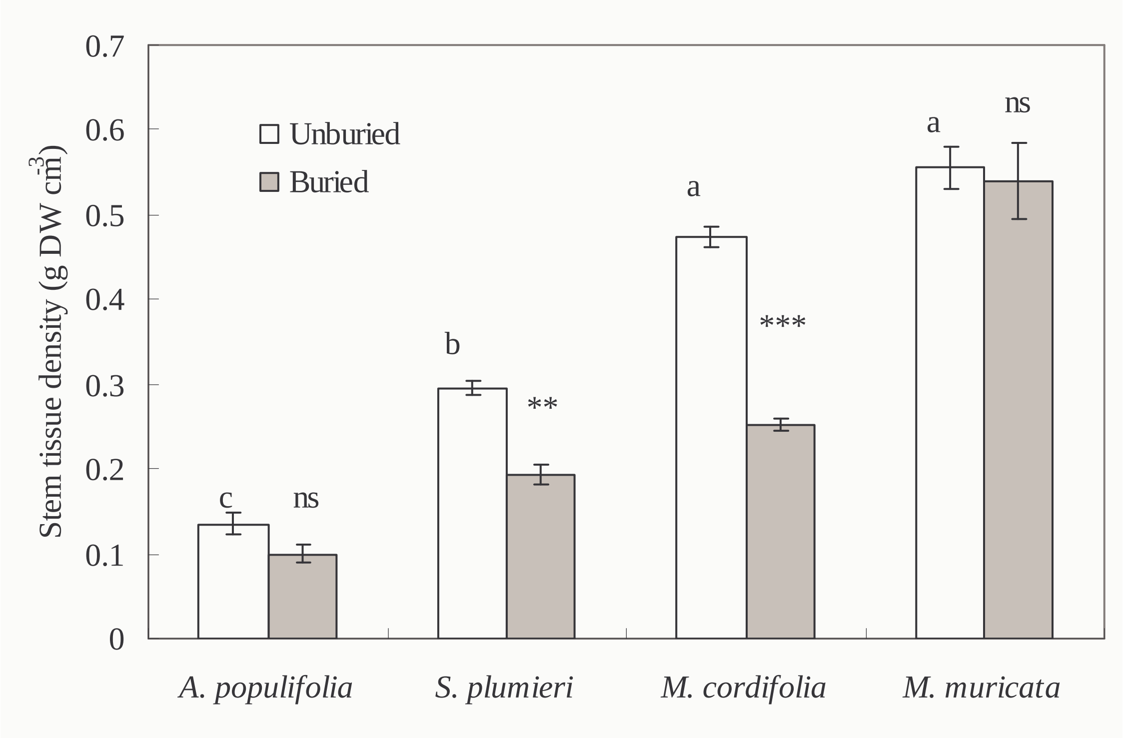


Figure 5.5: Stem tissue density below the lowest leaf of shoots under unburied or burial conditions, for four species of dune plants. Error bars are one standard error (n >= 7). Letters represent significant differences between species found with *post hoc* comparisons of Kruskal-Wallis ANOVA by ranks. Similarly, *'s and ns represent the significance levels (***. $P < 0.001$; **, $P < 0.01$; ns, $P \geq 0.05$) for paired comparisons of unburied and buried treatments within each species

Under unburied conditions stem tissue density (the dry weight production cost of stem volume) was 74 to 43% lower in the mobile-dune species, *A. populifolia* and *S. plumieri*, respectively, than the average stem tissue density of the stable-dune species (Figure 5.5). Under burial conditions, *S. plumieri* and *M. cordifolia* showed a reduction in stem tissue density of newly produced stem while *A. populifolia* and *M. muricata* showed little response (Figure 5.5).

Root ingrowth

The majority of roots growing near the soil surface under stands of *A. populifolia*, *S. plumieri*, and *M. muricata* are of the aboveground species (Figure 5.6, 5.7b). The majority of roots growing under the nitrogen-fixer, *M. cordifolia*, are roots of other species, with *M. muricata* roots most abundant. The species richness of roots increases with the typical stable-dune species, *M. muricata* and *M. cordifolia* (Figure 5.7c). The root ingrowth rate was highest in soil under the stable species, and significantly lower for *S. plumieri* (Figure 5.7a).

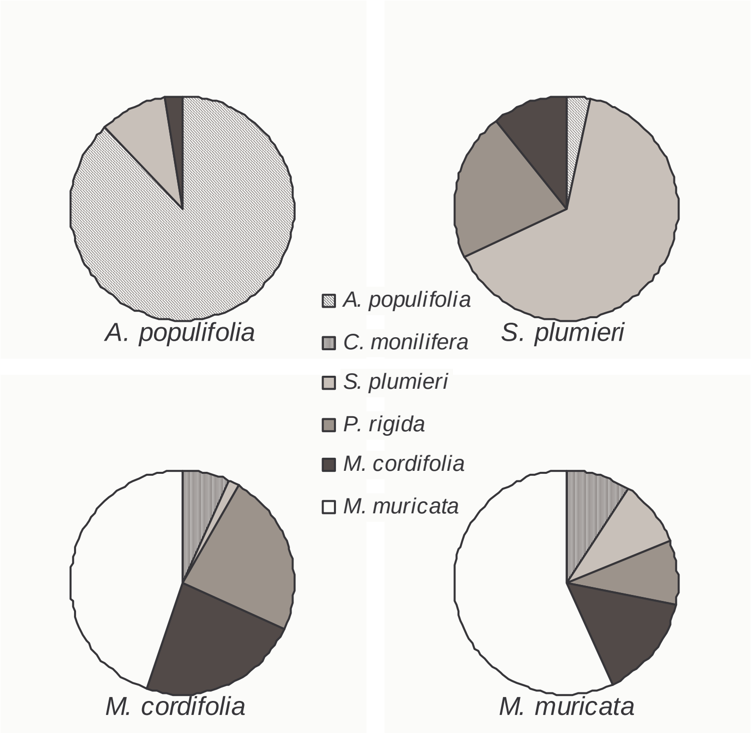


Figure 5.6: Rooting frequency of species growing under stands of four dune species.

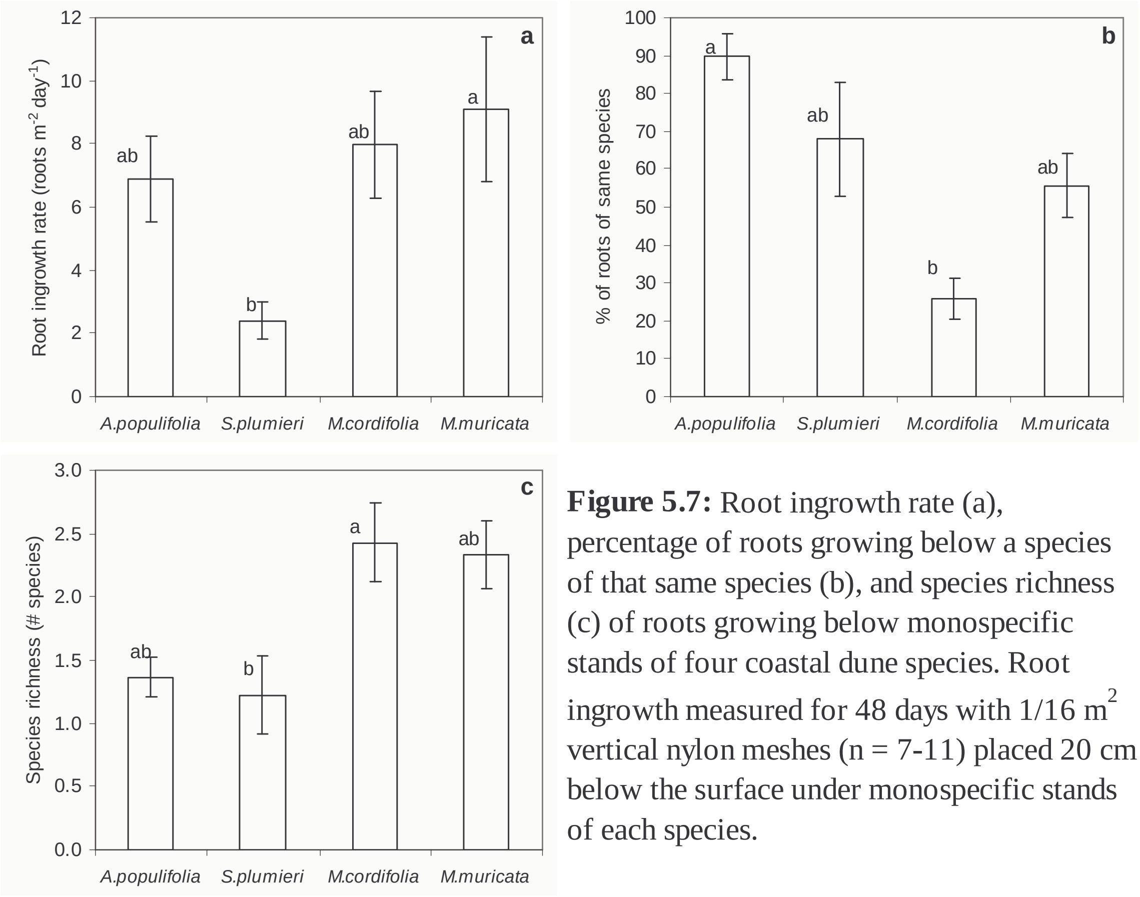


Figure 5.7: Root ingrowth rate (a), percentage of roots growing below a species of that same species (b), and species richness (c) of roots growing below monospecific stands of four coastal dune species. Root ingrowth measured for 48 days with 1/16 m^2 vertical nylon meshes (n = 7-11) placed 20 cm below the surface under monospecific stands of each species.

DISCUSSION

Generally, the low levels of nutrients in coastal dune sands are limiting to plant growth (Willis, 1965, Chapter 2.2) and the N: P ratios of the species studied suggest, that nitrogen may be limiting to *A. populifolia* and *S. plumieri* and phosphorus limiting to *M. muricata* and the nitrogen-fixer, *M. cordifolia* (see Güsewell (2004) for a review of nutrient limitation and N: P ratios). Thus, the availability of nitrogen or phosphorus may limit the ability of all four species to respond to burial and to maintain high shoot photosynthetic capacities. The carbon cost of stem production was related to the observed responses of the species to burial. Low tissue production costs allow plants adapted to high burial regimes to achieve the high growth rates needed to survive burial. Further plant characteristics relating to carbon- and nitrogen-use-efficiency are discussed in Chapter 8.

Response to fertiliser addition

The soil below stands of each species had similar low soil nutrient contents, and the N:P ratios of leaf tissue were likely to be nitrogen limited for the two mobile-dune species, and phosphorus limited for the two stable-dune species (Chapter 2.2; Table 2.2.2). This finding suggests that the surveyed mobile- and stable-dune species have different nutrient dynamics, and possibly different fertiliser responses. The total leaf area per shoot of *A. populifolia* and *S. plumieri* was increased by the application of fertiliser (a significant time-by-fertiliser addition interaction, Figure 5.2, Table 5.2), confirming that the mobile-dune plants are nutrient limited. However, *M. cordifolia* and *M. muricata* showed no response to fertiliser addition, for both total leaf area per shoot and stem elongation (Figure 5.1, 5.2 and Table 5.2). The nitrogen-fixing ability of *M. cordifolia* (Van Ryssen & Grobbelaar, 1970) may explain its lack of response to fertiliser addition. These findings are similar to those of agricultural nitrogen-fixers, such as soybean (Gutierrez-Boem *et al.*, 2004). Under normal conditions soybean yield is not thought to be greatly affected by nitrogen fertilisation, as the increased soil nitrogen levels and plant absorption, result in an equivalent inhibition of nitrogen-fixation, and little net increase in growth (Gutierrez-Boem *et al.*, 2004).

Vitousek & Walker (1989) found that the presence of *Myrica faya* in Hawai'ian forests increases the available nitrogen levels in the soil and positively affects the growth of seedlings of other species. The survey of root penetration through nylon sheets, found that the roots of *M. muricata* made up 45% of the roots penetrating the sheets below *M. cordifolia*, while roots of the mobile-dune species made up less than 2% of these roots. Thus, the presence of a high biomass of two nitrogen-fixers, *Acacia cyclops* (pers. obs. 2006) and *M. cordifolia* in the stable-dune habitat, the lack of response to fertiliser addition in *M. muricata* and the high quantities of roots of this species under stands of *M. cordifolia* suggests the possibility of an adequate nitrogen supply for *M. muricata* from nitrogen-fixing species.

Replacement of photosynthetic leaf area after burial

Growth subsequent to burial can be divided into two components necessary for survival: the shoot must replace its photosynthetic leaf area and elongate its stem. All species displayed a similar recovery of photosynthetic leaf area subsequent to burial, however the rates of leaf area production differed among species (Figure 5.2). A number of different responses of photosynthesis to burial have been described in the literature. Buried dune plants often increase photosynthetic rates e.g. *Ammophila breviligulata*, *Calamovilfa longifolia* (Yaun *et al.*, 1993), *Agropyron psammophilum*, *Panicum virgatum* (Perumal and Maun, 1999) and *Ulmus pumila* (Shi *et al.*, 2004). Alternatively, buried plants may show a reduction in photosynthesis e.g. *Cakile edentula* (Zhang, 1996). In contrast, this study found that buried plants replaced photosynthetic leaf area, by producing new leaf area (Figure 5.2) rather than changing photosynthetic rates (Figure 5.3). Similarly, the two mobile-dune species respond to fertiliser by increasing leaf area per shoot (a significant time-by-fertiliser interaction, Figure 5.2) and the size of individual leaves (Figure 5.4) but maintain the same rates of photosynthesis (Figure 5.3). Like the burial responses, this result contrasts with other studies on dune plants, that often find increases in photosynthetic rates in response to increased nutrient availability e.g. *Ammophila arenaria*, *Elymus mollis* (Pavlik, 1983) and *Cakile edentula* (Zhang, 1996). It seems likely that the photosynthetic gain for a greater investment of nitrogen per unit leaf area will be low as these species have high rates of photosynthesis, and relatively high photosynthetic nitrogen use efficiencies (Field and Mooney, 1986; Evans, 1989). Thus, there may be a greater gain in total shoot photosynthetic capacity by investing nitrogen in greater leaf areas rather than photosynthetic rates. Vos *et al*. (2005) suggest that plants, such as maize, respond to nutrient supply by increasing leaf nitrogen concentration and photosynthetic rate, while others, such as potato, adjust leaf size rather than photosynthetic rate. In response to increased nutrient availabilities, it appears that coastal foredune plants have been selected to adjust total leaf area and maintain constant nitrogen contents per unit leaf area rather than changing leaf photosynthetic rate per unit area, in a manner similar to the potato (Vos *et al.*, 2005).

Root distribution

Root ingrowth was sampled in the wet sand layer immediately below the surface concentration of litter. This sampling strategy may represent a bias for species for which rooting occurs at deeper depths. This may be the reason for the low root growth of *S. plumieri* (Figure 5.7a), however it is thought that the majority of soil nutrients are found in this layer. Alternatively, deep roots may access the permanent groundwater level, and any of the leached nutrients present (Ripley, 2002). The lower diversity, and higher proportion of roots belonging to the aboveground species, for the mobile-dune species, has important implications for the low biomass theory (see Chapter 9). This data suggests that there is less competition for belowground resources in the soil surface layers for mobile-dune species. REF Day

Differing stem elongation responses to burial

Under conditions of continual burial, a shoot must elongate its stem in order to keep newly produced leaves above the sand surface. This study found that dune plants may possess at least three different types of stem growth response to burial: (1) An inherently high rate of stem elongation with an enhanced response to burial, e.g. under non-burial conditions *A. populifolia* had high stem elongation rates relative to other species but was able to further increase these rates after burial. (2) An inducible stem elongation response, e.g. *S. plumieri* and *M. cordifolia* showed negligible or low rates of elongation under non-burial conditions, but had higher elongation rates in response to burial, the magnitudes of stem elongation being consistent with the *in situ* burial regimes of these species, and the height of their leaves above the sand level (Table 5.1). (3) No response, e.g. *M. muricata*, the species that is exposed to little burial and has the leaves at the greatest height above the sand (Table 5.1), survived moderate burial, but displayed no change in stem elongation rates.

Interaction between sand burial and nutrient limitation

The application of fertiliser allowed buried shoots of both mobile-dune species to recover and exceed the total leaf area of unburied shoots (Figure 5.2, Table 5.2). The stem elongation responses of both mobile-dune species were enhanced by burial for a short period, while addition of fertiliser to buried shoots allowed this response to continue until these shoots had reached the heights of the unburied plants (Figure 5.1, Table 5.2). In contrast, the total leaf area of the shoot, shoot height, leaf size and photosynthetic rates of the stable-dune species showed no change in response to fertiliser or a combination of burial and fertiliser addition. Thus, the high rates of fertiliser addition but lack of response to fertiliser of stable-dune species, suggest that nutrient limitation differs between the mobile-dune and stable-dune species, possibly due to differing abilities to acquire and/or use nutrients. The combination of high levels of sand burial and nutrient limitation in the mobile-dune habitat appears to be a vital factor in the determining the growth of the mobile-dune species, but this interaction does not appear to be important for the stable-dune species. Zhang ref

Characteristics linked to high production rates

Species stem tissue density under unburied or burial conditions correlated extremely well with the observed burial responses of the different species. The species showing a significant but small (*A. populifolia*) or no response (*M. muricata*) of stem elongation to burial had little ability to reduce stem tissue density (Figure 5.5), while *S. plumieri* and *M. cordifolia* showed inducible stem elongation responses to burial and a concurrent reduction in stem tissue density. The stem tissue density of a species was also negatively correlated with the maximal stem elongation rate (Figure 5.1 and 5.5). Thus, cost of stem tissue production (dry weight) and the ability of a species to reduce the cost of producing stem have the potential to determine the response of a species to burial.

Coastal dune plant species display fast stem elongation rates when buried; however, this study shows that the type of response is dependent on the *in situ* burial that a species is exposed too. The mobile-dune species were nutrient limited and this affected the ability of these species to respond to burial. This study suggests that the interaction of burial and nutrient limitation and associated characteristics is a vital aspect of the growth of coastal dune plants that has been overlooked in the past. In these circumstances the species were able to achieve the necessary fast production rates by possessing characteristics allowing favourable changes in the carbon economies of stem production.

Which responses are of further interest?

The distribution of *A. populifolia* and *S. plumieri* on the dunes results in these species encountering burial. After burial both species have a high stem elongation rate that allows them to maintain leaf area above the sand surface. *A. populifolia* displayed a small inducible stem elongation response to burial, but maintained a high rate of stem growth under all conditions. Given the nutrient limited growth of this species its high constitutive rate of growth is of interest. What nutrient and carbon related traits allow this species to grow fast? *S. plumieri* had a different growth pattern, with no stem growth under stable conditions, and an inducible rate of growth under burial conditions. What is the origin of the carbon and nitrogen used in this growth response? The post burial stem elongation responses of *A. populifolia* and *S. plumieri* are explained by reduced carbon costs of stem production, but what are the nitrogen costs of stem and leaf production? And, Do these costs allow the mobile-dune species to have fast, cheap production after burial? *M. muricata* shows no response to nutrient addition, but has a high rooting density under stands of the nitrogen-fixer *M. cordifolia*. Could this species be accessing the nutrients created by the nitrogen-fixer?

Thus, these three species have aspects of their growth of further interest. These aspects are investigated:

1) The lack of nutrient limitation in *M. muricata* in Chapter 6,

2) The inducible burial response of *S. plumieri* in Chapter 7,

3) The plant characteristics that allow fast growth under nutrient limiting conditions are investigated in Chapter 8 and Chapter 9.

Chapter 6

The effects of nitrogen-fixation on the growth of two species of dune plants found in different areas of the dune zonation

Do nitrogen-fixers facilitate growth of neighbouring plants? — Growth of *Scaevola plumieri* and *Metalasia muricata* in presence of the nitrogen-fixer, *Myrica cordifolia* — Growth of dune plants in a nitrogen limited environment, with and without nitrogen-fixers

INTRODUCTION

In a highly disturbed and stressed environment, such as coastal dunes, “reactive” growth is important response to disturbance (Maun, 1998). However, the growth of coastal dune plants is generally nitrogen limited (e.g. Willis, 1965). In this context, nitrogen-fixing leguminous, or actinorhizal plants may have a growth advantage relative to other plants. Soil salinity has an inhibiting effect on the nitrogen-fixing symbiosis (Cordovilla *et al.*, 1994; Erickson and Young, 1995; Sande and Young, 1992), and thus nitrogen fixation may be limited to the more vegetated stable areas some distance from the sea. Interestingly, only a few nitrogen-fixing species are important components of dune vegetation, in the Eastern Cape (Lubke, 1983; Lubke and van Wijk, 1998). It is not surprising that a leguminous nitrogen-fixer, *Acacia cyclops,* is a highly successful invasive stable-dune species in this area (Lubke and van Wijk, 1998). The actinorhizal nitrogen-fixer, *Myrica cordifolia,* is also successful in dune environments in which *A. cyclops* has not invaded (e.g. Figure 1.1).

The facilitation of the growth of plants growing near nitrogen-fixers

The positive effects of nitrogen-fixers on other plants have been well established. The North American coastal dune species, *Myrica pensylvannica*, was shown to facilitate the growth of two other dune species (Shumway, 2000). The leaf litter of the invasive *Myrica faya* added large quantities of nitrogen to nutrient impoverished volcanic soils in Hawaii, increasing the growth of the co-dominant *Metrosideros polymorpha* (Vitousek and Walker, 1989). Generally, nitrogen-fixing species remobilise little leaf nitrogen prior to leaf senescence, thus potentially adding significant amounts of nitrogen to the soil (See Chapter 8, Table 8.2; Tateno, 2003; Vitousek and Walker, 1989). In the Southern African context, *Acacia cyclops* changes the vegetation dynamics of coastal fynbos, in part, through soil enrichment (Witkowski and Mitchell, 1987). In that study, under stands of *A. cyclops* there were increased soil nitrogen levels, leaf litter phosphorus concentrations, and considerably higher soil phosphorus levels than surrounding areas. Thus, due to greater litterfall and higher litter phosphorus concentration under *A. cyclops*, the habitat was enriched in phosphorus and nitrogen (Witkowski and Mitchell, 1987). Finally, the actinorhizal *Alnus incana* has been shown to transfer nitrogen to *Pinus sylvestris* via ectomycorrhizal connections (Ekblad and Huss-Danell, 1995; Arnebrant *et al.*, 1993). This transfer was of limited importance to *Pinus sylvestris* (Ekblad and Huss-Danell, 1995), but it is plausible that nitrogen transference may be of greater importance in other nitrogen limited ecosystems. Thus, it seems likely that the high biomass of nitrogen-fixers in the stable-dunes may result in locally different nutrient dynamics and growth (Alpert and Mooney, 1996), whether through direct facilitation, or increased soil nutrient inputs.

A study in Florida (Greaver, 2005), found that two species, *Ipomoea pes-caprae* and *Coccoloba uvifera*, that were found along the entire dune gradient, had higher leaf nitrogen contents and N:P ratios further back in the dune zonation. Based upon N:P ratios it was concluded that these species shift towards nitrogen and phosphorus colimitation further from the sea, consistent with the results of this study (Chapter 2.2). Greaver (2005) attributes this gradient to greater amounts of mycorrhizal associations further from the sea. An alternative hypothesis could be that the ^{15}N depletion of backdune individuals could result from nitrogen-fixer facilitation, whether direct, or via the nitrogen enriched (and ^{15}N depleted) leaf litter of a nitrogen-fixer (Caldwell and Virginia, 1989). A species of *Chamaecrista*, a leguminous nitrogen-fixer, was present in the backdune at this site (Greaver and Sternberg, 2007, Ecology Archives).

Is dune plant growth, in the Eastern Cape, facilitated by the presence of *Myrica cordifolia*?

In this thesis, some previous observations may suggest an important role for nitrogen-fixers in determining the growth and distributions of *Metalasia muricata* on coastal foredunes. These observations are summarised below:

1) The lack of a fertiliser growth response for *M. muricata* and the nitrogen-fixer *M. cordifolia*, while the growth of *Arctotheca populifolia* and *Scaevola plumieri* were nutrient limited,
2) The presence of a high proportion of roots of *M. muricata* under stands of the nitrogen-fixer, *M. cordifolia*, Figure 5.6,
3) The N:P ratios of leaf tissue suggest that *M. muricata* is likely to be co-limited by nitrogen and phosphorus (Table 2.2), and
4) The presence of a high biomass of the nitrogen-fixers, *M. cordifolia* and *A. cyclops,* in the stable-dune habitat in many coastal dune sites (Figure 1.1).

Thus, *M. cordifolia* could play a role in increasing the available nitrogen to plants growing within or adjacent to stands of this species. However, the soil nitrogen content was similar in sieved soil sampled from underneath all four species studied in the previous section (Table 2.2). Thus, facilitative interactions may occur directly from plant to plant, or these interactions may be highly localised to areas such as immediately below the surface of decomposing plant matter (excluded from the soil analysis presented in Table 2.2).

Testing for nitrogen limitation, and growth stimulation by a nitrogen-fixer

Vitousek and Walker (1989) used three criteria to test for the effect of a nitrogen-fixer on other plants: 1) nitrogen must be limiting to the growth of the plants, 2) the nitrogen-fixer must change nitrogen inputs into the ecosystem, and 3) these inputs must be available to other species. For this study, the first and last criteria were tested. The second criterion was assumed to be the valid, as *M. cordifolia* was seen to be nodulated (pers. obs.), the senesced leaves of *M. cordifolia* are nitrogen rich (Chapter 8), and a large body of literature exists confirming this for most ecosystems (see above).

Greaver (2005) termed species whose distributions range across the dune zonation, trans-dune species. These species provide a useful opportunity to study environmental gradients or heterogeneity, without the problems of comparing different species (Greaver, 2005). *S. plumieri* is clearly a trans-dune species (Figure 1.1), found in isolation of other species, but also in close proximity to *M. cordifolia* stands (Plate 1.3b and c). At least two mechanisms may result in higher nutrient availabilities for mobile-dune individuals of *S. plumieri*, relative to stable-dune individuals in the absence of a nitrogen-fixer. The decomposing detritus at the tide/storm water mark may represent a large source of nutrients which *S. plumieri* plants may access through vigorous rooting. A similar phenomenon has been observed for *Cakile maritima* and *Salsola kali* (Pakeman and Lee, 1991a). The second reason is that lower biomasses on the mobile-dunes may result in lower competition for nutrients, and greater availability of the resource. This topic is dealt with extensively in Chapter 9. A second hypothesis was that the growth of stable-dune plants of *S. plumieri* is stimulated by the presence of a nitrogen-fixer. Thus, it is suggested that *S. plumieri* could have different growth in different areas of the dune zonation, due to different nutrient availabilities, and the presence of nitrogen-fixers

Increased growth associated with greater nutrient availability may be allocated to leaves, stems, roots, or reproductive tissue. Nitrogen addition, the presence of a nitrogen-fixer, or spatially heterogeneous soil nitrogen, may allow a plant to allocate growth to processes other than those required to survive the most severe environmental factor. For instance, a partially buried shoot with greater nitrogen resources may have increased leaf or reproductive growth, as there may be sufficient resources for stem elongation to be a weak nitrogen sink. Thus, changes in allocation may be an important response to changes in nitrogen availability, and any of the treatments applied in this study.

Other factors

Myrica biomass may also have negative effects on adjacent vegetation. *Myrica pensylvanica* and *Myrica cerifolia*, from the eastern United States have been shown to have facilitative, competitive and inhibitory effects on plants growing within stands of these two species (Collins and Quinn, 1982; Tolliver *et al.*, 1995). At moderate light intensities soil and leaf litter from *Myrica cerifolia* enhanced *Pinus* seedling shoot growth, while reducing the amount of roots, a possible nutrient related facilitation. But, the leaf leachate reduced the germination of *Pinus* seeds, a possible allelopathic response (Tolliver *et al.*, 1995). Low light levels reduced *Pinus* seedling growth, further indicating that light competition may also play a role in limiting the invasion of *Pinus* trees into *Myrica* dominated dune areas (Tolliver *et al.*, 1995).

AIMS

The nitrogen limitation of the growth of dune species was tested by applying an ammonium-nitrate fertilisation treatment or no treatment to individuals of *S. plumieri* and *M. muricata*. The possible facilitation effect of *M. cordifolia* was tested by repeating the same nutrient addition treatments for plants of both species growing within stands of *M. cordifolia*, or a distance from these stands. An additional set of nitrogen-addition treatments were performed on *S. plumieri* shoots on the low biomass, seaward slope of the foredune.

The hypotheses for this section were that:

1) Nitrogen was limiting to the growth of *S. plumieri* and *M. muricata*,
2) Individuals of these species growing in stands of *M. cordifolia* would not show an increase in growth in response to nitrogen addition, or the increase would be reduced in comparison with control plants to which nitrogen had been added,
3) The trans-dune species, *S. plumieri*, would show a gradient of production consistent with greater competition for nutrients in the higher biomass landward areas of the dunes, but not within stands of *M. cordifolia*,
4) The allocation of biomass to the production of leaf, stem or reproductive structures is resource dependent.

METHODS

Study site

This study was conducted at the Old Woman's River site. The exotic invasive nitrogen-fixer, *A. cyclops*, is present at this site. For this reason, experiments were limited to the areas of this site greater than 10 metres distant from large clumps of this species.

Nitrogen addition treatments

On 11 November 2006, one by one metre quadrats containing shoots of *S. plumieri* (n = 11) were treated with a surface application of 32 g of lime ammonium nitrate

(Wonder LAN, 280 g N kg^{-1}) and a second application of 32 g 20 cm below the sand surface. Whole plants of *M. muricata* (n = 10 to 11) were treated in a similar manner, with fertiliser being applied to the ground area of the entire plant. For each nitrogen treatment a comparable unfertilised shoot/plant was selected at greater than 2 m distance, such that there was little chance of nutrient contamination. The low organic matter of dune soils and fast rates of leaching may result in little lateral movement of nitrogen. This assumption was supported by a clear growth response of shoots of *S. plumieri* after nitrogen addition which was limited to a local area (<2 m radius) around the point of addition (pers. obs.). These treatments were performed landward of the foredune in the absence or presence of a high biomass of the nitrogen-fixer, *M. cordifolia*, for both *M. muricata* and *S. plumieri*. An additional set of treatments was conducted on shoots of *S. plumieri* growing on the seaward side of the foredune, in the absence of *M. cordifolia*. This experiment is comparable and was conducted simultaneously to the burial manipulation experiment, for *S. plumieri*, at varying biomass of Chapter 9.

Measurements

The application of nitrogen and initial measurements took place on 11 November 2006 with subsequent measurements on 28 December 2006 and 9 March 2007. The leaf area and stem production of shoots of *S. plumieri* were measured in a similar manner to those described in the previous section (Chapter 5). Fifteen short (3cm) stem segments of *M. muricata* were marked with coloured wire, such that on subsequent occasions the amount of biomass distal to the marker represented growth. The duration of the experiment was short enough that the no biomass was lost above the marker, due to senescence. On the first and final measurement dates, three of the marked stem segments on each plant were randomly selected and harvested. The stem, reproductive, and leaf dry weight were measured for the harvested shoots, after 3 days in a 60°C oven.

The canopy size of *M. muricata* was measured as the volume of the plant’s canopy. The volume was assumed to represent an ellipsoidal hemisphere, and thus the formula for the volume was:

$$Volume = \frac{1}{6} * \pi * l * w * height$$

Where l was the maximum length of the plant, w the width of the plant perpendicular to the maximum length, and the height measured at the tallest point. The canopy size was a highly significant predictor of other functional characteristics such as basal stem diameter ($R^2 = 0.88$, n = 16, $P < 0.001$, survey on untreated plants).

A separate survey was made of the relationship between flowering and canopy size of *M. muricata*. This survey preceded the experiment by a year, and represents the majority of un-manipulated *M. muricata* plants at the site. For each plant, the density of flowers was counted within a 0.06 m^2 quadrat placed randomly on the canopy, and the volume of the canopy was measured as above.

Analysis

The effects of the presence of a nitrogen-fixer (for *M. muricata* and *S. plumieri*), or position on the dune zonation (for *S. plumieri*) were tested separately as these relate to different hypotheses (hypotheses 2 and 3, respectively). As the variables were allometrically related, and hypotheses of the allocation of biomass were to be tested, ANCOVA models were used to model the relationship between: growth and plant initial size (*M. muricata*), and between leaf and stem growth (*M. muricata* and *S. plumieri*). In all analyses, the natural logarithm of each continuous variable was used in the analysis to linearise the allometric relationships, and reduce the difference in variances between treatments (Warton *et al.*, 2006). The ANCOVA procedures outlined in Appendix 1 (Figure 12.3) were used. The basic procedure was to fit a maximum factorial linear model for each growth variable (a linear model including all factors and all possible interactions). A minimum adequate sub-model was then found using the suggestions of Crawley (1993), but replacing the likelihood ratio test with the more recent information-theoretic approach (Johnson and Omland, 2004), using the *step()* function of the R program. This procedure uses Akaike's Information Criterion (AIC) to sequentially compare fitted models with sub-models lacking single effect or interaction terms. The model or sub-model with the smallest AIC values is chosen as the least complex model that best represents the data (R Development Core Team, 2006). If this model had significant factor-by-covariate interactions, then no further ANCOVA analysis was done (see flow chart in Appendix 1, in Figure 12.3, for an outline of this procedure).

The difference in the proportion of marked shoots flowering or seeding at the end of the experiment, between shoots to which nitrogen was or was not applied, was tested using a Monte Carlo randomisation procedure (Manly, 1997). The combined data of both treatments was randomly partitioned, without replacement, into two categories of the same size as the original data set. This procedure was repeated 1000 times. The probability that the observed difference would have occurred if the underlying process was random is given by: the number of random samples that had a proportional difference greater than or equal to the observed difference plus one and divided by the number of randomisations. These tests were not exact, and were sensitive to small changes in the numbers of plants that were flowering. Thus, the results of these simulations are only given as indications of possible treatment differences.

RESULTS

Growth and allocation relates to size

A number of *M. muricata* plants experienced herbivory during the experimental period. Removal of these plants from the analysis would have compromised the replication of some treatments, therefore herbivory was included as a factor in all analyses of *M. muricata*.

The initial size of *M. muricata* plants was an important non-linear determinant of the *RGR* of this species (an exponent significantly different to one represents changes in *RGR* with initial size; Figure 6.1; $R^2 = 0.96$, n = 40, $P<0.001$, final size=1.82*initial size$^{0.86}$). For this reason, the effects of nitrogen-fixers and nitrogen addition on canopy size at the end of the experiment were tested with an ANCOVA taking into account the initial size. This analysis avoided the use of *RGR* which was size biased for this data set (see Figure 6.2a). The allometric relationship between the initial and final size of *M. muricata* results in a 1.5 to 4.3 times increase in the size of the plant over the course of the experiment, from the largest to smallest measured plants (an extreme of 12.7 was measured in one small plant).

The decrease in the relative growth rate of the plant canopy with initial size (Figure 6.2a), coincides with a large increase in reproductive allocation (Figure 6.2b) and effort (Figure 6.2c). Reproductive allocation was not included as a factor in the linear models of *M. muricata* canopy growth, as this is a non-normal parameter, and including this parameter resulted in complex models with few degrees of freedom.

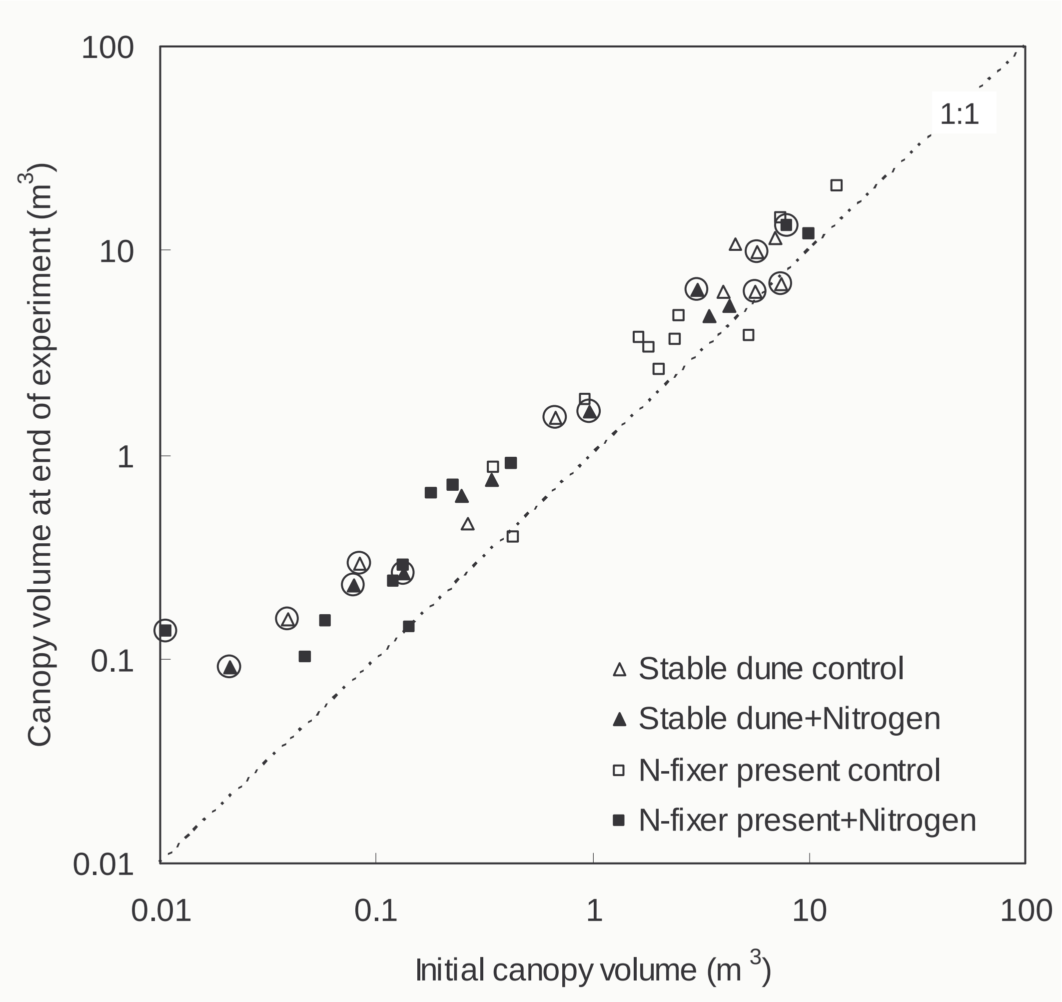


Figure 6.1: The growth of *M. muricata* canopy volume relative to initial canopy volume, under a nitrogen addition treatment, and in the presence of nitrogen-fixers. The line of no net growth (dotted), and herbivorised plants (circled) are shown. ANCOVA statistics are given in Table 6.1.

Leaf to shoot allocation of the biomass production of marked shoots of *M. muricata* and *S. plumieri* were allometrically related (data not shown; *M. muricata*: $R^2 = 0.92$, $n = 38$, $P < 0.001$, leaf growth = 2.07*stem growth$^{0.83}$: *S. plumieri*: $R^2 = 0.90$, $n = 64$, $P < 0.001$, leaf growth = 15.2*stem growth$^{0.55}$). Thus, for both species the productivity of the shoot largely determines the relative leaf to stem allocation of biomass. The different allometric exponents, between species, result in large changes in leaf to stem ratio with shoot productivity, for *S. plumieri*, and smaller changes for *M. muricata*. For example, the leaf to stem ratio of *S. plumieri* varies from 14.8 to 2.1 from the least to most productive shoots measured, while the leaf to stem ratio of *M. muricata* varies from 1.3 to 1.8 (with an extreme value of 3.2).

Does size affect the responses of species to nitrogen, nitrogen-fixers or dune position?

Due to allometric relationships, ANCOVA was used to test for changes, across treatments, in the allocation of biomass between natural logarithm transformed leaf and shoot production. The stem production of *S. plumieri* did not significantly interact with any of the tested the factors, although the interaction of stem production and nitrogen application was included in both minimum adequate models (Tables 6.1 and 6.2). Thus, the treatments did not significantly affect the allometry of leaf to stem allocation for *S. plumieri*, or treatment effects were not greatly affected by the size of the shoot. Similarly, the final canopy size of *M. muricata* was not significantly affected by the interaction between initial size and a treatment factor, although the interaction of stem production and nitrogen application was included in the minimum adequate model (Table 6.1), similar to the leaf to stem allocation of *S. plumieri*. The consistent inclusion of treatment-by-covariate interactions in the minimum adequate models, prevents further ANCOVA analysis (see Figure 12.3). Thus, while size had no significant effect on the allocation or growth of *S. plumieri* or *M. muricata*, such an effect could not be discounted.

The stem production of *M. muricata* shoots, as a predictor of leaf production, did have significant interactions with nitrogen application and the presence of a nitrogen-fixer (Table 6.1). The implications of this complex interaction are difficult to determine. Graphically, stable-dune plants to which nitrogen had been applied appeared to have a lower leaf to stem ratio than other treatments, after the allometry of stem production had been taken in to account (data not shown).

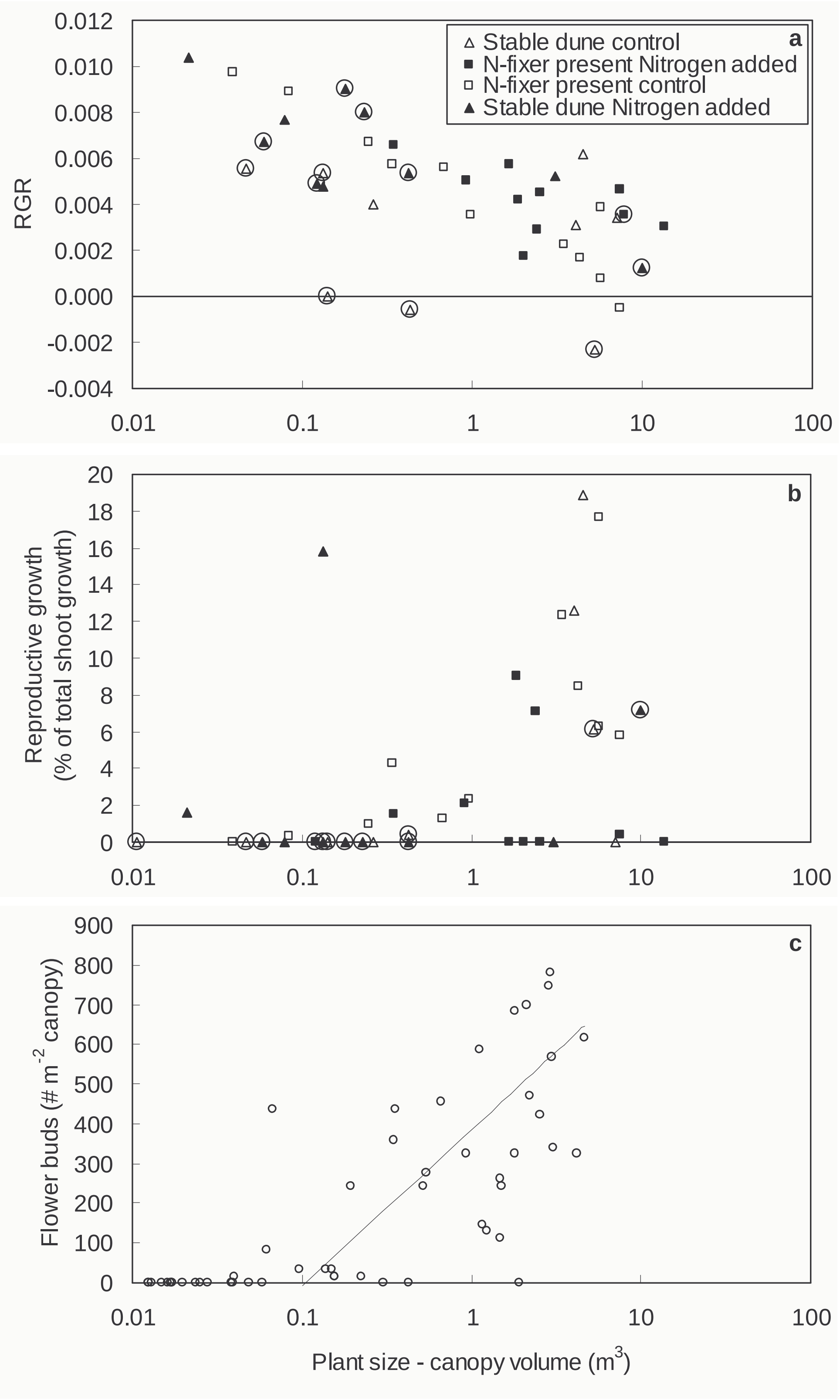


Figure 6.2: Canopy size relative growth rate (a), percentage of the mass of produced shoots that was reproductive (b) of experimental plants of *M. muricata*, and the reproductive effort of natural *M. muricata* individuals (c) across a range of sizes. Point symbols are the same as Figure 6.2. An SMA line was fit to the plants bearing flowers (panel c: Flower bud density = 171*ln(canopy volume)+385, $R^2 = 0.44$, n = 33, $P < 0.001$).

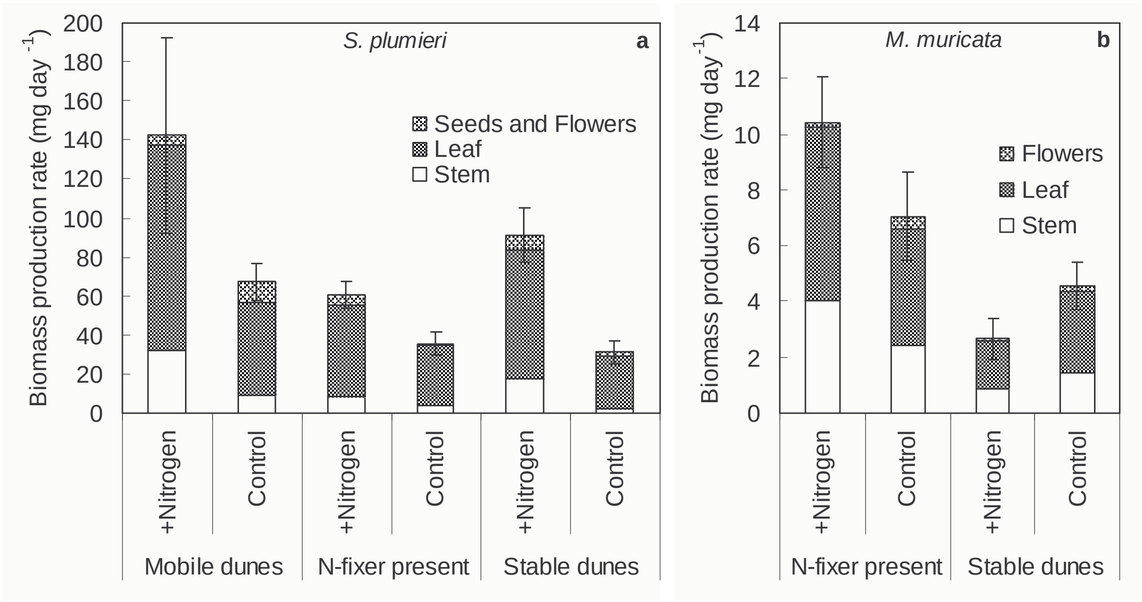


Figure 6.3: Biomass production rates of leaf, stem, and reproductive components of marked *S. plumieri* (a) and *M. muricata* (b) shoots. Statistics testing for the effect of the presence of nitrogen-fixers on the growth of both species, or position on the dune zonation on *S. plumieri* are given separately, in Table 6.1 and 6.2, respectively.

Is nitrogen limiting to the growth of *S. plumieri* and *M. muricata?*

The application of nitrogen had a significant effect on the growth of both species for the majority of response variables tested (Figures 6.3, 6.4, and 6.5, Tables 6.1 and 6.2). In two specific cases the application of nitrogen did not have a stimulatory effect. There was a significant interaction between the presence of a nitrogen-fixer and nitrogen application for *M. muricata*, such that stable-dune plants showed a decrease in growth after nitrogen application, but not in the presence of a nitrogen-fixer (Figure 6.3b and Table 6.1). The second exception occurred when nitrogen application had no effect on the proportion of seeding *S. plumieri* shoots in the mobile-dunes, and no effect on the proportion of branching shoots in the presence of a nitrogen-fixer (Figure 6.4b and d).

Table 6.1: Significant effects and coefficients for minimum adequate linear models testing for the effects of presence of a nitrogen-fixer, nitrogen addition, herbivory, initial canopy size, and stem production, on components of the growth of *M. muricata* and *S. plumieri*. The sign and significance (bold text) of coefficients are given. These notes can be interpreted as the sign of the effect of the listed conditions on the variable in question. For example, for *M. muricata* shoot production **+N Nitrogen-fixer** represents a significant and positive effect of nitrogen application in the presence of a nitrogen-fixer. n.i. = not included in the minimum model

	M. muricata						*S. plumieri*							
Effect	Leaf mass production		Total shoot production		Canopy growth		Total shoot production		Leaf area production		Stem elongation		Leaf mass production	
	P	Coef.	P	Coef.	P	Coef.	P	Coef.	P	Coef.	P	Coef.	P	Coef.
Nitrogen-fixer presence (Nfix)	n.s	-Nfix.	***	+Nfix	n.i.		n.s.	+Stable	n.s	+Stable	n.s	+Stable	n.i.	
Nitrogen application (N)	*	-N.	n.s.	**-N**	**	+N	***	**-Unfert.**	***	**-Unfert.**	***	**-Unfert.**	***	-Unfert.
N*Nfix	n.s.	+N. Nfix.	**	**+N and Nfix**	n.i.		n.s.	-Cont.Stable	n.i.		n.i.		n.i.	
Initial Canopy size (S)	-		*	+	***	+	-		-		-		-	
Herbivory (H)	n.s.	**-Herb.**	**	-Herb.	**	**-Herb.**	-		-		-		-	
H*N	n.s.	-Herb. N.	n.s.	-N and Herb.	***	**+Herb. N**	-		-		-		-	
S*N	-		n.s.	+N and Size	n.s.	+N Size	-		-		-		-	
Nfix*H	*	**+HerbNfix**	n.s.	-Nfix Herb.	n.i.		-		-		-		-	
Nfix*S	-		n.s.	-Nfix Size	n.i.		-		-		-		-	
H*S	-		n.s.	**-Herb. Size**	n.i.		-		-		-		-	
Nfix*N*S	-		*	-Nfix N Size	n.i.		-		-		-		-	
Nfix*H*S	-		**	**+NfixHerb Size**	n.i.		-		-		-		-	
ln Stem production	***	+	-		-		-		-		-		***	+
N*ln Stem production	**	**+large N.**	-		-		-		-		-		n.s.	+large Cont
H*ln Stem production	n.s.	+largeHerb.	-		-		-		-		-		-	
Nfix*lnStem production	n.s.	+largeNfix.	-		-		-		-		-		-	
H*N* lnStem production	n.s.	+largeHerbN	-		-		-		-		-		-	
Nfix*N*ln Stem prod.	0.053	-NlargeNfix.	-		-		-		-		-		-	

Table 6.2: Significant effects and coefficients for minimum adequate linear models testing for the effects of presence of position on the dune zonation, fertiliser application, and stem production, on components of the growth of *S. plumieri*. The sign and significance (bold text) of regression (slope) coefficients are given. The significance of regression coefficients can be interpreted as the reliability of the effect, and the sign as the positive or negative influence of that factor.

Effect	Total shoot production		Leaf area production		Stem elongation		Leaf mass production	
	P	Coefficient	P	Coefficient	P	Coefficient	P	Coefficient
Position (P)	**	-Stable	**	**-Stable**	**	-Stable	***	-Stable
Nitrogen application (N)	***	-Unfert.	***	**-Unfert.**	***	+Unfert.	***	-Unfert.
P*N	n.s.	-Unfert. Stable	n.i.		**	**-Stable Unfert.**	*	**+Stable and Unfert.**
log Stem production (S)	-	-	-	-	-	-	***	+
N*ln Stem prod.	-	-	-	-	-	-	n.s.	+ large Unfert.

[1] – not tested or not applicable; n.i. not included in the minimum model ; n.s. not significant at a 0.05 level of significance; * $0.05 < P < 0.01$; ** $0.01 < P < 0.001$, *** $P < 0.001$. **Bold** text represents model coefficients that were significantly different to 0.

Does position on the dune zonation affect the growth of *S. plumieri*?

The total shoot growth, stem elongation, leaf area production, and proportion of shoots with seeds were significantly higher in mobile-dune plants in comparison with stable-dune plants (Figures 6.3 and 6.4; Table 6.2). The growth of unfertilised mobile-dune shoots of *S. plumieri* was comparable to the values measured for stable plants to which nitrogen had been applied (Figure 6.3a, and Figure 6.4a, b, and c).

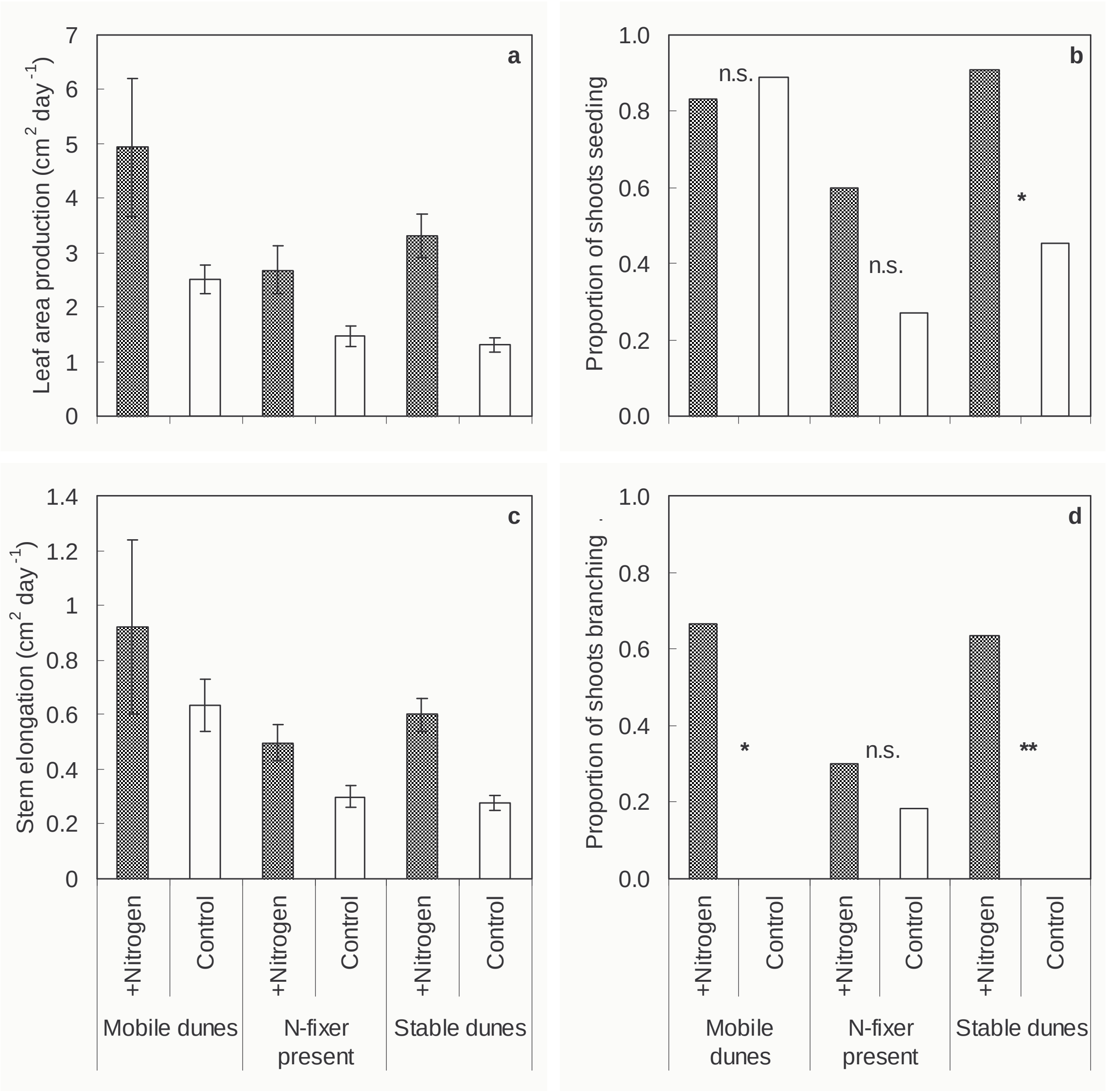


Figure 6.4: Leaf area production (a), stem elongation rate (c), and proportion of shoots bearing seed (b) or branches (d) for *S. plumieri*. Statistics for panels (a) and (c) are shown in Tables 6.1 and 6.2. Significant differences in seeding or branching proportions, between plants that had nitrogen applied or not are indicated, based upon the comparison of the observed differences with those generated by a random process (see Methods for details). * = $0.01 < P < 0.05$; ** = $0.001 < P < 0.01$; n.s., not significant.

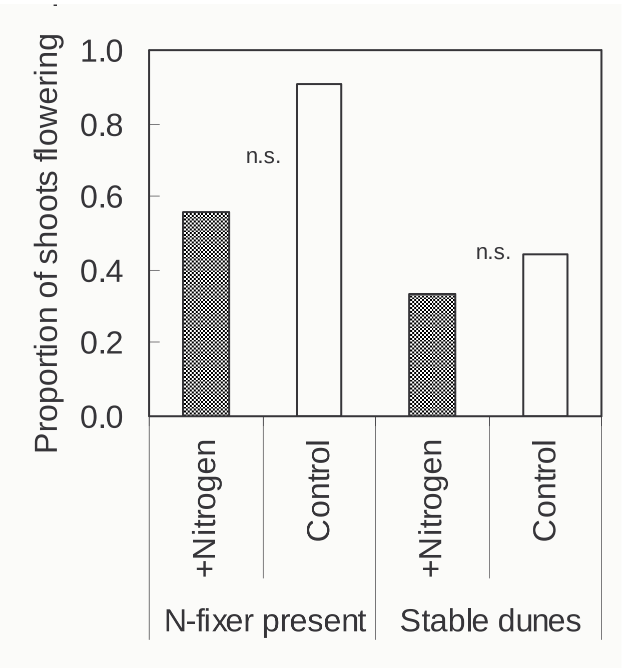


Figure 6.5: Proportion of marked *M. muricata* shoots flowering at the end of the experiment. Significant differences in proportions, between plants that had nitrogen applied or not are indicated, based upon the comparison of the observed differences with those generated by a random process (see Methods for details). n.s. = not significant.

Does the presence of a nitrogen-fixer increase growth?

The presence of a nitrogen-fixer significantly increased the shoot growth of *M. muricata*, but not *S. plumieri* (Figure 6.3, Table 6.1). While not significant, the effect of the nitrogen-fixer was included in the minimum adequate models of 3 out of 4 of the measured response variables for *S. plumieri* (Table 6.1). The effect of the nitrogen-fixer in these three models was negative, not positive as suggested in hypothesis 2. The presence of a nitrogen-fixer had no effect on the canopy growth of *M. muricata* (Figure 6.1, Table 6.1), but showed a significant positive effect on the growth of marked shoots (Figure 6.3b, Table 6.1). This positive effect was dependent on two significant higher order interactions with herbivory and size (positive effect), or nitrogen addition and size (negative effect; Table 6.1).

DISCUSSION

Allometry and allocation

Initial plant size did not appear to affect the response of *M. muricata* to nitrogen addition or the presence of a nitrogen-fixer (Figure 6.1). Similarly, the leaf mass production relative to stem mass production of *S. plumieri* was also unaffected (Figure 6.3a). The allocation of production between leaf and stem mass was affected in *M. muricata*, however the implications of this were unclear. Thus, in general, the effects of nitrogen-fixer presence, position, and nitrogen addition are independent of plant size or allocation. This result allows a more simple interpretation of the results of the analyses presented in Tables 6.1 and 6.2. However, two interesting size related phenomena were observed, independent of the treatments.

A large decrease in the *RGR* of *M. muricata* with size corresponded to a significant increase in reproductive effort (Figure 6.2a, b, and c). For the largest plants, this corresponded to an allocation of 19% of growth to reproductive structures (Figure 6.2b), or up to 900 inflorescence's per square metre of canopy (Figure 6.2c). Thus, it is suggested that the reason for the size related decrease in *RGR* is the opportunity cost of allocating carbohydrates to non-photosynthetic reproductive structures in larger plants (Bloom *et al.,* 1985).

Contrary to expectations, the allocation of the biomass to stem, or leaf growth for *S. plumieri*, was not dependent on the addition of nitrogen or presence of a nitrogen-fixer (Table 6.1 and 6.2). However, the allocation between these two organs was strongly dependent on the total production of the shoot (ca. stem plus leaf production). This is an example of apparent plasticity, where allocational differences are related allometrically to size (Figure 6.2 and 6.3). The increases in shoot total production resulted in a shift from a leaf to stem ratio of 2.1 to 14.8. This shift may be explained by the following mechanisms: 1) in order to survive leaf osmotic accumulation *S. plumieri* shoots must constantly produce leaves, and so that at low total production, most of shoot biomass is allocated to leaf production, but 2) since burial occurs at episodic intervals, stem growth must occur so that when burial happens the leaves are well above the sand surface. Thus, at high production rates, greater amounts of biomass are allocated to stem production. This allocation trend may represent a fundamental adaptation of this highly burial tolerant species to maintaining leaf area in conditions of episodic burial (See Chapter 7 for an analysis of characteristics allowing *S. plumieri* to tolerate burial).

Is nitrogen limiting to the growth of *S. plumieri* and *M. muricata*?

Nitrogen was limiting to the growth of both species, although for some response variables this was dependent on the level of another factor (Table 6.1 and 6.2). There were significant interactions of position with nitrogen addition for the stem elongation rate and leaf mass production of *S. plumieri*. These interactions resulted in unfertilised stable-dune shoots having the least stem elongation and conversely larger leaf mass production. This was not the case when stable-dune shoots were compared to those growing in the presence of *M. cordifolia*. This finding is consistent with *S. plumieri* shoots avoiding burial by having high rates of stem growth in plants growing closer to the sand surface (Chapter 7), and greater allocation to stem growth in mobile-dune plants (previous paragraph).

M. muricata showed a variable response to nitrogen applications, in some instances contradicting the results found in the previous section (Chapter 5). Canopy growth showed a positive response to nitrogen addition, and this response was increased when the plant was exposed to herbivory (Table 6.1), but under unfertilised conditions herbivory had a negative effect. The total shoot production of marked unfertilised shoots was significantly reduced by nitrogen application, while that of shoots in the presence of a nitrogen-fixer were increased (Figure 6.3). These results are discussed below.

Does the presence of a nitrogen-fixer increase growth?

In a manner similar to the variable nitrogen limitation of the growth of *M. muricata*, this species showed a variable response to the presence of a nitrogen-fixer (Table 6.1). Thus, canopy growth was not affected by the presence of a nitrogen-fixer (Figure 6.1), while total shoot production was highly significantly affected (Figure 6.3). The different results for total shoot production and canopy growth are difficult to reconcile, beyond the suggestion that these two aspects of plant growth may reflect different processes. For instance, an increase in shoot production may result in more dense canopy, rather than outward canopy growth. It is noted that all linear model coefficients that are significantly different to zero (the most reliable) indicate a positive effect for nitrogen-fixers (Table 6.1). Thus, the following conclusions are made: 1) the presence of a nitrogen-fixer has a facilitative effect on the shoot growth of *M. muricata*, including conditions of herbivory or nitrogen addition, 2) but, when nitrogen is added to stable-dune plants this results in a decrease in growth, in contrast to (1), 3) the overall increase in plant size was independent of the growth rate of individual shoots, and nitrogen addition positively affects the increase in size of the whole plant.

S. plumieri had little response to the presence of a nitrogen-fixer (Figure 6.3), but consistently showed a non-significant increase in growth in the absence of a nitrogen-fixer.

Therefore, it is concluded that the growth of *M. muricata* and *S. plumieri* are regulated by different nutrient dynamics. *M. muricata* showed little nitrogen limitation in the stable-dunes, and is likely to access nitrogen from adjacent nitrogen-fixers. This facilitation could occur directly or indirectly. *S. plumieri* is highly nitrogen limited in all environments, but shows little ability to gain from an association with *M. cordifolia*.

Does position on the dune zonation affect the growth of *S. plumieri*?

S. plumieri shoots growing in the mobile-dune environment had considerably greater growth, under unfertilised conditions and after nitrogen application (Figure 6.4). Only for the proportion of shoots bearing seeds did nitrogen not limit growth (as a proportion, this variable is constrained to a maximum of one and thus little limitation can exist as it approaches one). These results are consistent with the hypotheses that plants growing in the lower biomass mobile-dune area have greater soil availability of nitrogen, or other sources of nitrogen are available to plants growing in this habitat.

Competition and facilitation on coastal dunes

Competition for light and nutrients has been suggested as important factors in Eastern Cape coastal dune ecology (Chapter 4 and 5). In addition, facilitation by stabilising coloniser plants may allow other species to grow in new areas (Chapter 4), and nitrogen-fixers may influence nutrient limitation in some species, on local scales (this section). It was proposed that structurally weak, fast-growing plants were excluded from areas of taller vegetation, resulting in a lack of competitive ability for light (Chapter 4). A number of mobile-dune species were nutrient limited, while species from further back in the zonation were not nutrient limited (Chapter 5).

The two species studied in this section displayed differing responses to the presence of a high biomass of a nitrogen-fixer. *S. plumieri* showed a neutral or negative response to the presence of a nitrogen-fixer, relative to equivalent stable-dune plants (Figure 6.3). This response suggests little or no facilitation was occurring, and rather that a possible competition interaction was present. *S. plumieri* is limited to growing to approximately 50-80 cm tall, but on average this species achieves heights of 26 cm. This corresponds to the observed average height of 30 cm for *M. cordifolia* (Table 5.1), although this species is capable of growing to 180 cm height (Chapter 4). Thus, it is proposed that the nature of the interaction between these two species is likely to be asymmetric. *S. plumieri* stabilises mobile-dunes, facilitating the invasion of *M. cordifolia*. Subsequently, *M. cordifolia* has little nutrient affect on *S. plumieri*, but if it grows tall, it out competes the shorter species, *S. plumieri*. Thus, the long-term presence of *S. plumieri* at a point is likely to be determined by resumption of mobile-dune activity, or factors that maintain low heights of *M. cordifolia* e.g. wind pruning.

M. muricata displayed the opposite response to *S. plumieri*, with a clear enhancement of growth due to the presence of *M. cordifolia* (Figure 6.3b). This corresponds to a high rooting frequency of *M. muricata* under homogenous stands of *M. cordifolia* (Figure 5.6). This facilitation did not result in this species being able to respond to burial in the experiments of Chapter 5, but did allow this species to better respond to herbivory (Table 6.1).

The nature of facilitation by nitrogen-fixers remains unclear. Nitrogen fixed by plants often enhances the soil nitrogen, partly because of increased nitrogen levels in leaf litter (Huss-Danell and Ohlsson, 1992). The lack of remobilisation of nitrogen from senescing leaves is thought to play a large role in the increase of litter nitrogen (Cote *et al.*, 1989; Tateno, 2003; Chapter 8). Alternatively nitrogen can also be transferred directly via ectomycorrhizae, although the quantitative importance of this interaction is unclear (Ekblad and Huss-Danell, 1995; Arnebrant *et al.*, 1993).

As the high biomass of nitrogen-fixers is limited to the stable-dune (Figure 1.1), and species response to the presence of a nitrogen-fixer is not universal, nitrogen-fixation is not likely to play a role in dune plant response to burial. However, stable-dune species may benefit from the presence of *M. cordifolia*, by enhancing growth. Thus, the enhancement of local nutrient conditions by nitrogen-fixers may be an important factor in stable-dune species growth. It remains unclear how *M. cordifolia* maintains a high biomass in the stable-dunes when other stable-dune species can grow taller. This could occur due to inhibition of germination and seedling establishment under the dense canopy of this species, as is the case for *Myrica pensylvanica* (Tolliver *et al.*, 1995).

SECTION 4: ADAPTATIONS THAT ALLOW PLANTS TO SURVIVE ON COASTAL DUNES

INTRODUCTION

Investigations of specific traits allowing species to survive on coastal dunes

INTRODUCTION

Burial and low soil nitrogen levels have been shown to limit the growth and distribution of dune plants. In particular, partial burial has been shown to occur frequently and of a magnitude such that a shoot must respond to burial in order to survive (Chapter 2.1). The burial response of mobile-dune species has been shown to be limited by available nutrients (Chapter 5), especially nitrogen (Chapter 6). Finally, a general characteristic of most species, stem tissue density, was shown to predict the potential for species to elongate stem, and potentially determine burial tolerance (Chapter 4). In this context, it is interesting to ask what other specific traits allow species to survive in conditions of high burial on nutrient limiting dunes? As stem tissue density demonstrates, traits relating to production and cost of production are particularly relevant in connecting disturbance with stress.

What resources does a plant use for a burial response?

The large inducible burial growth response of *Scaevola plumieri* is similar to the widely studied stimulation of growth of *Ammophila* species in response to burial (e.g. Eldred and Maun, 1982). Numerous hypotheses have been postulated to explain this response in *Ammophila*, but few of these have been tested simultaneously using comparable measures of production. Thus, **Chapter 7** attempts to test as many of these hypotheses as possible, and includes a number of novel suggestions. A physiological model is developed to explain stimulated growth after burial, and provide specific mechanisms for the blanket term “reactive growth”. A quantitative review of the literature is also presented.

How do plants maximise production whilst minimising nitrogen use?

The explicit link between production (needed for burial responses) and nitrogen use (limiting production) is made in **Chapter 8**, for three dune species. The high nitrogen use efficiency (*NUE*) of *Arctotheca populifolia* and *Scaevola plumieri* is compared to that of *Myrica cordifolia* which is not limited by nutrients, possibly as it is a nitrogen-fixer. This section reviews the concepts of photosynthetic, leaf and whole plant *NUE*, and determines the optimality of the chloroplast-level components of photosynthetic nitrogen use efficiency.

How does biomass affect plant growth?

The low nutrient availability that mobile-dune species encounter is partly determined by the biomass of competing shoots at a site. Specifically, the aboveground biomass for a site influences the amount of available nutrients, per unit biomass, and therefore limits growth. Theoretically, plants can increase nutrient availability by decreasing the amount of biomass present. This could be mediated through mechanisms for maintaining a low biomass within an individual, or by environmental factors, such as burial, which may filter slow growing shoots from areas of higher biomass. A low biomass model is developed and tested in **Chapter 9**.

Are there ways of minimising the growth needed for a burial response?

By reducing the amount of stem that is required to outgrow burial a plant would be able to grow in a more extreme environment. An alternative mechanism to a reduction in the cost of stem production would be optimisation of the geometry of growth after burial. Specifically, plants must grow in the correct direction to maintain leaf area above the accreting sand surface. A novel hypothesis is developed in **Chapter 10** which indicates that not all plants must grow directly upwards in order to minimise growth in response to burial.

Chapter 7

What are the external and physiological mechanisms that result in a stimulation response of plant growth to burial?

A descriptive and a physiological model of stimulation burial responses — An quantitative analysis of the literature — A survey and experiments determining the carbon and nitrogen budget of the burial response of *Scaevola plumieri*

INTRODUCTION

Burial is a vital factor in dune ecology as the ability of a species to respond to partial burial determines the position of the species along the dune burial gradient (Chapter 3). The burial responses of a large number of species have been studied, and many specialist mobile-dune species show an increase in growth in response to varying degrees of burial (Maun, 1998), similar to the partial burial response of *S. plumieri* (Chapter 3). This stimulation could occur via a large number of mechanisms (e.g. the mechanisms in Table 7.1). However, little work has been done to assess the *relative* importance of each of these proposed mechanisms on the net growth response of any particular species (Maun, 1998). In addition, no quantitative analysis has been undertaken of the numerous burial responses reported in the literature. Growth is the net product of competing resource economies within a plant, thus the growth response of a shoot represents the net result of the action of numerous mechanisms. Ideally, an experimentally tested carbon and nitrogen budget for the response of a species to partial burial is needed. This budget should provide tests and estimates for the relative contributions of as many of these mechanisms as possible.

A descriptive model of plant response to burial

Maun (1998, 2004) presented a descriptive model of general plant responses to burial. Maun's (1998) model is summarised as follows, plants can have:

1) a negative response to burial resulting in death, if the plant is not adapted to burial,

2) a negligible response to burial at lower burial depths, but death at higher burial depths, and
3) a stimulated growth bounded by a maximum level of burial above which the plant cannot survive.

While this model adequately describes the observed effects of burial on a plant, it gives a poor explanation of the mechanisms behind the growth response. To account for this the stimulated growth of a buried plant is attributed to four factors in the "multifactor hypothesis" of Maun (1998): 1) greater soil volumes, 2) increased mycorrhizal activity, 3) increased soil resources, and 4) reactive growth. In all likelihood the three initial mechanisms contribute to the burial response of the plant, while the last can be viewed as a blanket term to cover internal physiological processes. The literature includes a number of suggestions of the physiological mechanisms of reactive growth, these are summarised in Table 7.1. To a large degree these mechanisms were not analysed in terms of their effect on biomass production, rather they were identified by observing which of the factors changed in response to a burial treatment. These mechanisms are generally tested in isolation of each other, or in possibly inappropriate conditions (the greenhouse). Thus, there is a need for experimental tests of these mechanisms, *in situ*, and measured in terms of biomass, carbon and nitrogen production. Such an experiment would allow a test of which, and by how much, these mechanisms result in reactive growth. A second partially descriptive model is presented by Antos and Zobel (1987, cited in Kent *et al.*, 2001), which describes the responses of different plant growth forms to burial, but also lacks an explicitly physiological basis.

Physiological mechanisms of plant response to burial

The majority of mechanisms explaining "reactive growth" relate to factors external to the plant (see mechanisms in Table 7.1). However, physiological explanations could potentially explain the majority of the response to burial. Previously suggested physiological mechanisms include: 1) shifts in allocation to the parts of the plant responding to burial (from reproductive, root and leaf production to stem and leaf production; Seliskar, 1994; Dech and Maun, 2006), 2) remobilisation of stored resources (Harris and Davy, 1988), and 3) increased photosynthetic rates (Yuan *et al.*, 1993; although it is not often explained where the nitrogen and other resources come from to allow this increase). A number of other mechanisms are suggested here: 1) remobilisation of resources in buried leaves, 2) decreases in stem tissue density after burial (greater cell size and water content would allow this), and 3) more generally, decreases in the cost of produced tissue (carbon and nitrogen) that would allow greater growth. Finally, the appropriate measures of growth physiology are in units of mass, while the measures that are relevant to responding to burial are stem length and production of photosynthetic capacity. Therefore, changes in the growth characteristics that increase stem length and the production of photosynthetic capacity, whilst maintaining the same biomass production would be particularly beneficial for a plant to survive burial in an environment that may limit biomass production. For instance, producing thinner stems would allow shoots with the same mass of stem production to produce considerably taller stems. Other examples would be reduced carbon and nitrogen costs of production, decreases in stem tissue density, and greater photosynthetic nitrogen use efficiency.

The stimulated growth of buried dune grasses and the reason for *Ammophila* decline

The growth of many dune grasses has been shown to be stimulated under burial conditions (e.g. the list of Maun, 1998 which includes two *Ammophila* species). A number of dicot species have also been shown to be stimulated by burial (Martinez and Moreno-Casasola, 1996; Dech and Maun, 2006), including *S. plumieri* (Chapter 5). A second body of literature documents the decline of *Ammophila* under stable conditions (e.g. Eldred and Maun, 1982; Disraeli, 1984). A direct link has been made between these two phenomena although a convincing series of mechanisms explaining both phenomena remains elusive. Loss of vigour under stable conditions and enhancement of growth under burial conditions could be viewed as extremes of a continuum where the same mechanisms are important. For instance, if burial allows the plant to achieve high growth rates due to an increase in nutrient availability, then subsequent stability will gradually result in a decrease in growth. The rate of decrease of the growth will depend on the nutrients available during the stable period, and the rate of nutrient loss from the plant. Thus, it is surprising that few studies connect these two phenomena with measurements of plants at various times since burial. *Scaevola plumieri* may show both of these phenomena, a stimulation of growth after partial burial, and a loss of vigour under stable conditions. Therefore it is of interest to measure growth as a function of time since burial for this species.

Table 7.1: Hypothesised mechanisms of a stimulated growth response to burial. To distinguish between mechanisms that affect carbon and nitrogen metabolism of the buried shoot the letters C and N were used, respectively. Similarly, I and E represent whether the mechanism is external influence or internal (physiological) adjustment. A number of mechanisms are from Eldred and Maun (1982).

Mechanism	External/ Internal	C/N	Reference[a]
Growth response			
Increase in number of internodes	I		Maun and Lapierre, 1984
Internode elongation	I		Maun and Lapierre, 1984
Resource acquisition			
Higher nutrient levels in fresh sand – adventitious root production	E	N	Dech and Maun, 2006 Fay and Jeffrey, 1992
Increased soil volume	E	N	Eldred and Maun, 1982
Greater moisture for roots of buried plants	E		Olson, 1958
Allocation			
Clonality or intershoot allocation[b]	I	C&N	Yu *et al.*, 2004
Remobilisation of resources in buried leaves	I	C&N	These authors
Release of stored resources	I	C&N	Harris and Davy, 1988
Changes in stem vs. leaf allocation	I	C&N	Unknown
Changes in shoot vs. root allocation	I	C&N	Dech and Maun, 2006
Reduced leaf area increases transpiration[c]	I	C&N	These authors
Changes in reproductive vs. growth allocation	I	C&N	Seliskar, 1994
Costs of production			
Greater photosynthetic capacity of unburied photosynthetic area, due to	I	C	Yuan *et al.*, 1993
- increased PSII efficiency		C	Perumal and Maun, 2006
- higher leaf light and temperature			
- greater leaf thickness			
Reduced stem tissue density	I	C&N	These authors
- Inc. cell size or inc. water content			
Decreased stem width	I	C&N	These authors
Facilitation / Competition			
Physical buffering of adjacent shoots resulting in less compact sand	E		Franks and Peterson, 2003
Release from competitors, or lowered density of plants, inc. light intensities/ leaf temp.	E	C&N	Eldred and Maun, 1982[d] Maun and Lapierre, 1984
Other			
Release from parasites	E		van der Putten *et al.*, 1988
Increase in beneficial mycorrhizal activity	E		Little and Maun, 1996
Reactive growth	I		Danin, 1996
Reduced soil temperatures	E		Olson, 1958
Increased aeration for new adventitious roots	E		Maun, 1998

[a] The references are not necessarily the first citation of these mechanisms

[b] Many plants are not clonal, but may reallocate resources between shoots. Indeed, if connected completely buried shoots allocate resources to partially buried shoots this could represent a large increase in available resources.

[c] Burial of leaves may result in increased potential for root water supply due to a decreased leaf area to root ratio this could result in greater transpiration and thus mass flow of nitrogen into leaves.

[d] Competition is reduced under burial conditions, potentially allowing greater nutrient availability. This mechanism may be relevant for higher density ecosystems that the single species *A. breviligulata* ecosystem studied by Eldred and Maun (1982). See Chapter 9 for more information.

Allocation of growth after burial: a model

The response of a shoot to burial can be divided into two convenient components: 1) changes in the access of the shoot to the resources present in the environment (external), resulting in changes in the net production of the shoot, and 2) internal (physiological) changes including shifts in the allocation of biomass between components (stem, leaves and reproductive tissue), and changes in the cost of production of the new tissue. To a large degree the physiological shifts result in a change in the use of produced biomass, and not necessarily a change in the total biomass produced. This distinction, although not totally exclusive, is potentially a useful criterion for differentiating between the two types of mechanisms that result in a growth response. The following model provides a general framework to assess the importance of the physiological component of the burial response.

Resource use can be defined in terms of the mass of either nitrogen or carbon used in the burial response (for carbon these are M_l and M_s, leaf and stem mass: g). Alternatively the effectiveness of the response is defined as the height (*SL*, stem length: cm) and leaf area (*LA*, leaf area: cm^2) produced by the shoot. Thus, resource use and production in response to burial are defined in different units, the connection being the mass cost of producing leaf area (*SLA*, specific leaf area: cm^2 g^{-1}) or stem length (*MSL*, mass specific length: cm g^{-1}). If *SLA* and *MSL* were constant across buried and unburied treatments, then it would be expected that changes in the leaf to stem mass ratio (*viz* allocation changes) would be proportional to those in the leaf area to stem length ratio:

$$\frac{LA}{SL} \propto \frac{M_l}{M_s}$$

Thus, a reduction in mass allocation to leaves would result in a proportional increase in amount of stem mass and length. By factoring *SLA* and *MSL* into this model:

$$\frac{LA}{SL} = \frac{SLA}{MSL}\frac{M_l}{M_s}$$

further insight is gained about the mechanisms by which a shoot could have higher stem length production with, or without, an increase in the total resources used. This formulation has not been directly considered in the burial response literature, although a number of these parameters have been measured. For instance, *SLA* has been shown to be affected by burial (Martinez and Moreno-Casasola, 1996), as have related parameters such as chlorophyll content (Disraeli, 1984; Perumal and Maun, 2006), photosynthetic rate (Permual and Maun, 2006; Yuan *et al.*, 1993), and leaf thickness (Yuan *et al.*, 1993; Permual and Maun, 2006). The components of *MSL*: stem width and stem tissue density have been measured less often (but see Chapter 4 and 5), although these relate more directly to stem elongation in response to burial.

Ideally, these considerations should be framed in terms of allometric relationships rather than ratios. However, considering the small correlations between the leaf and stem mass of buried shoots ($R^2<0.2$; data not shown), this may not be practical.

Components of stem mass specific length

Stem mass specific length is determined by two components: stem width and stem tissue density. Stem tissue density has been shown to be reduced in buried stems of many species (Figure 4.13b), and is low in the stems of species that typically survive recurrent burial (Figure 4.5). Stem tissue density and the implications of reducing this parameter have been discussed extensively in Chapter 4. Stem width is another potential characteristic that could be adjusted in response to burial. Indeed, the increase in stem length of a stem with reduced width is proportional to the inverse of the width squared. This is demonstrated by the following equations. The formula for the volume (V: cm^3) of a cylindrical stem is:

$$V = L\pi\left(\frac{W}{2}\right)^2$$

where L is the length of the stem (cm), and W the width (cm). The dry mass of the stem (M_s) is then the volume multiplied by the dry stem tissue density (ρ_{dry}: g cm^{-3}):

$$M_s = \rho_{dry}V = \rho_{dry}L\pi\left(\frac{W}{2}\right)^2$$

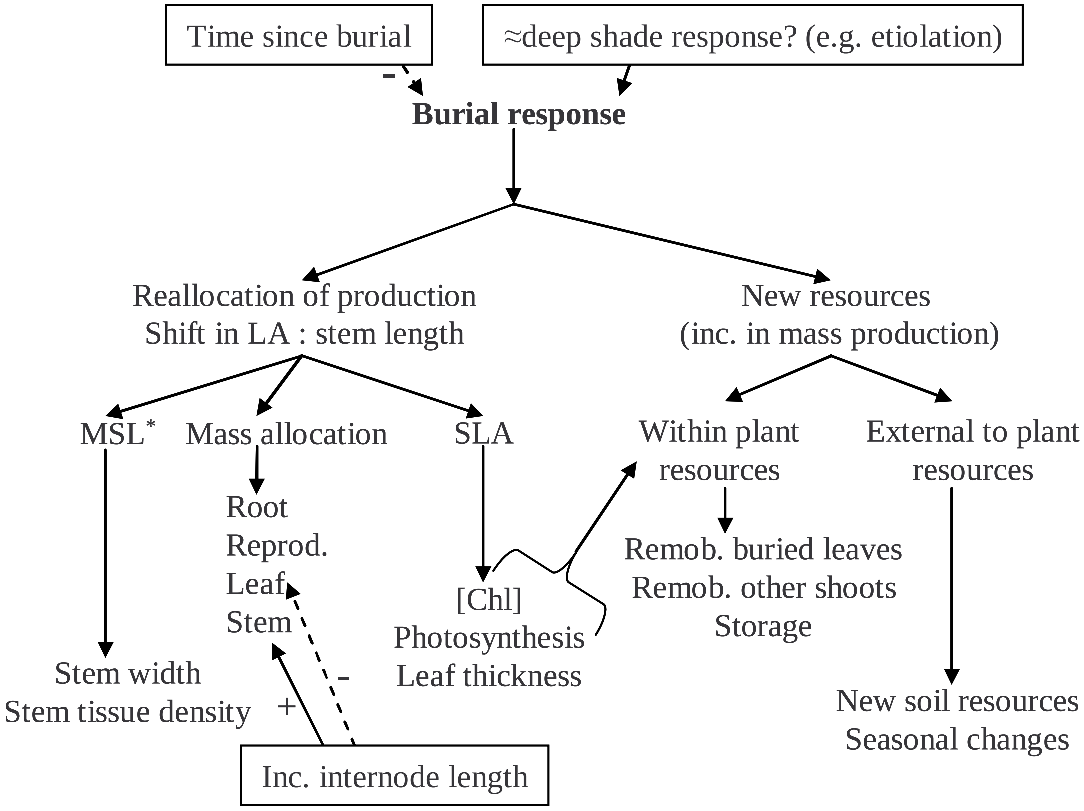
The mass specific length of the stem (*MSL*) is the length divided by the mass, which is found by rearranging the previous formula:

$$MSL = \frac{L}{M_s} = \frac{L}{\rho_{dry}L\pi\left(\frac{W}{2}\right)^2} = \frac{1}{\rho_{dry}\pi\left(\frac{W}{2}\right)^2}$$

This model can be parameterised using the ρ_{dry} measured for *S. plumieri* (0.29134 g cm^{-3}), incorporating a factor of 100 (to account for *W* being measured in mm which are then squared). Hence,

$$MSL = \frac{100}{0.29134 * 3.1415\left(\frac{W}{2}\right)^2} = \frac{109.3}{\left(\frac{W}{2}\right)^2}$$

Thus, the change in produced stem length (at a given stem mass) for changes in stem width is very steep, due its squared hyperbolic nature, and the large slope. For example, 1 g of a 10 mm wide stem would be 4.4 cm long, while a 1 mm reduction in width would result in the stem increasing to a length of 5.4 cm! Combined with the fact that partially buried stems have to support less weight, temporary or long-term reductions in stem width or tissue density would have large positive consequences on the stem growth of buried plants. However, this has not been assessed in the literature (Chapter 4).



*MSL = mass specific length

Figure 7.1: Hypothesised mechanisms of a shoot's response to burial**.** Changes in resources and production are measured in units of mass, while responses are measured in units of leaf area and stem length, connected by the mass specific leaf area and stem length. Factors that are hypothesised to influence the response mechanisms are shown (within boxes). Hypothesised negative effects on the resources available for growth (dashed lines) and positive effects are shown (solid lines).

Synthesis

An overall model (Figure 7.1) of the mechanisms that could potentially result in burial-stimulated growth was formed by combining the allocation model, developed here, with the list of possible mechanisms presented in Table 7.1. The burial response is divided into two components: changes in allocation, and increases in total resources or total production. Three other factors are suggested to influence these mechanisms: 1) an increase in internode length could be the mechanism of the leaf to stem allocation shift, 2) the time since burial, and as a result the decrease in the available resources, could explain aspects of the burial response and decline in vigour of stable-dune shoots, and 3) the burial response could be comparable to the responses of shoots placed in deep shade, e.g. etiolation (Voesenek *et al.,* 1998). The latter suggestion, may explain how plants could develop burial responses, as this does not have to occur in isolation to other aspects of plant physiology.

To assess these hypothesised mechanisms of burial response two different types of techniques were used. Of the many studies that measure burial responses there is no quantitative review of these types of experiment (but see Maun 1998, Kent *et al.,* 2001, and Ievinsh, 2006 for qualitative reviews of this literature). A large number of studies report comparable data on the total biomass, root to shoot ratio, and photosynthetic rates of plants growing under unburied and buried conditions (e.g. the list of references in Table 7.2). Thus, the stimulation of growth after burial, changes in biomass allocation, and photosynthetic rate can be analysed for a wide range of species, to determine how general and important these responses are for a burial response to occur. Secondly, a survey and a series of experiments were undertaken to test specific hypotheses relating to the carbon and nitrogen used by *S. plumieri* to respond to burial.

LITERATURE ANALYSIS

The response of dune plants to burial can derive from two sets of factors: internal physiological changes, and shifts in external resource or stress factors. Thus, the reported stimulation of burial in dune plants can be attributed to either of these sets of factors. Distinguishing whether resource levels or physiology are of importance is difficult, but an increase in the *total* biomass of buried plants relative to unburied plants is clearly indicative of increased resources or a reduction in stress levels. Changes in shoot height, leaf area, cover, or mass of single organs or shoots can be the result of changes in either set of factors. Thus, a reasonable distinction can be made between these two processes by analysing the extensive burial literature specifically for burial responses where total biomass production is enhanced. Indications of changes in physiology could also be detected through analysis of standard growth parameters such as root to shoot ratio and photosynthetic rate.

Seven questions were asked about the burial responses of plants presented in the literature:

1) Does the total biomass of a plant increase after burial? This would be consistent with a net increase in resources. An alternative explanation is that the biomass of a tagged shoot may increase, as a result of unburied shoots allocating resources to it. By only considering studies where total plant biomass was reported the hypothesis of a resource related stimulation of growth can be assessed.
2) Is there a consistent allocation shift from root to shoot biomass in buried plants?
3) Are allocation shifts enhanced in species that are capable of stimulated burial?
4) Are increases in photosynthetic rate characteristic of burial responses?
5) Are increased photosynthetic rates dependent on the time since burial occurred?
6) Are there consistent differences between greenhouse (mostly seedling) and field (adult plants) studies of burial responses?
7) Half of the available data for these responses derive from Sykes and Wilson (1990) and this study may, or may not, contradict the findings of other studies (Maun, 1998, pp. 734; Shi *et al.*, 2004 pp. 553), thus: Does the data from this study differ from the other data when factors like differences in burial depth are taken into account?

The positive enhancement of total biomass by plants at different burial depths should be distinguished from the three types of burial responses presented by Maun (1998, 2004). The difference being that the burial responses of Maun (1998, 2004) can refer to all aspects of growth, not just total biomass. Thus these three types of burial response are indicative of changes in physiology and/or resources, while total biomass enhancement is more indicative of changes in resource levels. An exception maybe that physiological changes in photosynthetic rate and efficiency would result in higher total biomass production. If a solely physiological shift was able to increase growth rate, why would unburied plants not have this physiology too? This question implies that for buried plants to have increased photosynthetic rates an external influence is necessary, and thus, some external factor is needed to adequately explain this type of reactive response. An example is that an increased photosynthetic rate implies a greater availability of nitrogen or greater efficiency of nitrogen use. In order for either of these to occur only in buried plants a second factor must be present to make such a shift possible (release of nitrogen in buried leaves or increased soil nitrogen availability).

METHODS FOR LITERATURE ANALYSIS

The available literature was reviewed for studies that included an estimate of the responses of total biomass, root to shoot ratio, or photosynthetic rate to burial. Sixteen studies were considered appropriate (Table 7.2), these included a total of 61 species. As some species occurred in more than one study, these were treated as separate confirmations of a burial response, thus totalling 70 measured burial responses (*Ammophila breviligulata* was measured in a number of studies, and *Cakile edentula* in two studies, and a number of species were measured in both the field and greenhouse). Of these burial responses, 55 had accompanying biomass allocation data and 22 had photosynthesis data (Table 7.2). To avoid the complications of having different biomass and photosynthetic rates between species and studies, the burial measurements were expressed relative to values for unburied plants. The majority of the literature uses the convention of measuring burial relative to the height of the apical bud of the plant, thus 33%, 50%, 67%, 100% and 133% of apical height (0.33H, 0.5H, 0.67H, 1H and 1.33H, respectively) were common burial treatment depths. Studies where burial was given as a height were transformed to a proportion by calculating the burial height relative to the mean reported height of the plant. A number of studies included experiments of repeated burial (Maun and Lapierre, 1984), and complete burial followed by re-exposure (Harris and Davy, 1988; Kent *et al.*, 2005; Zhang and Maun, 1992), this data was not included in the analysis. The burial responses of five desert and salt marsh plants were included in the analysis (e.g. Shi *et al.*, 2004; Brown, 1997; Zheng *et al.*, 2005), as it was thought that there was little difference in the mechanisms of burial response between coastal and desert dune plants. However, studies of the burial response of marine plants (Cabaco and Santos, 2007; Marba and Duarte, 1994), plants covered by gopher mounds (Williams *et al.*, 1986), and volcanic tephra (Zobel and Antos, 1987) were not included in this review.

The large amount of allocation data in each study was reduced by considering just the 1H burial treatment (or 0.67H treatment where a 1H treatment was not included). In the case of Sykes and Wilson (1990) many of the shoot to root ratios of the 1H burial treatment included anomalous values of 0.1, for this reason values for the 0.67H treatment were used. Root to shoot ratio (*R:S*), was the most common way of reporting allocation, although shoot and root mass fraction are preferable for a number of reasons (Poorter and Nagel, 2000). As the allocation to the shoot mass of the plant was of interest, the shoot mass fraction (*SMF*) was calculated by the following formula:

$$SMF = \frac{1}{1 + R:S}$$

Where possible, the calculation of the *SMF* fraction was limited to root and shoot data, excluding reproductive tissue. In general, buried plants should preferably allocate mass to both components of shoot mass (leaf and stem), while reducing reproductive and root mass allocation. Since few studies included species that had reproductive tissue during the experiment, the inclusion of the reproductive component would complicate the analysis unnecessarily. The time transient of photosynthetic rate after burial was used to determine if enhancement of photosynthetic rate was time dependent.

Analysis

The lack of reported of measures of variance in a number of studies precluded the use of standard meta-analysis techniques (e.g. those used by Gurevitch *et al.*, 1992). Thus, the aim of the literature analysis was limited to finding general trends in the available data, and test whether these trends were consistent with the hypotheses proposed earlier.

The non-parametric Wilcoxon signed rank test can be modified to allow a number of different types of tests: 1) a test of the difference of a single sample from a hypothesised value (in this case one), 2) a test of the difference between two samples where the samples are paired in nature (dependent), and 3) a test of the difference between two independent samples (R Development Core Team, 2006). The first test was used to assess whether buried treatments were different to the unburied treatment, for a range of burial depths (0.33H, 0.5H, 0.67H, 1H and 1.33H). Specifically, the stimulation of photosynthesis, shoot mass fraction, and total biomass of buried treatments relative to the unburied treatment was tested by comparing the median of a set of data to a value of one (no difference to the unburied treatment). To assess whether an enhanced burial response was explained by an allocational shift to shoot mass, the experiments that had enhanced and negative total biomass responses to burial were analysed separately. As photosynthetic rate was limited to data from 15 species, there was insufficient data to test whether an increase in photosynthetic rate corresponded to species with stimulated growth after burial. There was sufficient data to test whether photosynthetic rate changed with time after burial. For this analysis the ratio of the photosynthetic rate of buried plants relative to unburied treatments was grouped into time categories of 25 days. Furthermore, the data from all available photosynthetic time transients was grouped into a table of three by three categories describing the change in photosynthetic rate over time, and whether burial caused enhanced photosynthesis. As up to 15 Wilcoxon tests were performed for each set of data there may have been unreasonable numbers of Type 1 errors. For this reason Bonferroni adjustments were made to the required level of significance (0.05 / number of analyses performed), and are mentioned where necessary.

Table 7.2: Studies used to examine the generality of a stimulation response to burial, and whether a shift in allocation to shoots, or an increase in photosynthetic rate corresponds to the ability of a species to respond to burial.

Reference	Number of species	Type of experiment	Total biomass data	R:S data	Photosynthesis data
Brown, 1997	1	Field	n	y	n
Cheplick and Grandstaff, 1997	1	Greenhouse	y	y	n
Dech and Maun, 2006	7	Field	y	y	n
Disraeli, 1984	1	Field	n	y	n
Gagne and Houle, 2002	2	Greenhouse	n	y	n
Langlois *et al.*, 2001	1	Greenhouse	n	n	n
Little and Maun, 1996	1	Greenhouse	n	y	n
Maun and Lapierre, 1984	1	Field	n	n	n
Martinez and Moreno-Casasola, 1996	6	Greenhouse	y	y	n
Perumal and Maun, 2006	10	Field and greenhouse	y	n	y
Shi *et al.*, 2004	1	Greenhouse	y	y	y
Sykes and Wilson, 1990	30	Greenhouse	y	y	n
Yuan *et al.*, 1993	2	Greenhouse	n	n	y
Zhang, 1996	1	Greenhouse	n	y	y
Zheng *et al.*, 2005	1	Greenhouse	y	y	n
This study	4	Field	n	n	y
	# of spp.	Number of burial responses measured:			
Field	18	18	11	11	11
Greenhouse	24	24	18	16	11
Sykes and Wilson	30	30	30	28	0
Total	61	70	59	55	22

RESULTS OF LITERATURE ANALYSIS

The greenhouse burial experiments of Sykes and Wilson (1990; Figure 7.2a), a literature survey of field studies (Figure 7.2b), and the remaining greenhouse studies (Figure 7.2c) demonstrated different responses of total biomass to burial, for different burial depths. For instance, the large study of Sykes and Wilson (1990) found that virtually all species showed a reduction in the total biomass produced by buried plants relative to unburied treatments (Figure 7.2a). At generally lower burial depths, the other greenhouse studies, and field studies showed that buried plants had mostly enhanced total biomasses relative to the unburied treatments (Figures 7.2b and c). Thus, it is difficult to determine whether the reduced responses of Sykes and Wilson's plants and enhanced responses of the other studies differ due to ecosystem and experimental differences or simply that the former study buried plants to deeper depths. However, it is clear that in both the field and greenhouse, lower burial depths have a consistent and significant stimulatory effect on the total biomass of buried plants (Figure 7.2b, c and d). It is suggested that all the data can be combined to show a continuum of responses to burial, where there is a stimulation of total biomass at low burial depths, decreasing to a severe reduction of growth at burial depths above the height of the plant (Figure 7.2d). A number of species show an extreme enhancement (2 to 8 times) of the total biomass of buried plants relative to unburied treatments (Figure 7.2).

The species that displayed allocation shifts from root to shoot biomass corresponded to the species which were able to enhance growth after burial (Figure 7.3a). The photosynthetic rate of buried plants was significantly enhanced during the period 25 to 50 days after burial (Figure 7.3b). The large variability of photosynthetic rates on the other dates indicated that there was little general temporal trend for species' photosynthetic response to burial (Figure 7.3b). In the broadest of terms, when the available time courses of photosynthesis were grouped into a positive or negative, and increasing or decreasing responses the majority of time courses showed a positive stimulation by burial which decreased over time (Table 7.3).

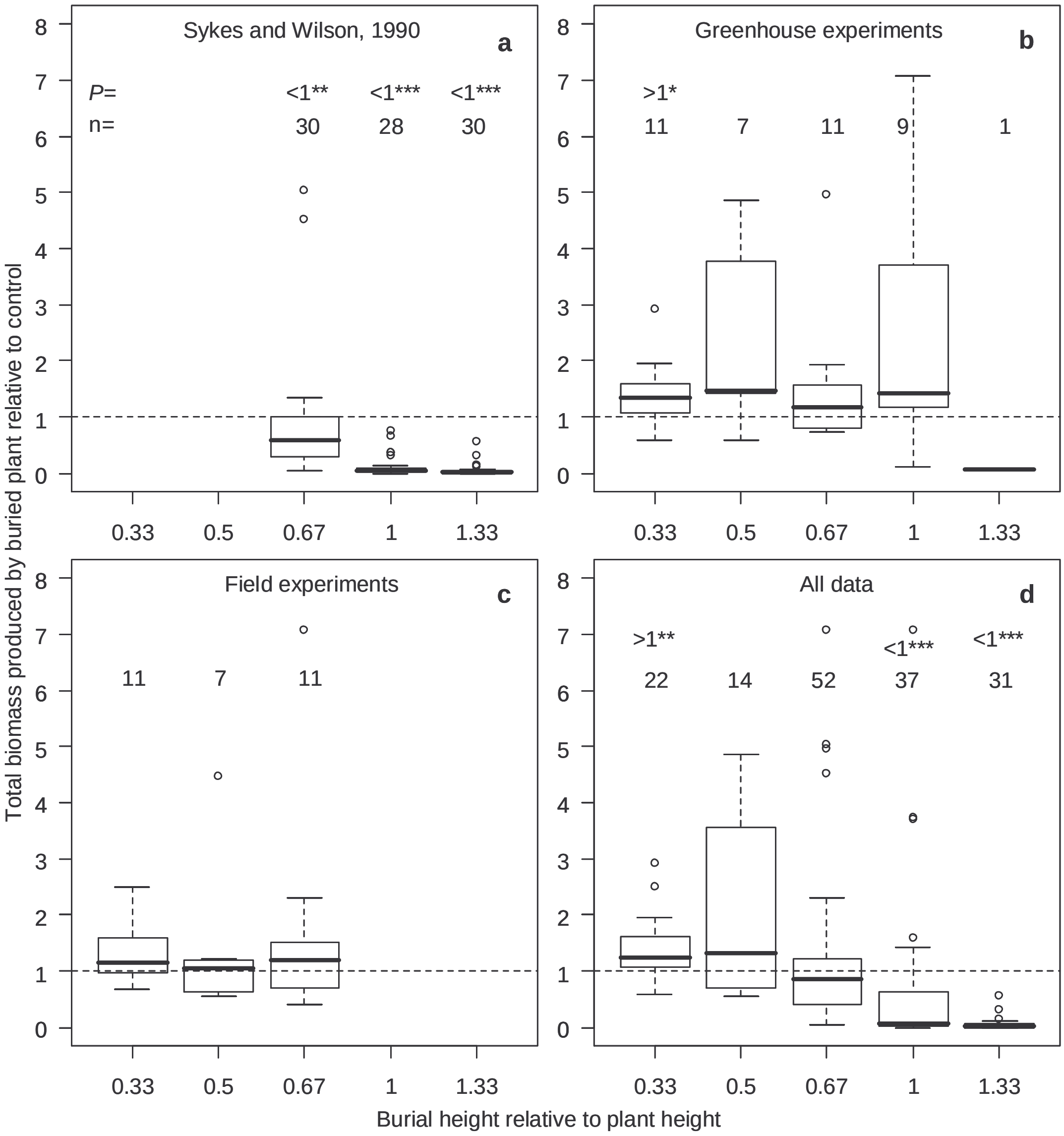


Figure 7.2: The effect of five burial treatments on the total biomass production of buried plants relative to unburied (control) treatments, grown: a) in the greenhouse by Sykes and Wilson (1990), b) for 2 field studies, c) for 5 greenhouse studies, and d) all studies combined. Data are from a literature survey of 7 studies, including 59 species. The dashed line represents no difference of the buried from the unburied treatment. The number of experiments and significance of a difference between the median and the unburied treatment value of one are given in the top of each graph. Bonferroni adjustments to the 15 analyses removed significance of the greenhouse data at a burial depth of 0.33H. * = $0.01 < P < 0.05$; ** = $0.001 < P < 0.01$; *** = $P < 0.001$; no symbol = not significant.

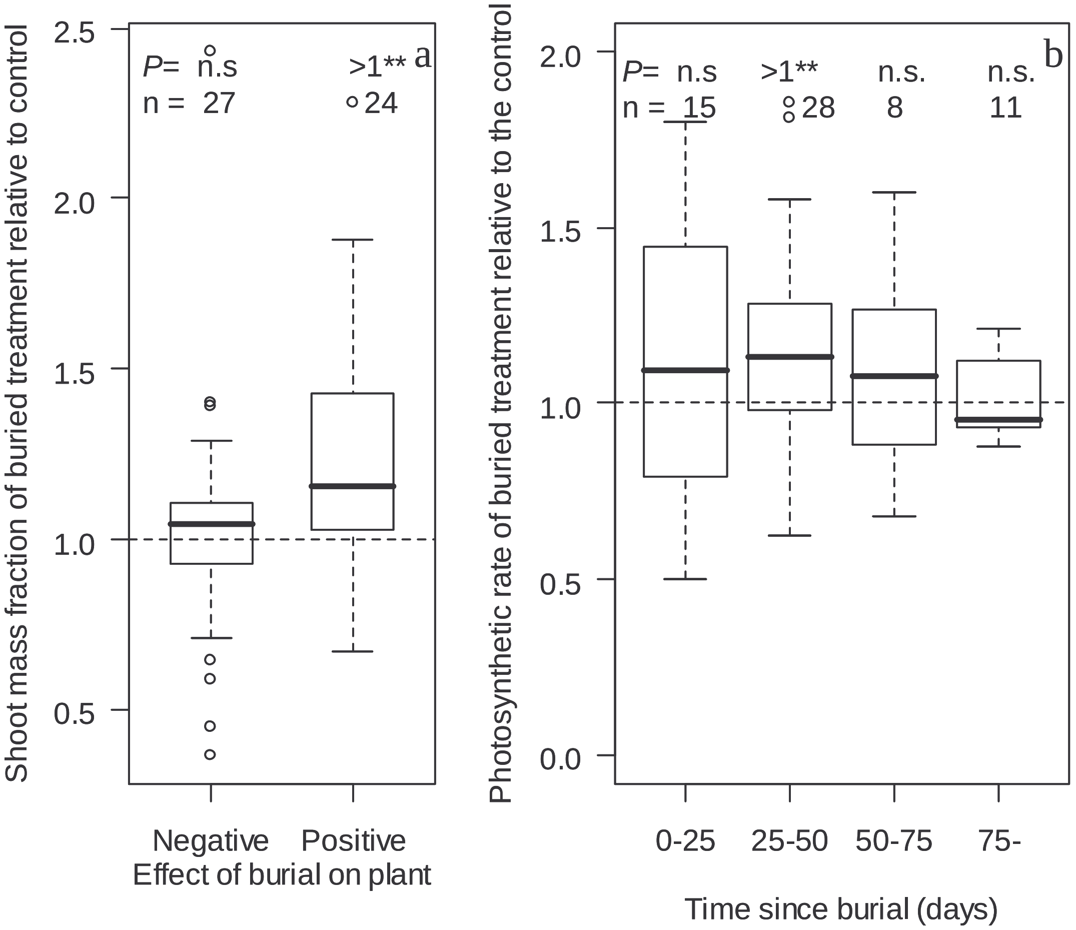


Figure 7.3: The effect of burial on shoot mass fraction of plants with different responses to burial (a) and the response of photosynthetic rate to burial at time zero, over time (b). Both parameters were calculated as the values for the buried treatment relative to the unburied (control) treatment (one). Data are from a literature survey of 16 studies, including 61 species. The dashed line represents no difference of the buried from the unburied treatment. The number of experiments and significance of a difference between the median and the unburied treatment value of one are given in the top of each graph. Bonferroni adjustments to the analyses did not remove any of the presented significant effects. ** = 0.001 < *P* < 0.01; n.s. = not significant.

Table 7.3: Photosynthesis responses of buried treatments over time for data presented in four studies (Perumal and Maun, 2006; Yuan *et al.*, 1993; Shi *et al.*, 2004; Chapter 5). The data represents the time courses of the photosynthetic responses of 16 species to burial, at a number of burial depths and for both field and greenhouse studies, a total of 38 time transients. Each transient was classified subjectively by the general effect of burial (3 levels) and the nature of the response over time (3 levels).

	Response over time			
Effect of burial:	Increasing	No trend	Decreasing	Row totals:
Positive	16%	0%	45%	61%
No effect	0%	21%	5%	26%
Negative	0%	3%	11%	13%
Column totals:	16%	24%	61%	

DISCUSSION OF LITERATURE ANALYSIS

Three clear trends were found for the combined data of the reviewed literature: 1) a stimulation of total biomass for plants buried at shallow depths, and a reduction of growth at deeper depths, 2) plants that showed stimulation in response to burial were able to change biomass allocation in favour of shoot growth, and 3) in general, the photosynthetic rate of partially buried plants was enhanced and decreased with time. Although this data set has serious limitations it could be concluded that both changes in external stress or resources, and internal physiological shifts were characteristic of many species response to burial. Thus, ascribing growth changes in response to burial to a single factor is not adequate to explain the mechanisms of growth response.

The reported lack of shift from root to shoot for the 30 species studied by Sykes and Wilson (1990) has been cited as contradictory to the majority of the data presented by other authors (Maun, 1998, pp 734; Shi *et al.*, 2004, pp 553). However, when included in the current data analysis the data of Sykes and Wilson (1990) contributed to the observed shift in root to shoot biomass for plants which showed stimulation of total biomass in response to burial (Figure 7.3b). Four reasons are given for this anomaly, firstly, a large number of the reported shoot to root ratios of Sykes and Wilson (1990), at burial depths of 1H and 1.33H had an anomalous value of 0.1 (possibly an error). These anomalies could seriously bias the reported differences, for this reason all values of 0.1 were excluded from this analysis. Secondly, at the 0.67H burial depth 21 out of 28 species showed a large (1 to 6 times) mostly non-significant increase in shoot to root ratio, consistent with a shift from root biomass to shoot biomass. This is important, as the binomial probability of 21 out of 28 responses showing an increase in shoot allocation is 0.0044, given equal chances of a response being greater or less than the unburied treatment. A third reason may be the difference in experimental burial depths between the majority of studies and Sykes and Wilson (Figure 7.2). Finally, Sykes and Wilson (1990) studied a wide range of species in comparison to the smaller range of more burial responsive species studied by other authors. Therefore, it is concluded that the data of Sykes and Wilson (1990) is in full agreement with the general trend of buried plants shifting biomass to the shoot at the expense of root biomass production. Thus, future between-species comparative studies would benefit from measuring burial responses of species from different areas of a dune zonation, and across a broader range of burial depths.

Limitations of current burial response data, and suggestions for future work

While the results presented here do not contradict any of the reported responses to burial, they illustrate the need for more work in this field. The studies used for this literature analysis represent virtually all of the literature including data on the burial responses of multiple species of dune plants. However, there were large biases in the number of studies focussing on: greenhouse rather than field experimentation (54 greenhouse to 18 field burial responses), and seedling rather than adult growth (10 of 70 burial responses were measured for adult plants). The response of seedlings to burial is of vital importance to dune plant ecology as this burial could represent a large recruitment limitation. However, more work must be done on field grown adult plants, before a convincing explanation of burial response can be tested.

An enhancement of photosynthetic rate has been demonstrated for a number of species after burial (e.g. eight perennial and herbaceous lake dune species: Perumal and Maun, 2006; two dune grass species: Yuan *et al.*, 1993). These type of responses amount to a large proportion of the total photosynthetic burial responses measured (~60%; Table 7.3). In addition to the majority of burial responses exhibiting an enhanced photosynthetic rate, the majority of these species also had a decrease in enhancement of photosynthetic rate with time (Figure 7.3b and Table 7.3). The temporary enhancement of photosynthetic rate after burial indicates that more studies should focus on measuring time courses of photosynthetic rates after burial. Until more studies generate such data, little can be said about the amount of carbon gained by a plant responding to burial with temporarily enhanced photosynthetic rates.

Finally, no experimental studies measured *in situ* rates of burial and combined these with burial manipulations that approximated the field conditions. For example, if burial occurs gradually, large once-off burial treatments would not be ecologically relevant. Alternatively, if most burial occurs in response to particular extreme events these type of experiments would be of greater relevance (see Chapter 2.1 for a description of the *in situ* burial rates at OWR). Disraeli (1984) and Eldred and Maun (1982) present an alternative way of measuring burial response, where plants encountered natural burial and biomass was measured after this occurred. While these type of studies are more ecologically relevant, explaining the mechanisms of burial response is difficult as environmental factors in the dune environment are highly autocorrelated.

Since the burial response literature has focussed to a large degree on the external influences of the environment on the burial response (Table 7.1 and see Table 1 of Eldred and Maun, 1982), a number of untested physiological mechanisms of burial response where tested for a characteristic South African dune species that showed stimulated growth in response to burial.

CARBON AND NITROGEN BUDGETS FOR THE BURIAL RESPONSE OF *SCAEVOLA PLUMIERI*

This study elaborates on the numerous experiments performed on the temperate grasses, *Ammophila breviligulata* and *A. arenaria*, that test specific hypotheses of the carbon and nitrogen sources of burial responses (e.g. Seliskar, 1984; Fay and Jeffrey, 1992; Voesenek *et al.*, 1998). However, few experiments have compared the many hypothesised mechanisms of burial response in a quantitative manner.

Species selection

The factorial experiment of Chapter 5 found that *A. populifolia*, *S. plumieri*, *M. cordifolia* and *M. muricata* had contrasting responses to partial burial and nutrient addition. Stem elongation of these species in response to partial burial differed markedly, with: *A. populifolia* having inherently fast growth with a small short-term inducible response to partial burial, *M. cordifolia* showing a limited inducible response to partial burial and *M. muricata* no response to partial burial. Thus, *S. plumieri*'s large inducible burial response, its nutrient limitation and its importance as a foredune forming species make a detailed investigation of burial response of this species particularly interesting. For these reasons, *S. plumieri* was chosen as the study species for this chapter. Important characteristics of the other species were investigated in other chapters. Thus, the primary aim of this chapter was to estimate a carbon and nitrogen budget for the burial response of *S. plumieri*, the most important dune binding species along the Southern African coast.

Hypotheses

Specific hypotheses were:

1) That growth in response to partial burial occurs in the following manner:

a) **Internode elongation/allocation hypothesis:** by extending the length of internodes, shoots would have greater stem elongation without increasing the rate of leaf production. This results in a longer length of stem that bears leaves, further reducing the risk of leaves being buried. Alternatively, more internodes (and leaves) are produced to further increase stem elongation rate. As a consequence there is an allocation trade-off between leaf area production and stem elongation, which compete for the same pool of resources, and

b) **Loss of vigour hypothesis:** production is negatively related to the time since the plant was buried, possibly as burial temporarily increases the amount of resources available to the shoot and the available resources decrease with the time since burial.

2) The carbon and nitrogen used in the response to burial derive from the:

c) **Sand nutrient hypothesis:** the strong adventitious rooting of the buried shoot, allows greater access to new resources in the fresh sand after partial burial,

d) **Nutrient limitation hypothesis:** that this species is nutrient limited, and can access the nutrients available to it in freshly blown sand after burial,

e) **Leaf resource remobilisation hypothesis:** the remobilisation of carbohydrates and nitrogen from buried (non-photosynthetic) leaves allows the plant to increase stem and leaf production rates,

f) **Allocation shift hypothesis:** production of leaf and reproductive tissue is a competing sink for the resources that could be used for a stem related burial response,

g) **Inter-shoot translocation hypothesis:** inter-shoot allocation in spatially heterogeneous burial conditions allows increased growth in response to burial,

h) **Source-sink hypothesis:** an adjacent completely buried shoot represents a source of resources for a connected partially buried shoot. The alternative hypotheses are that the completely buried shoot is a sink for, or unrelated to, the resources of a connected partially buried shoot,

i) **Production cost hypothesis:** reductions in the costs of producing leaves and stem results in greater leaf area or stem length for a given resource input, or the following alternative hypothesis j,

j) **Increased photosynthetic capacity hypothesis:** the increase in the nitrogen content and photosynthetic rates of leaves above the sand surface may allow increased production rates relative to the photosynthetic leaf area,

k) **Increased nutrient supply due to increased transpiration:** burial may result in an effective shift to a higher root mass relative to the remaining unburied leaves. This could have consequences of greater transpiration, soil water mass flow and, thus, increases in the supply of nitrogen via the transpiration stream (e.g. Plhak, 2003), and

l) **Seasonal growth hypothesis:** the seasonal growth of *S. plumieri* may limit burial responses differentially during different periods of the year.

3) Mechanisms relating to the avoidance or nature of burial were the:

m) **Stem buffer hypothesis:** a tall initial height and bare stem buffer the shoot from burial events covering photosynthetic surfaces. Specifically, this allows the shoot the option of sensing and responding to burial before its leaves are buried, thus potentially maintaining its full leaf area under burial conditions, and

n) **Shade hypothesis:** the burial response is similar to shade induced growth, and thus that artificial burial (complete shading of ~90% of the shoot) would induce a response similar to burial.

These hypotheses were investigated in two separate field studies: 1) A survey of the production characteristics of 143 *S. plumieri* shoots of which 18 were buried by natural sand accumulation events, and 2) two sets of experimental manipulations of shoots of *S. plumieri* to test the contribution of various factors to the carbon and nitrogen budget of the burial response.

METHODS

Survey of the response of *S. plumieri* to natural burial

Fifty *Scaevola plumieri* dunes were selected in sequential order along 2 km of coastline west of Cintsa West. Dunes were chosen where the stand of *S. plumieri* shoots was distinct from other stands, and could be assumed to consist of one or few individuals (see Barker *et al.*, 2002). Three shoots were marked in the centre of each dune before the start of spring (15 July 2006) with lengths of coloured wire (n = 143 shoots as seven shoots were not found on subsequent inspections). The wire was positioned below the 4th leaf such that on a subsequent occasion the number of leaves above the marker could be counted and the number of produced leaves calculated. The experiment was terminated at the end of spring (5 November 2006). On each shoot the following production characteristics were measured on the final date: leaf production, total leaf number, length and width of the 8th leaf (representative of other mature leaves), the length of leaf bearing stem, the length of the three internodes below the lowest leaf, and the height of the lowest leaf above the sand surface.

The height of leaves above the level of the sand was taken as an indication of the minimum time since burial of the leaves had last occurred. This estimate was calibrated using the following information: *S. plumieri* shoots consistently produce flowers annually, and the flower support structures are evident on these stems in later years. By measuring the length of stem between the first set of flower “scars” to the next set of flower “scars”, the yearly stem production of thirty-three shoots of varying total length was measured, at Cintsa West. These shoots were selected on the basis that they showed no indication of having been buried during the period in which the visible stem was produced (as indicated by a lack of burial induced internode elongation). The length of stem produced per year for these unburied shoots was 14.6 ± 4.3 cm (mean ± SD). This transformation was used to estimate the minimum time since burial for all the shoots studied in the survey. More information from this survey can be found in Chapter 9 which specifically deals with non-burial determinants of production.

Experimental manipulation of factors determining the response of *S. plumieri* to partial burial

Treatments

By experimentally manipulating the conditions in which buried shoots grew, and monitoring the growth of these shoots relative to unburied treatments, the relative contributions of different mechanisms to the growth response of *S. plumieri* were assessed. Two similar sets of experiments were done to test as many hypotheses as possible (see list of mechanisms in Table 7.1). The two experiments differed only in the time of the experiment, and are referred to as Experiment 1 and Experiment 2. The experimental manipulations and the related hypotheses are given below:

Experiment 1:

Unburied (n = 9): unburied shoots marked and monitored with no other treatment, these shoots represent a control treatment to the buried and other unburied treatments.

Buried (n = 9): shoots were placed in PVC (12 cm OD) tubes, and the majority of their leaves buried in homogenous freshly blown beach sand. The five smallest apical leaves where left above sand level (ca. 15-20 leaves per shoot). These represented comparative shoots to the other burial treatments. This level of burial was chosen as it represents the most extreme form of burial that this species typically encounters. In Chapter 2.1 it was shown that this species encounters burial regularly, to depths within the range of normal shoot height above the sand surface. Thus, it was considered reasonable that responses to partial burial were more ecologically important than complete burial. For the rest of this chapter *burial* generally refers to a partial burial event including loss of the majority of the photosynthetic leaf area.

Leaf resource remobilisation hypothesis: Shoots remobilise resources from buried leaves which are no longer photosynthetic, and allow the growth response after burial.

Buried – buried leaves (n = 8): shoots were buried similarly to the buried treatment, however all buried leaves were removed, so as to prevent remobilisation of resources out of the non-photosynthetic buried leaves.

Unburied – "buried" leaves (n = 8): equivalent to the previous treatment, except that the plants were not buried.

Sand nutrient hypothesis: The freshly blown sand surrounding a buried shoot represents a source of nutrients that the shoot accesses with adventitious root production. This may enable the buried shoot to grow faster. Alternatively, if a buried shoot showed little ability to access nutrients in the sand then this finding would invalidate the hypothesis.

Buried – sand nutrients (n = 7-8): shoots were treated similarly to the buried treatment, however, acid washed sand was used to bury the plants. One hundred litres of previously collected beach sand was washed with 200 ml conc. sulphuric acid and diluted with sufficient water to saturate the sand, this subsequently rinsed for 4 hours with tap water) to remove available nutrients and return the soil pH to the level of the tap water.

Buried + fertiliser (n = 8): shoots were similar to the buried treatment with the addition of 15g of slow release N:P:K fertiliser (Wonder 3:2:1 28 SR*, 140g N kg^{-1}, 93.3g P kg^{-1}, 46.7g K kg^{-1}, Agroserve Pty., South Africa) distributed through different levels of the fresh sand column. An additional fertiliser application was made to the surface of the sand surrounding burial tube.

Shade hypothesis: Burial responses are similar to shade induced growth (e.g. etiolation), and thus it was hypothesised that artificial burial would induce a response similar to burial. Such a treatment could demonstrate that the burial response is dependent on the shoot being surrounded by sand.

Shaded (n = 7): shoots were placed in similar PVC tubes to the burial treatment, the tubes were lined with tinfoil (aluminium foil), no sand was added, and layers of black cloth and tinfoil were placed over the majority of the shoot such that five leaves remained exposed to the sun, the rest being in complete darkness. Thus, the experimental conditions of the shaded shoots approximate burial as closely as possible, but lack a sand medium. The inclusion of tinfoil within the PVC tube, and around the shoot, was thought to prevent light entering the burial tube, as well as maintaining the tube at moderate temperatures, similar to the buried treatment.

Stem buffer hypothesis: *S. plumieri* avoids burial of its photosynthetic leaf area by the maintenance of a bare stem “buffer” between the sand surface and the start of a shoot’s leafing zone.

Half buried (n = 8): shoots were buried with short PVC tubes half way up their bare stems, but no leaf area was buried.

Experiment 2:

Unburied (n = 6) and *buried* (n = 7) treatments were repeated for between- and within- experiment comparative purposes.

Nutrient translocation hypothesis: Inter-shoot allocation of resources may allow shoots of *S. plumieri* to increase the growth of buried shoots at the expense of adjacent unburied shoots.

Buried + fertiliser (n = 4): A 1 L plastic container containing 20g of Horticote a long release full nutrient fertiliser mixed with sand was placed in the wet soil layer 20 cm below the shoot that was to be buried (‘Horticote 13:6:11 (30) plus’, 130g N kg^{-1}, 60g P kg^{-1}, 110g K kg^{-1}, 12g Mg kg^{-1}, 200 mg B kg^{-1}, 500mg Cu kg^{-1}, 2000mg Fe kg^{-1}, 600mg Mn kg^{-1}, 200mg Mo kg^{-1}, 150mg Zn kg^{-1}, Efecto, South Africa). The container was oriented upwards to prevent any spread of nutrients and was presumed to allow local nutrient absorption only by the nearest shoot.

Buried + connected shoots fertilised (n = 9): prior to burial, excavations were made to find the nearest and second nearest shoot connected to the experimental shoot. A fertiliser container (see above) was then placed below the stem connecting the nearest and second nearest shoot. This treatment was intended to allow increased nutrient uptake by the adjacent shoots, but not that of the buried shoot unless by inter-shoot allocation.

Source-sink hypothesis: Resource relations between interconnected shoots are altered by manipulating the burial states of the connected shoot. A completely buried shoot may be a carbon sink for an adjacent partially buried photosynthetically active shoot. Alternatively, the completely buried shoot may not influence the partially buried shoot's growth, or may "take one for the team" and provide a source for the partially buried shoots growth. A complete shade treatment would allow the shaded shoot to respond to this treatment, without accessing soil resources.
Buried + connected shoot completely shaded (n = 4) – The closest connected shoot to the treated shoot was placed in a closed tinfoil lined PVC tube, such that the entire shoot was in complete darkness.

Experiment 1 was started on 12 March 2005 and harvested 151 days later on the 13 August 2005. Experiment 2 was started on 2 October 2005 during the onset of the flowering season and harvested 144 days later on the 26 February 2006. Thus, the two experiments were done during winter and summer, respectively. The repetition of unburied and burial treatments for both experiments allowed seasonal differences in the response to burial to be investigated. This is of particular interest as the species shows seasonal growth (Pammenter, 1983, this study), while burial can occur year round or seasonally (Chapter 2.1). Conducting the experiments in different seasons also allowed an investigation of the effect of the reproductive state of the shoot on the burial response.

Site and shoot selection

Both of the experiments were conducted on *S. plumieri* shoots in the stable-dune zone at Old Woman's River. Shoots were selected if they were a sufficient distance (2 m) from another treated shoot so as to prevent contamination between treatments. In order to reduce variation at the start of the experiment, only shoots with 13 to 17 leaves and between 30 and 50 cm in height were chosen. Nutrient addition treatments were placed as far from the other treatments as possible.

Shoot measurements

Before applying the treatments and on subsequent occasions a series of measurements were made on all shoots. The rates of leaf production and loss were measured by counting the leaves above and below a permanent wire marker. The height of the lowest leaf, highest leaf and tip of the shoot apices were measured relative to a constant level marked on the experimental tubes or wooden stakes. The length stem below the lowest leaf with five leaf scars was measured and used to as a measure of average internode distance (= average stem height production per leaf). An estimate of the total leaf area of the shoot was made by measuring the length and width of the 3rd, 5th, 8th, 10th, 15th and 20th leaf from the shoot apex. The area of individual leaves was determined from a previously established regression ($R^2 > 0.97$) of length multiplied by width predicting leaf area. The areas of the leaves that were not measured were then estimated by linear extrapolation. The total shoot leaf areas estimated by this technique were highly correlated with actual leaf area measurements made a month later, after the shoots were harvested ($R^2 = 0.81$, n = 62).

Harvest measurements

At the start of both experiments, eight random shoots were harvested to generate the leaf area to length multiplied by width relationships. At the end of the experimental periods all treated shoots were harvested, placed in moist, cool containers, and brought back to the laboratory. Individual leaf areas, leaf dry weights and internode distances were measured for each shoot. By reference to the wire marker indicating the start of the experiment, each shoot was divided up into leaves and stem produced prior or subsequent to the start of the treatments, and dry weight recorded. In addition, the roots, seeds and flowers where separated and weighed. Dry weights were measured after the material had reached a constant weight in a 60°C oven. To determine the amounts of nitrogen used in production, shoots were divided into three sub-samples: the youngest fully expanded leaves, the remainder of the leaves and the stem produced since the start of the experiment. Sub-samples from five treatments from Experiment 1, each with five replicates, were analysed for total nitrogen (Kjeldahl digestion, Matrocast Laboratories, Capetown). The leaves that were removed to test the leaf remobilisation hypothesis were also dried, and analysed for total nitrogen. The freshly blown sand used to bury plants, and acid washed sand used to bury the burial – nutrients treatment were also sampled, dried and analysed for total nitrogen.

Measurement of shoot total photosynthetic capacity

The photosynthetic rates of all large leaves (>6 cm^2) were measured on seven shoots, at OWR between 2 and 4 PM on the 31 May 2005. An ADC LCA-2 infra-red gas analyser (ADC, Hoddeston, England) was used to determine the photosynthetic rates of individual leaves, measured under ambient humidity and temperature conditions. Cuvette conditions were 37.5±1.1% RH, a leaf temperature of 26.3±0.4^{o}C, and PAR of 1795±3 μmol m^{-2} s^{-1} (mean ± 1 SE). All leaves in full sunlight were measured, whereupon the shoot was rotated so that other leaves were illuminated prior to measurement. After a 2 to 5 minute acclimation period these leaves were then measured. Thus, these measurements represent the total maximum light saturated photosynthetic capacity of the shoot, and not actual *in situ* photosynthetic capacity. This method was thought to be adequate to estimate the loss of shoot total potential photosynthetic capacity in response to burial, rather than immediate loss of shoot integrated photosynthetic rate with leaves at a range of light saturations. As the average shoot measured in Experiment 1 had 14 leaves, the photosynthetic rates of five shoots of this size were measured. A further two shoots of 20 leaves were also measured to test the generality of the relationships found for shoots of 14 leaves. After the photosynthetic measurements the leaves were marked, harvested and individual leaf areas measured in the laboratory. Total shoot photosynthetic capacity was determined by multiplying photosynthetic rate by the leaf area of the leaves measured. The photosynthetic rates of leaves that were too small to fit in the cuvette were extrapolated from the data of the other measured leaves.

Measurement of leaf photosynthetic rate

On two occasions during Experiment 1 mid-day (from 11:30AM to 2:30PM) photosynthesis measurements were made on the 8th or 9th leaf of shoots from each treatment (31 May 2005 and 13 August 2005). From the previous set of measurements it was confirmed that the 8th or 9th leaf of shoots with 14 leaves consistently had the highest photosynthetic rates. Leaves were chosen based upon which leaf was illuminated at the time of measurement. The same ADC LCA-2 IRGA was used for these measurements as above. The CO_2 and H_2O span and zero of the IRGA's were checked, or set, prior to measurements using standard gas cylinders and a dew point apparatus. Cuvette conditions were 45 ±0.6 % RH, a leaf temperature of 22 ±0.2 ^{o}C, and PAR of 1800 ±16 μmol m^{-2} s^{-1} for 31 May 2005, and 52 ±0.6 % RH, a leaf temperature of 24 ±0.2 °C, and PAR of 1845 ±10 μmol m^{-2} s^{-1} for 13 August 2005 (mean ± 1 SE).

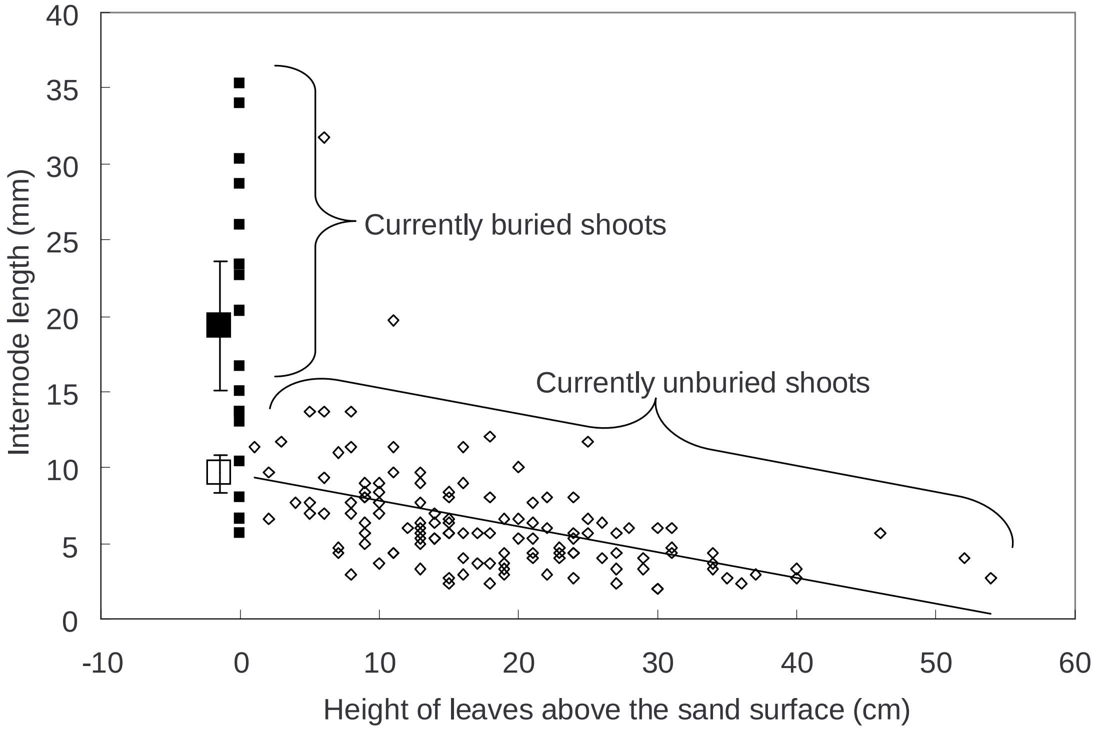


Figure 7.4: Example diagram of how differences between partially buried and unburied shoots in a growth variable (in this case internode length) were determined for the growth survey at Cintsa West. An OLS regression line was used to predict the relationship between the variable and the height of the leaves above the sand (a rough estimate of the time since burial) for currently unburied shoots (open symbols). The y-intercept and 95% confidence interval were calculated (indicated on the diagram by the large open symbol). The mean value for partially buried shoots was calculated along with a 95% confidence interval of the mean (solid symbols). When predicting the value of a variable from a bivariate relationship OLS regression is preferable to SMA procedures (see Appendix 1).

Analysis

The length of stem below the lowest leaf to the sand surface was found to be a good indication of the time since the leaves of the shoot were last buried (demonstrated above). By transforming stem lengths to represent time since burial (length/14.6 cm yr^{-1}) the changes in other growth variables could be related to the approximate time since leaves were last buried (e.g. Figure 7.4, where the height of the leaf above the sand surface relates roughly to the time since burial last occured), and thus test the "Loss of vigour hypothesis". The mean value of a growth variable for buried shoots could be compared to the 95% confidence interval of the y-intercept of the regression line of the unburied shoots. The y-intercept corresponds to the mean for the unburied shoots when the height of the leaves has been taken into account (e.g. Figure 7.4). The confidence intervals could be statistically compared using a t-test.

As the manipulation Experiments 1 and 2 were designed based upon *a priori* hypotheses, these hypotheses were tested individually by comparing a treatment with a relevant control as implemented in Dunnett's test. In the case of the buried leaf nutrient remobilisation hypothesis (Experiment 1) the unburied, buried, unburied – leaves and buried – leaves treatments were compared by factorial ANOVA, with burial and leaf removal as factors. Similarly, the effect of burial and season was tested using a factorial ANOVA with season (≈ experiment) and burial as factors.

RESULTS

Survey of *S. plumieri* response to burial

The highly variable production characteristics of *S. plumieri* shoots in response to burial were surveyed to test hypotheses with the benefit of large sample sizes (n = 143). The data, in part, corresponds to some of the competition hypotheses tested in Chapter 9.

Internode distance, the length of stem bearing leaves, the area of individual mature leaves, and the leaf number, total leaf area, and stem length produced during the experiment were significantly related to the height of the lowest leaves above the sand (and hence roughly the time since last burial; Figure 7.5). Buried shoots displayed similar leaf areas, produced the same number of leaves, and length of stem bearing these leaves to those predicted by linear regression of unburied plants. The internode distance and length of stem produced by buried shoots was significantly greater than equivalent unburied shoots (Figure 7.5).

In relative terms, the height of the leaves above the sand surface had the greatest effect on stem growth variables, reducing stem production by 100% once the leaves were 50 cm above the sand level, estimated as roughly 3 to 4 years since burial had occured (Figure 7.5a and f). Leaf area and leaf number production were reduced by 40 to 80% between buried shoots and shoots where the leaves were 50 cm above the sand level (Figure 7.5c, d and e).

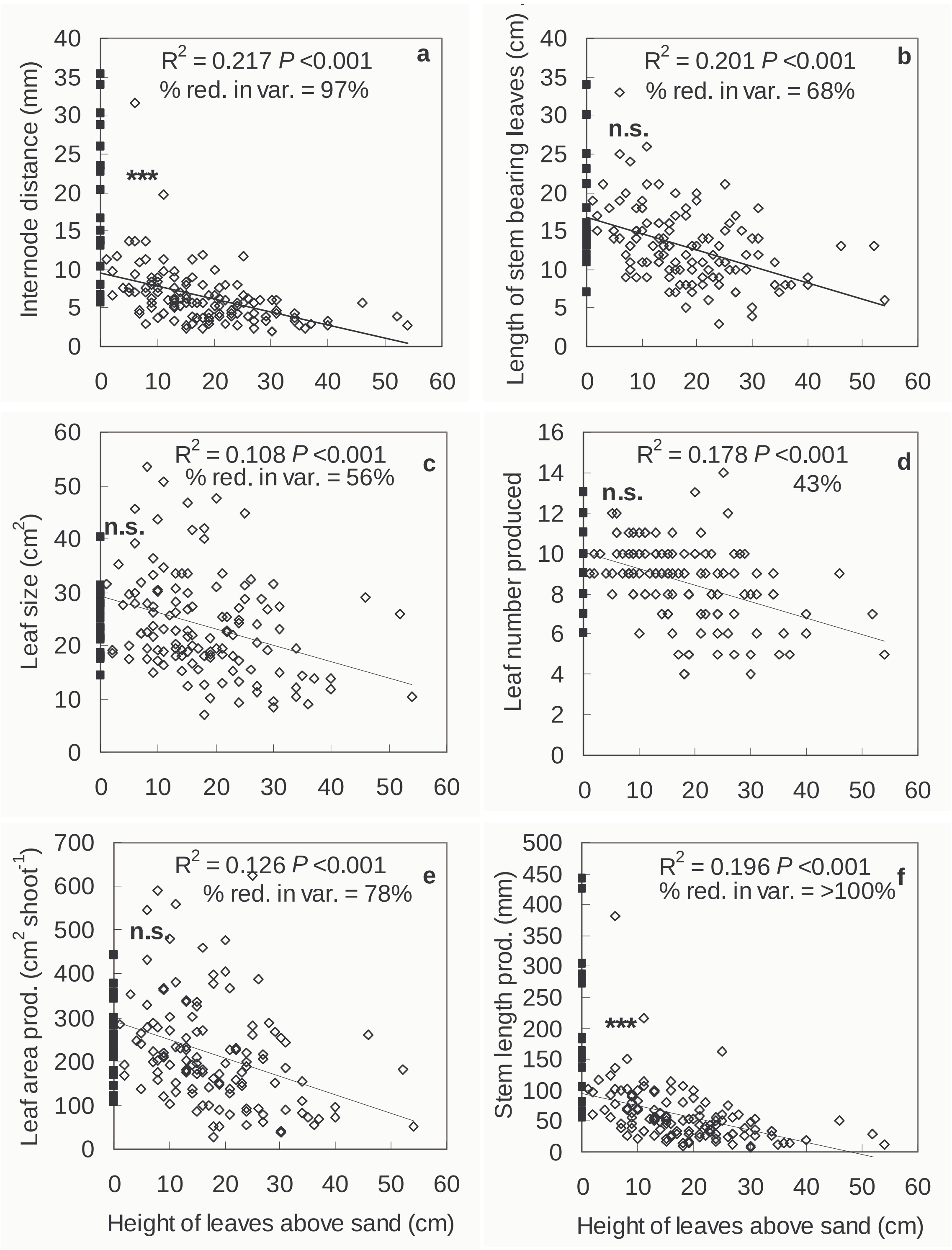


Figure 7.5: The responses of *S. plumieri* shoots to burial (closed squares) in comparison to plants that did not experience burial (open diamonds) for the survey at Cintsa West. The height of leaves above the sand is correlated with the time since the shoot was last buried ($R^2 = 0.88$), with roughly14.6 cm of stem production per year. Response characteristics are: internode distance (a), length of stem bearing leaves (b), leaf size (c), the number of leaves produced (d), leaf area produced (=number*leaf area; e), and the length of stem produced (f). Lines represent the ordinary least square regression lines for unburied plants. *** = $P < 0.001$; n.s. = not significant for the difference between buried and unburied shoots when the height of leaves above the sand, or time since burial, has been taken into account. The percentage average reduction in the variable over the range of measured heights is given.

The leaf area production and stem production of unburied shoots were proportionally, and closely, related to each other, over a wide range of total productivities (Figure 7.6). When buried, shoots showed a significant shift favouring stem production (Unburied: 80% leaf production, Buried: 58% leaf production, *P* < 0.001).

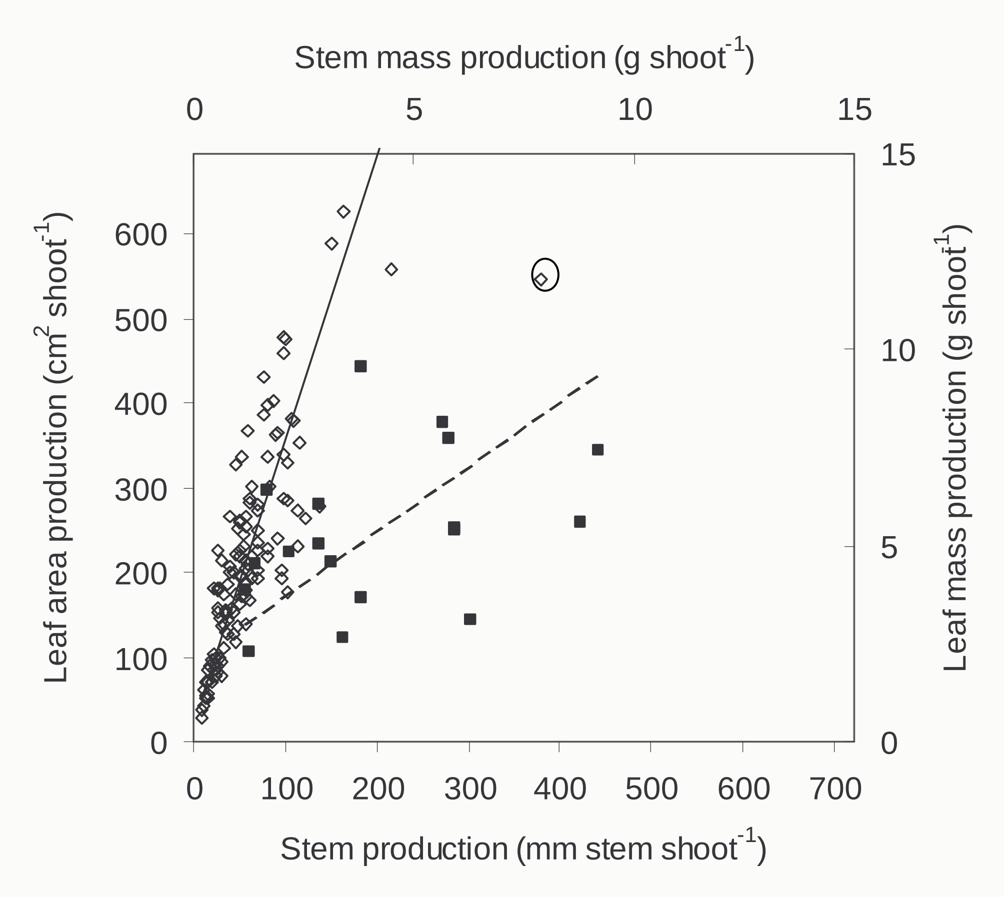


Figure 7.6: Relationship between leaf and stem production of buried (closed squares, dashed line) and unburied (open diamonds, solid line) shoots of *S. plumieri*. Lines represent SMA regression lines, the circled point was excluded from the analysis (buried n=18, R^2=0.14, *P*=0.132; unburied n=124, R^2=0.69, *P*<0.001). For comparative purposes both stem and leaf area production have been converted to mass units on the second axes. The mass conversion was based upon measured SLA and stem mass specific length values (*SLA*: 46.3 ± 2.2 cm^2 g^{-1}; *MSL*: 0.21 ± 0.06 g cm^{-1}.

Flowering was not significantly affected by burial (*P* = 0.071), however 44% of the buried shoots were not flowering, while only 27% of unburied shoots were not flowering. Among, the unburied shoots, flowering shoots were significantly more vegetatively productive (leaf and stem production) than non-flowering plants (*P* = 0.003).

Experimental manipulation of the burial response of *S. plumieri*

Shoots of many of the burial treatments in Experiment 2 suffered non-experimental disruption due to high winds. This resulted in reduced sample sizes for a number of the treatments (sample sizes were eight or more at the start of the experiments).

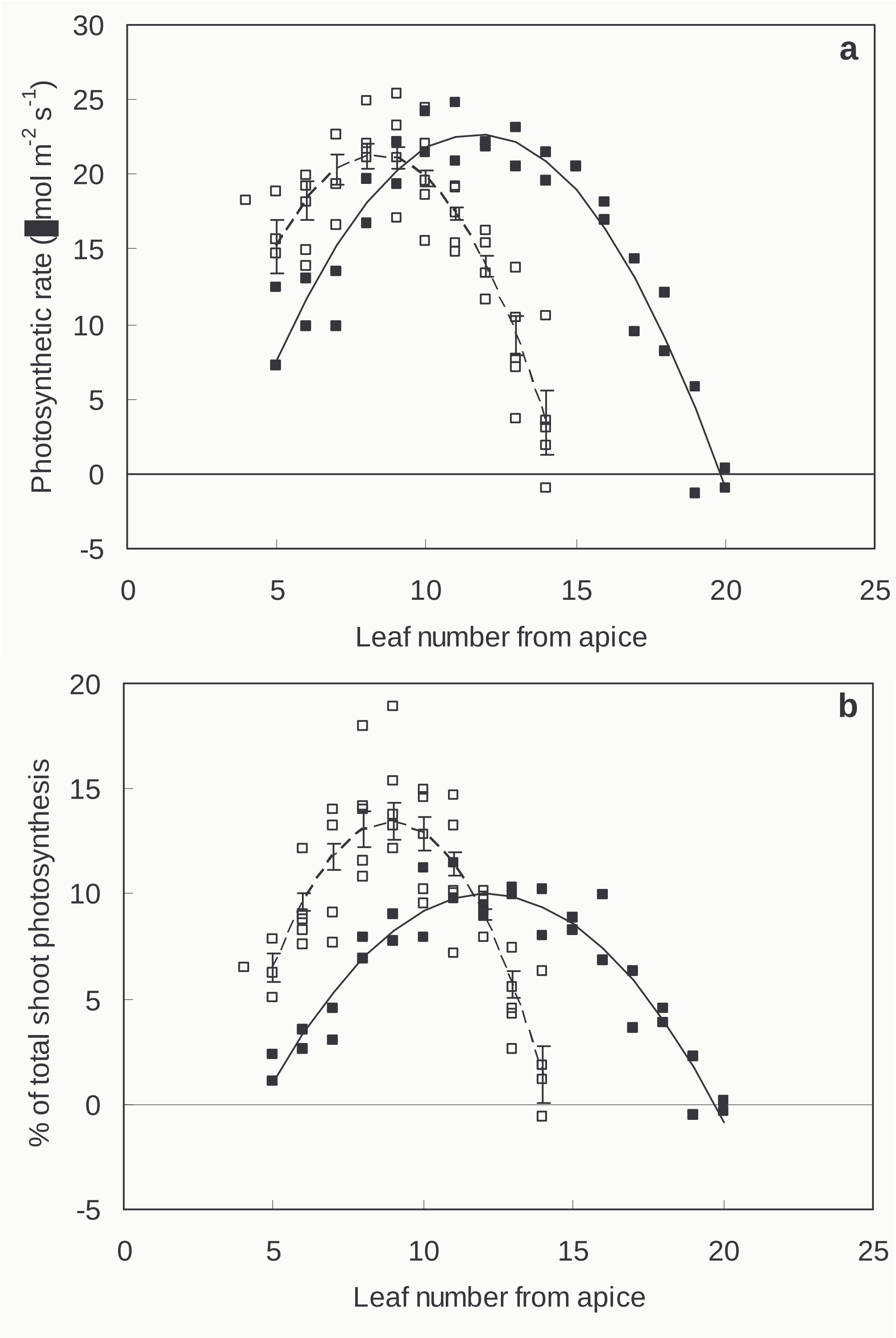


Figure 7.7: The photosynthetic rates (a) and percentage of shoot total photosynthetic rate (b) of the 5th to 20th leaf from the shoot apice. The data represents five shoots of 14 leaves (open symbols) and two shoots of 20 leaves (closed symbols). For the smaller shoots the dashed line represents the mean and standard errors of the lines fitted to each shoot.

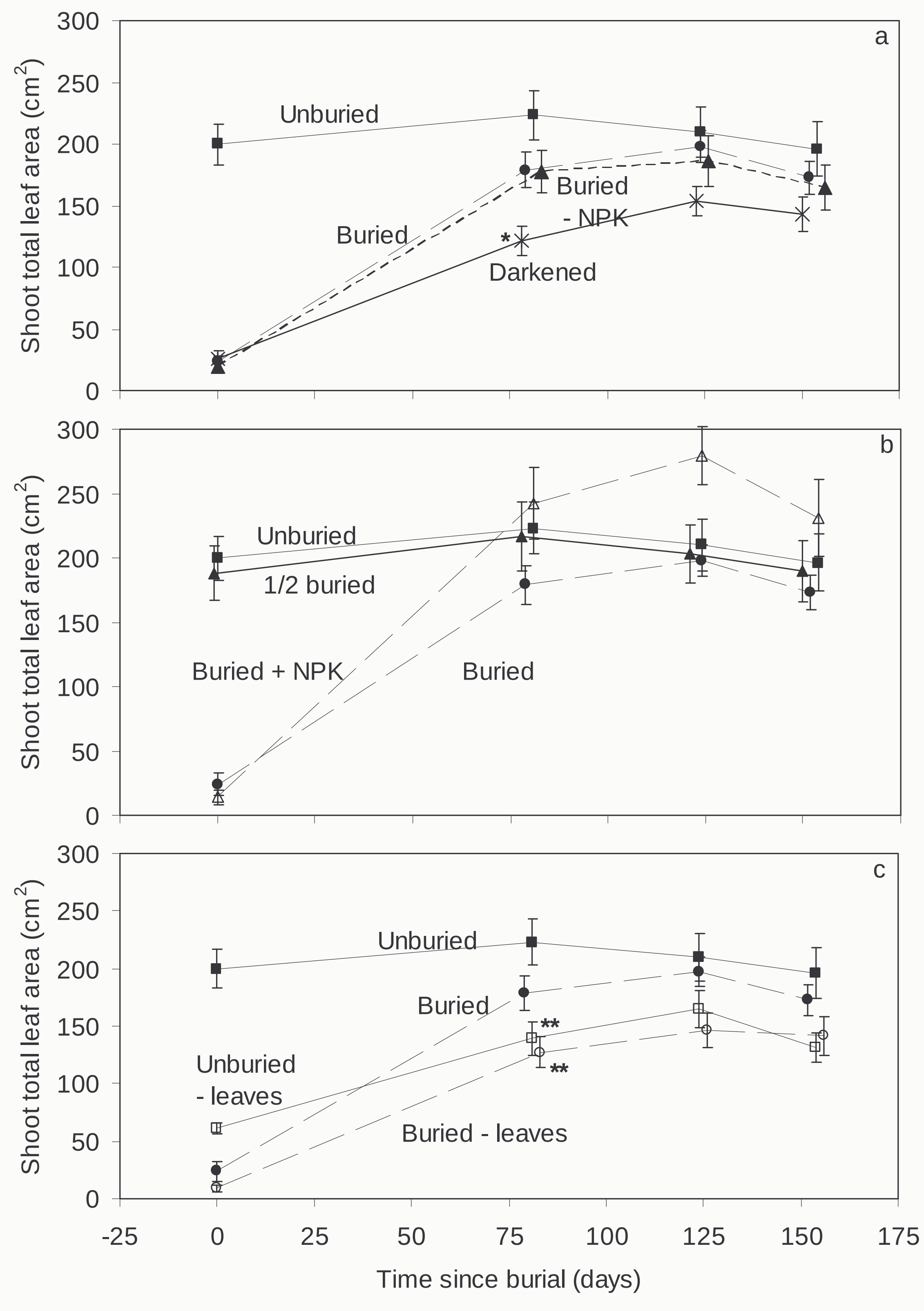


Figure 7.8: Replacement of shoot photosynthetic capacity since burial for unburied and buried shoots (a, b and c), shaded or shoots buried in acid washed sand (a), shoots buried to half the height of the bare stem or buried shoots to which a full nutrient fertiliser was added (b), and buried and unburied shoots which had the lowest buried leaves removed (c). Points represent means and standard errors of the mean (n ≥ 5). Points have been shifted by ± 2 days to enhance clarity. * = 0.01 < P < 0.05; ** = 0.001 < P < 0.01; no symbol = not significant for the difference between the unburied shoot and other treatments at 81 day since burial (Dunnett's control group test).

Loss of photosynthetic capacity after burial

After burial, all treatments had between 85 and 96% of leaf area below the sand surface (or in complete shade in the case of the shade treatment). The three unburied treatments had no leaves covered by sand, although 70% of the shoot leaf area was removed in the unburied – leaves treatment.

Burial of the lowest leaves would remove only a small amount of the total shoot photosynthetic capacity (Figure 7.7b). However, the burial treatments covered all leaves below the fifth leaf, thus removing all large and high photosynthetic rate leaves from the total shoot photosynthetic capacity (Figure 7.7a and b).

Buried shoots from most treatments replaced the total shoot leaf area lost by burial, after 81 days (Figure 7.8). The darkened and buried and unburied treatments where “buried” leaves were removed did not replace the lost leaf area until the following set of measurements. This was indicated by the significant difference between the total leaf area of these treatments relative to the unburied treatment (Figure 7.8). By the third measurement date all treatments were not significantly different to the unburied treatment, although the buried shoots with added nutrients had considerably greater total shoot leaf areas (Figure 7.8).

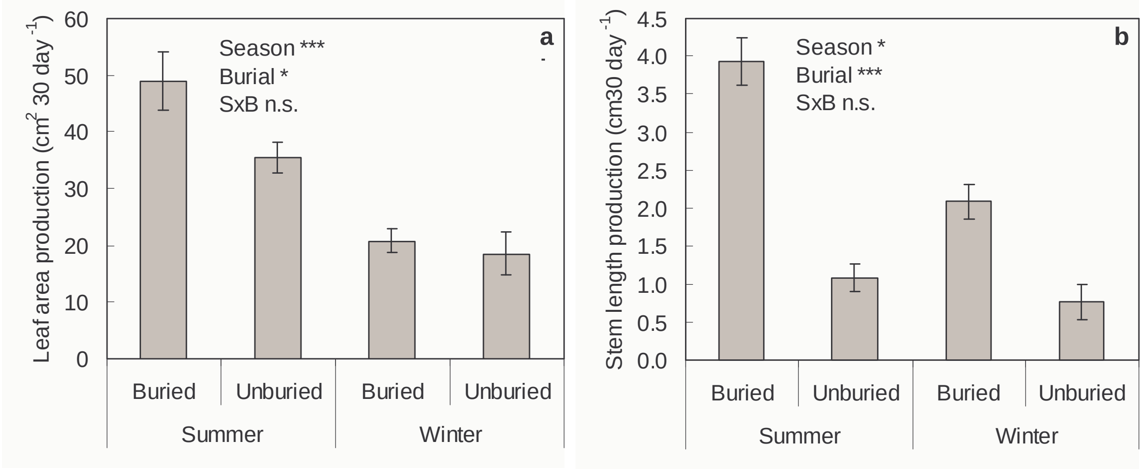


Figure 7.9: Leaf area (a) and stem length (b) production for buried and unburied shoots of *S. plumieri*, measured in Experiment 1 (winter) and Experiment 2 (summer). Bars represent means and standard errors of the mean. Factorial ANOVA results are given where symbols indicate the following degree of significance: * = $0.01 < P < 0.05$; *** = $P < 0.001$; n.s. = not significant.

Changes in production with burial (Seasonal growth hypothesis)

Buried shoots had increased production of leaf area and stem relative to unburied shoots (Figure 7.9a and b). The burial stimulation response was consistent across both seasons, but shoots had greater production during summer (Figure 7.9a and b).

Similarly, the production of leaf and stem mass was significantly increased by burial, and also during the summer experiment (Figure 7.10b and c). This pattern was also evident in the production of the total vegetative mass of buried shoots, which included root and branch mass (Figure 7.10e). However, the mass of reproductive tissue (seeds and flowers) was larger in the summer unburied shoots than the summer buried shoots, accounting for the decreased vegetative growth in unburied shoots (Figure 7.10f). Thus, when the mass of reproductive structures was taken into account, there was no significant burial effect on the production of total shoot mass. The buried shoot production was greater than unburied shoot production for both experiments, although not significant (Figure 7.10f).

Sand nutrient and nutrient limitation hypothesis

The majority of buried shoots showed vigorous adventitious rooting within the new soil volume within the PVC tubes. Thus the new soil volume could represent a pool of nutrient that the buried shoot is able to access. What is the size of the soil nitrogen pool added by the burial treatment? Do the adventitious roots access these new nutrients? Finally, this is only relevant if the buried shoot is nutrient limited.

The amount of nitrogen present in the sand used for the burial treatments was calculated from the following measurements:

- 4.2 kg of sand was used to bury a shoot (in a 2.85L PVC tube of 12 cm diameter),
- there was an average of 244 mg kg^{-1} of nitrogen in the sand used to bury the plants,

Thus the soil volume by which the treated plants were buried contained 1.03 g of nitrogen. The buried shoots produced approximately 3.3 g of shoot mass over the winter experimental period, with stem and leaf nitrogen contents of 0.25 and 0.58 mmol N g^{-1} dry mass. Thus, shoot production was 23 mg N shoot^{-1} over the experimental period, which is 45 times less than the nitrogen in the added soil volume. Naturally buried shoots would have access to much greater volumes of sand than the limited PVC tube used in these treatments, thus these estimates represent an underestimate of the nitrogen present in the soil volume accessed by naturally buried shoots.

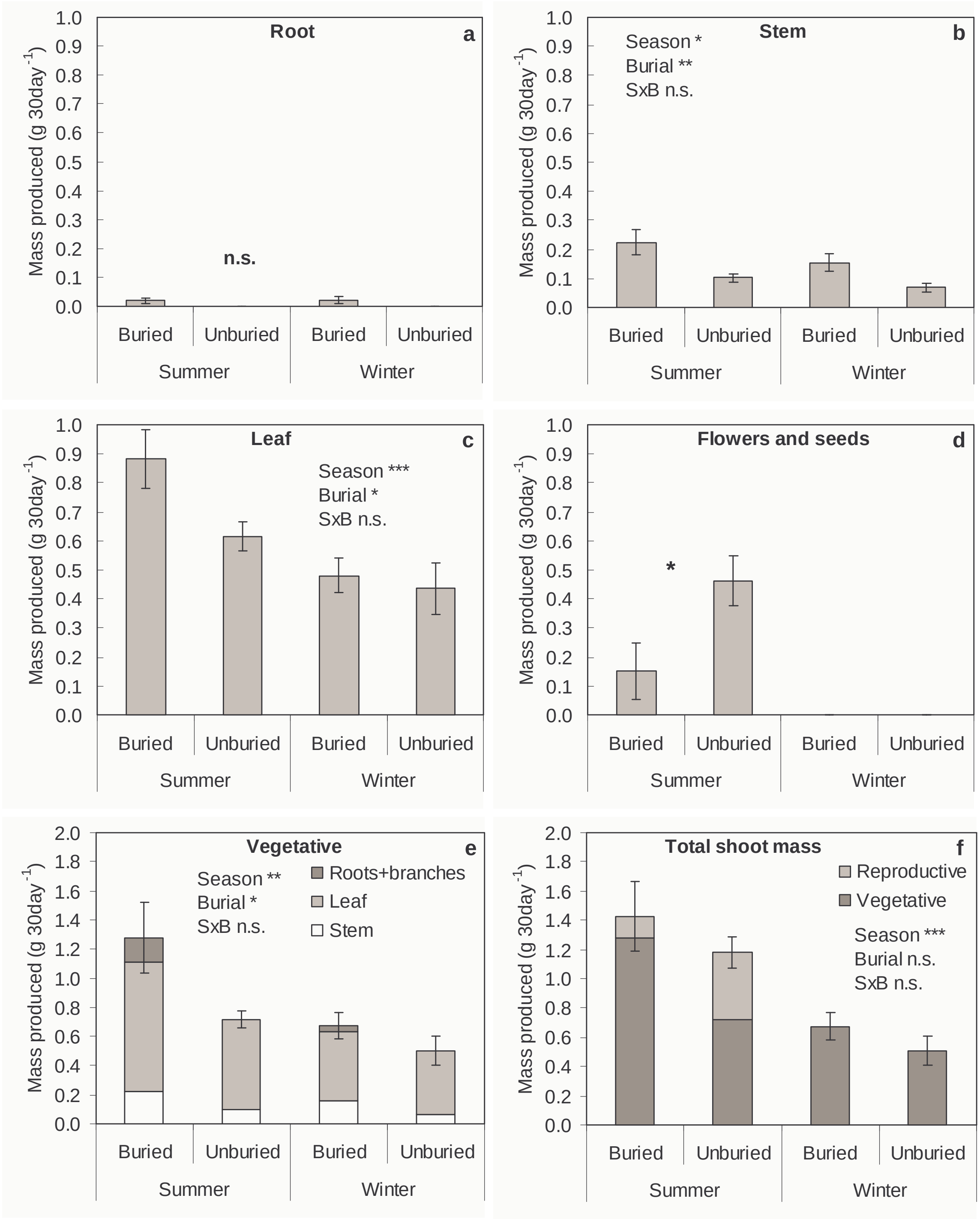


Figure 7.10: The mass production of buried and unburied treatments for experiment 1 (winter) and experiment 2 (summer). Error bars represent the standard error of the mean. For each biomass component the results of a factorial ANOVA are shown, in the case of reproductive biomass a t-test was performed. * = 0.01 < *P* < 0.05; ** = 0.001 < *P* < 0.01; *** = *P* < 0.001; n.s. = not significant.

Washing sand with dilute acid reduced soil nitrogen by 14%. This was assumed to be equivalent to the available nitrogen portion of the total pool. The shoots buried in the acid washed sand had a non-significant 13% reduction in growth relative to buried plants (Figure 7.11). The addition of a full nutrient slow release fertiliser into the sand surrounding buried shoots resulted in a large (88%) significant increase in biomass production (Figure 7.11).

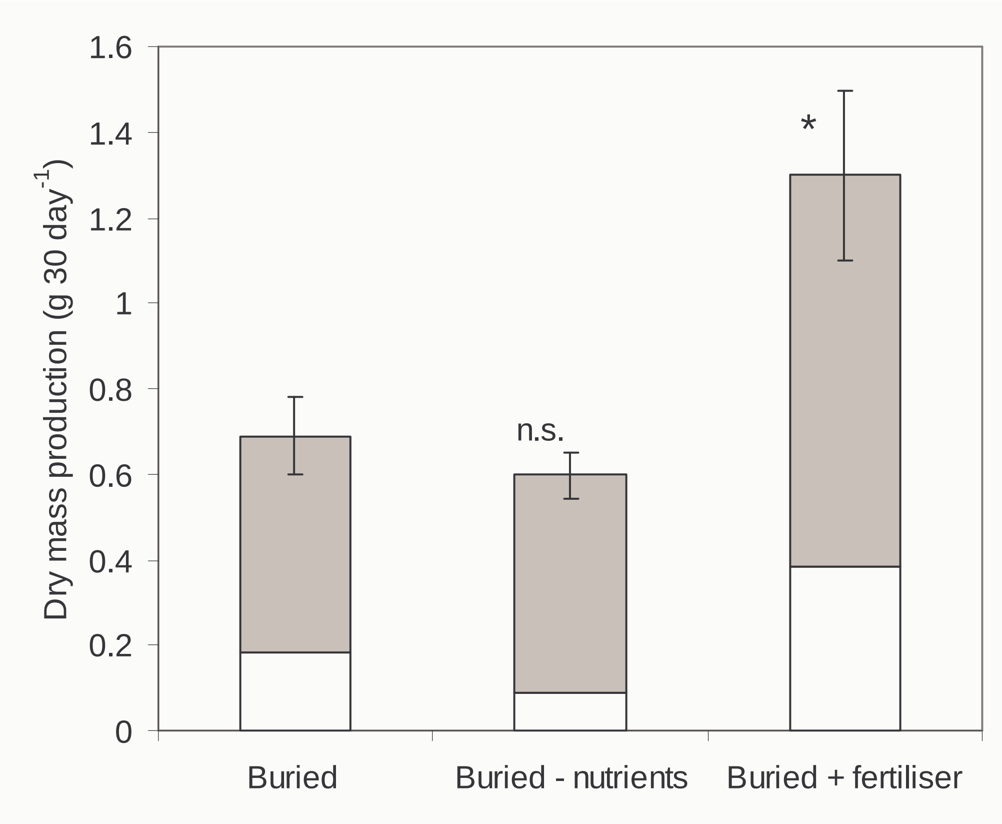


Figure 7.11: Total dry mass production of buried shoots with freshly blown sand (n = 9), acid washed sand (n = 7) and NPK fertiliser (n = 5) added to burial tubes. Error bars are ± one standard error of the mean. n.s. and * represent non-significant and significant differences ($P < 0.05$), respectively, for t-test comparisons between buried and the marked treatment.

Leaf resource remobilisation hypothesis

The removal of 8 to 12 buried leaves represented a 66 to 80% reduction in the total pool of leaf nitrogen for the unburied – leaves and buried – leaves treatments (removed leaves were analysed for nitrogen and compared to the total nitrogen in shoots harvested prior to the experiment). *S. plumieri* showed vigorous root scavenging of nutrients in decomposing buried leaves (pers. obs.), in addition to the removal of 52 to 80% of leaf nitrogen by remobilisation at senescence (data presented in Table 8.2, or Harte and Pammenter, 1983, respectively). Thus, the leaf removal treatments represent a large reduction in the available pool of nutrients potentially used for a burial response.

The removal of buried leaves reduced growth of the buried shoot by 50% (Figure 7.12). The dry mass production of stem of *S. plumieri* was significantly reduced by the removal of buried leaves, but stimulated by the burial (Figure 7.12; factorial ANOVA results for the effect of: leaf removal $P = 0.001$, burial $P = 0.034$, and the interaction $P = 0.099$). Leaf and total shoot mass production were not affected by burial, but were significantly decreased by removal of the buried leaves (Figure 7.12; leaf production: leaf removal $P = 0.019$, burial $P = 0.927$, and interaction $P = 0.236$; total shoot production: leaf removal $P = 0.007$, burial $P = 0.698$, and interaction $P = 0.189$).

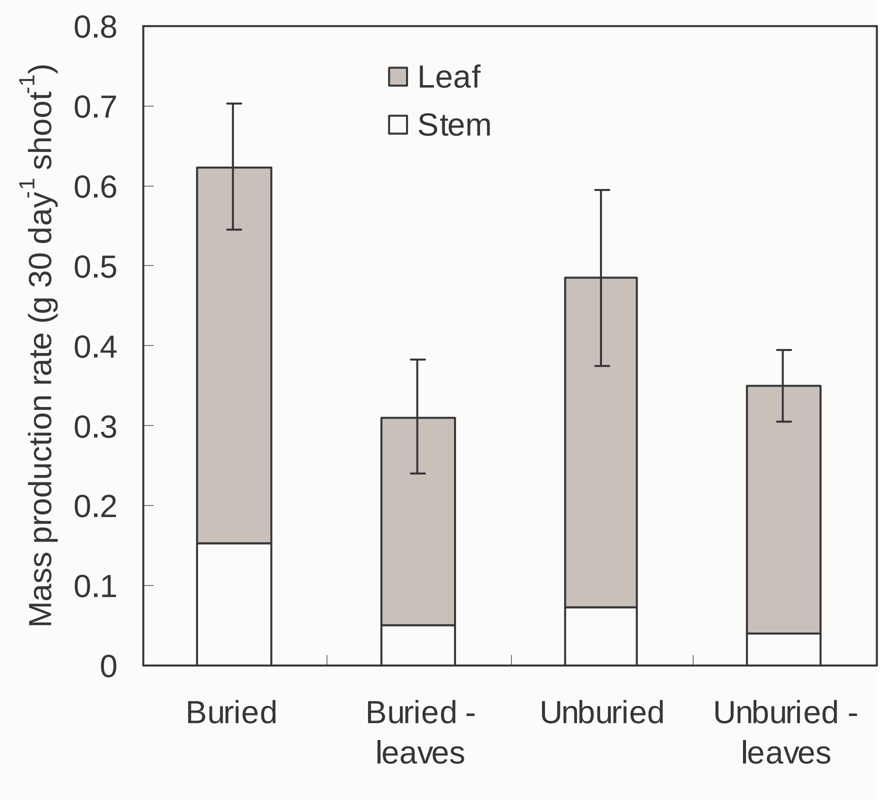


Figure 7.12: Total dry mass production of shoots of *S. plumieri* exposed to factorial treatments of burial and leaf removal (n ≥ 8). Error bars are means ± one standard error.

Intershoot translocation hypothesis (Source-sink hypothesis)

There was no significant effect of any of the three intershoot treatments on the growth of partially buried shoots (Figure 7.13; one-way ANOVA, $P = 0.560$). Unfortunately it is unclear whether this is a consequence of there being no fundamental difference between treatments, reduced sample sizes, experimental problems with the shoot's accessing the fertiliser, or high variability in the amount of growth. The indication was that fertiliser addition to an adjacent shoot did not affect the growth of a connected partially buried shoot. However, full shade, a simulation of complete burial without the effects of the sand medium, resulted in an increase in the growth of the adjacent shoot (Figure 7.13; however no effect was significant).

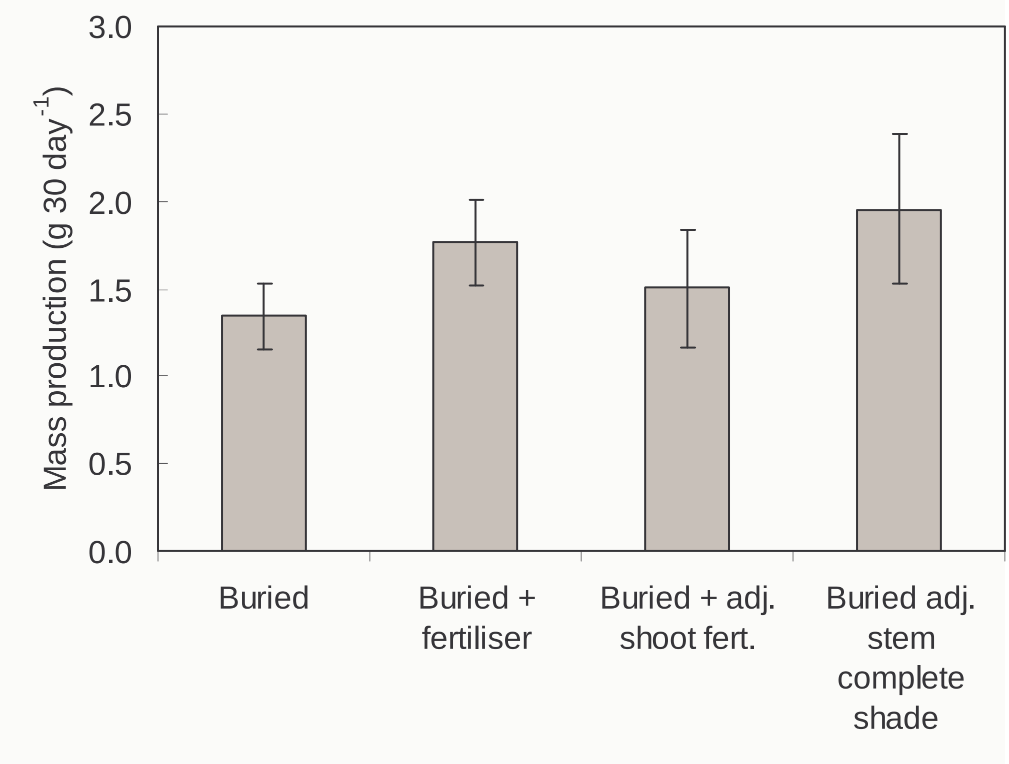


Figure 7.13: The effect of adjacent shoots resource status on the growth of a connected partially buried shoot. Treatments were partially buried shoot, partially buried shoot with fertiliser applied to the base of the stem, partially buried shoot with fertiliser applied to the stem connecting the adjacent shoot and further shoots, and a partially buried shoot adjacent to a connected shoot completely covered by a full shade treatment.

Allocation hypothesis

Allocation from reproductive to vegetative production was shown to account for the difference in vegetative production between treatments during summer (Figure 7.10f). In addition to the change in reproductive biomass allocation, there was a significant shift in allocation of mass from leaves to stems in buried treatments for both seasons (Figure 7.14a). This shift was more pronounced when presented as the ratio of leaf area production to stem length production (Figure 7.14b).

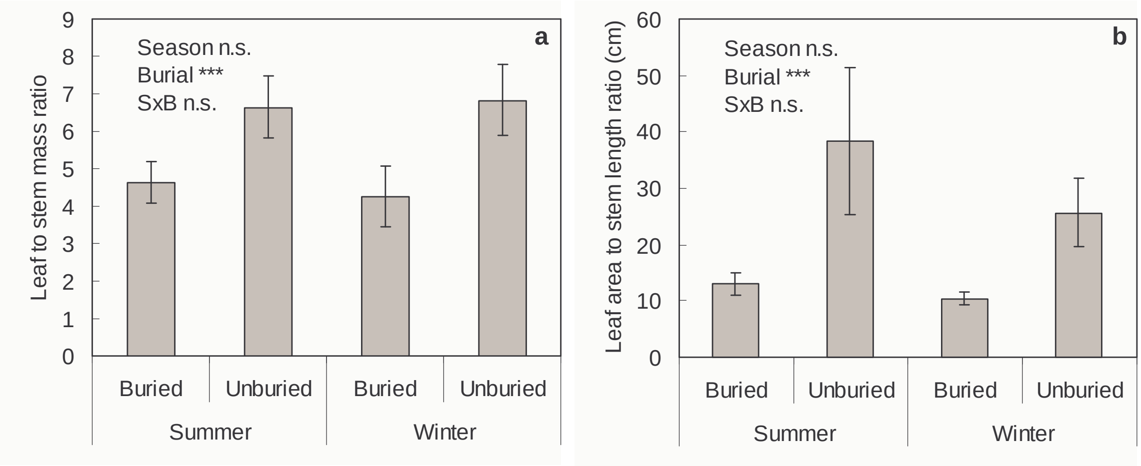


Figure 7.14: The allocation ratios of leaf and stem mass or the functional measures of leaf area and stem length for buried and unburied shoots measured in Experiment 1 (winter) and Experiment 2 (summer). Bars represent means and standard errors of the mean. Factorial ANOVA results are given as indicated by: *** = $P < 0.001$; n.s. = not significant.

Production cost hypothesis

The allocation of leaf and stem mass to production is roughly proportional to the production of leaf area relative to stem length (see equation on pp. 172). The mass costs of leaf area and stem length link these two sets of production measures. The stem length produced per unit mass was reduced between unburied and buried shoots (not significant), however this parameter was greatly decreased in the buried shoots which had buried leaves removed (Table 7.4). Specific leaf area was significantly increased by burial, particularly in shoots that had the buried leaves removed (Table 7.4). Stem nitrogen content was significantly reduced in shoots that had buried leaves removed, particularly in the burial treatments, including the buried nutrient addition treatment (Table 7.4). Leaf nitrogen and photosynthetic rate were little affected by any treatment (Table 7.4). In general, the mass and nitrogen costs of producing stem length and leaf area were reduced in the buried and extreme burial treatment where the buried leaves were removed (stem nitrogen content, *SLA* and *MSL*; Table 7.4).

Table 7.4: Dry mass and nitrogen costs of stem and leaf production for six treatments. Three statistical tests are presented for each production variable: a factorial ANOVA testing for an effect of leaf removal on burial response, a t-test comparing the burial and unburied treatment, and a t-test comparing buried and fertilised buried shoots. Significant effects are shown in bold

	Treatment	Mass specific stem length cm g^{-1}	*SLA* cm^{-2} g^{-1}	Stem N mmol g^{-1}	Leaf N mmol g^{-1}
	Unburied	5.8 ±0.5	41.5 ±0.9	0.32 ±0.02	0.51 ±0.03
	Unburied - "buried" leaves	7.5 ±0.8	43.7 ±1.3	0.24 ±0.03	0.54 ±0.01
	Buried	6.3 ±0.5	43.8 ±0.8	0.26 ±0	0.53 ±0.04
	Buried - buried leaves	8.4 ±1.1	46.5 ±1.1	0.25 ±0.01	0.58 ±0.03
	Buried + NPK	5.8 ±0.4	42 ±0.5	0.25 ±0.02	0.59 ±0.02
	Time zero harvest	na	na	0.33 ±0.02	0.65 ±0.04
Statistical Comparisons:		*P*-value	*P*-value	*P*-value	*P*-value
1) Factorial ANOVA:	Burial	0.311	**0.019**	0.183	0.375
	Leaf removal	0.528	0.840	**0.055**	0.825
	Burial*Leaf removal	**0.033**	**0.025**	**0.020**	0.251
2) t-test:	Unburied to Buried	0.514	0.076	**0.022**	0.689
3) t-test:	Buried to Buried + NPK	0.403	0.070	0.821	0.260

Does a change in stem width or stem tissue density account for the observed changes in mass specific stem length?

The mass specific stem length is determined by stem width and stem tissue density (see equations on pp.174). Changes in stem tissue density were not considered here (see Chapter 4).

To investigate the effect of stem width on the mass specific stem length, all burial and unburied treatments were combined and plotted in Figure 7.15. A single curve of the form given in the Introduction (pp. 174) fits all the data well, with no difference in slope (*P* = 0.337) or elevation (*P* = 0.969) between treatments. The burial treatments were significantly shifted along the common relationship (*P* = 0.038), to higher mass specific stem length, at lower widths. The theoretically derived slope of the relationship (109.3; see Introduction) was not significantly different to the fitted regression slope (113.8).

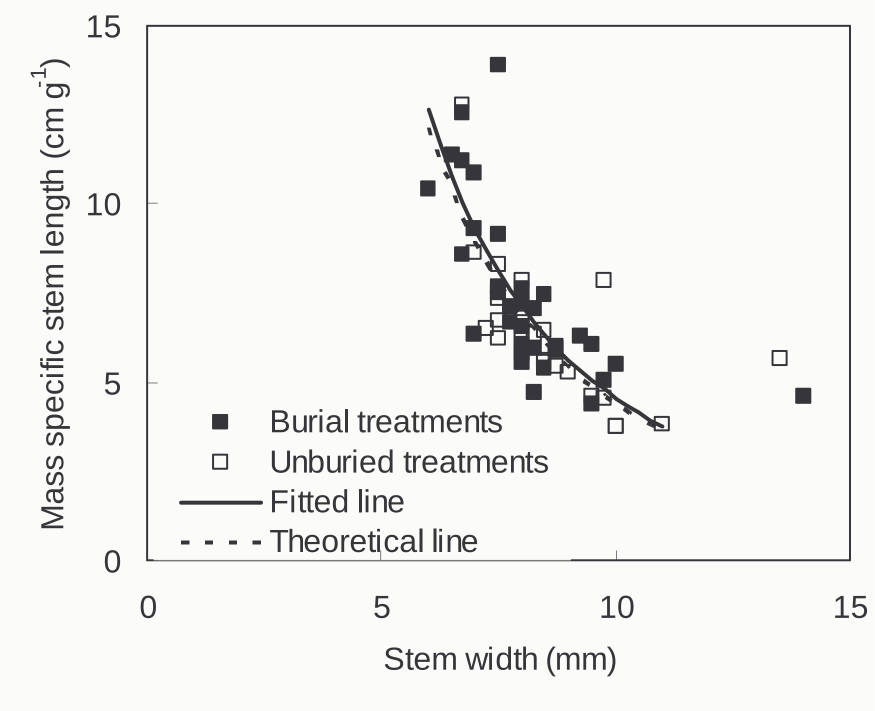


Figure 7.15: The mass specific stem length related to stem width of the 9th internode for combined burial and unburied treatments. $R^2 = 0.571$, $P < 0.001$. The solid line represents the SMA regression fit for linearised data, while the dashed line represents the theoretically derived relationship between the two parameters.

Photosynthesis

The photosynthetic rates of leaves on shoots from widely different treatments were similar (at most 20% difference; Figure 7.16a). However, the photosynthetic rate did differ by treatment ($P = 0.001$) and by date ($P < 0.001$). There was no significant interaction between treatment and date ($P = 0.892$), indicating that differences between treatments were maintained over the course of the four month interval between photosynthetic measurements. Although, this indicates that the given treatments were consistently higher or lower over the two dates of measurement, *post hoc* tests found few differences between individual treatments (Figure 7.16a; namely, only the buried and buried – leaves treatments had significantly lower photosynthetic rates than the shoots buried to half the height of the bare stem). In general, this analysis indicated that burial treatments and treatments where resources were removed had lower photosynthetic rates than unburied treatments or treatments where resources were added (<7% average difference).

The transpiration rate per unit leaf area was not significantly affected by treatment ($P = 0.100$), or the treatment by date interaction ($P = 0.805$), but date did have a significant effect on transpiration ($P < 0.001$; Figure 7.16b). The transpiration rate for buried shoots was 4% lower than the unburied shoots for both measurement dates.

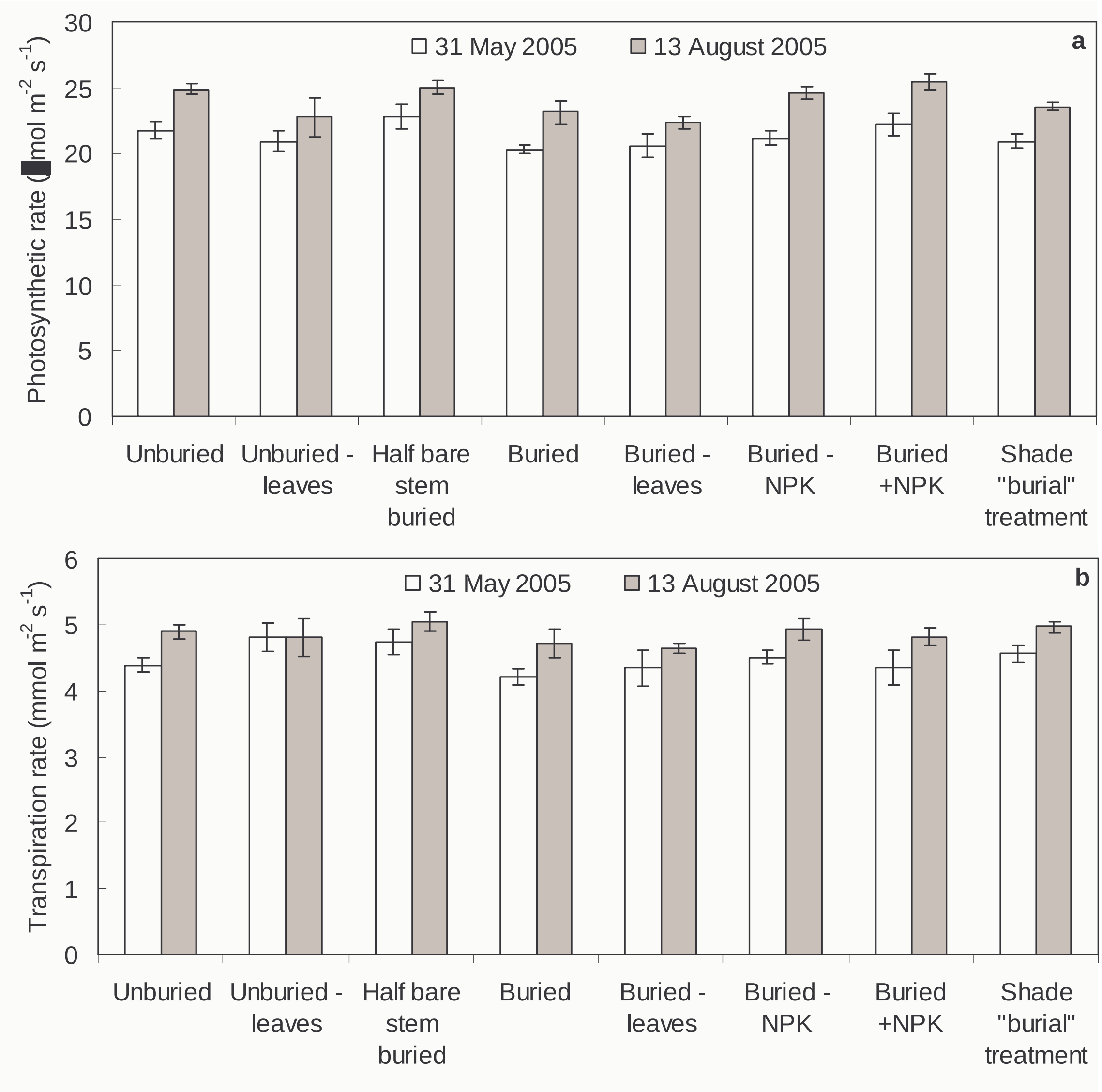


Figure 7.16: Photosynthetic (a) and transpiration (b) rates of shoots in eight treatments, measured on two dates. Bars represent means with standard errors of the mean (n ranged from 3 to 9 with a mean of 7).

What stimulates the burial response? Complete shade? Burial of bare stem?

An increase in internode length was the most characteristic response of buried shoots (pers. obs.). A clear response to burial was seen for the buried and shade treatments, where the length of internodes increased after application of the treatments (Figure 7.17). The shade treatment resulted in a doubling of internode length, while the buried treatment resulted in greater than tripling of internode length (Figure 7.17). The total stem length produced by buried and shaded shoots was significantly greater than that of unburied shoots, however burial of bare stem resulted in no difference from the unburied shoots (data not shown).

The mass of stem produced by the burial treatment was significantly higher than that of the other three treatments (Figure 7.18). The production of leaf mass was significantly reduced in the shade treatment relative to the other three treatments (Figure 7.18). All four treatments produced a similar total mass per shoot (Figure 7.18).

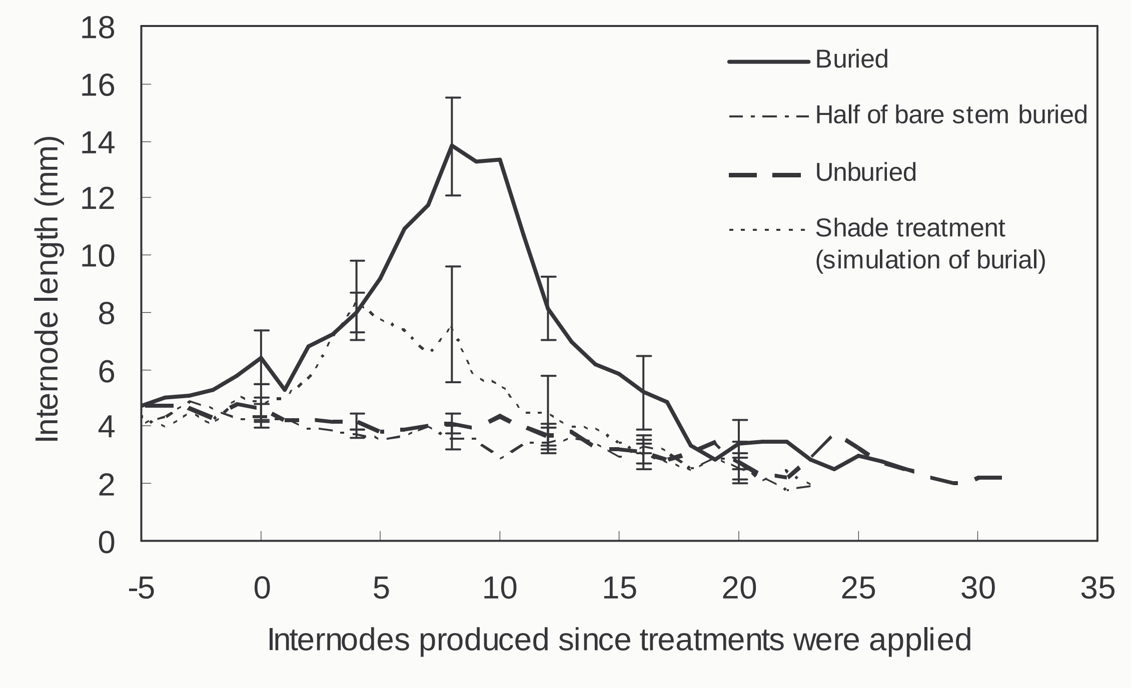


Figure 7.17: Lengths of internodes produced after treatments were applied (at zero). Standard errors of the mean are shown for each treatment.

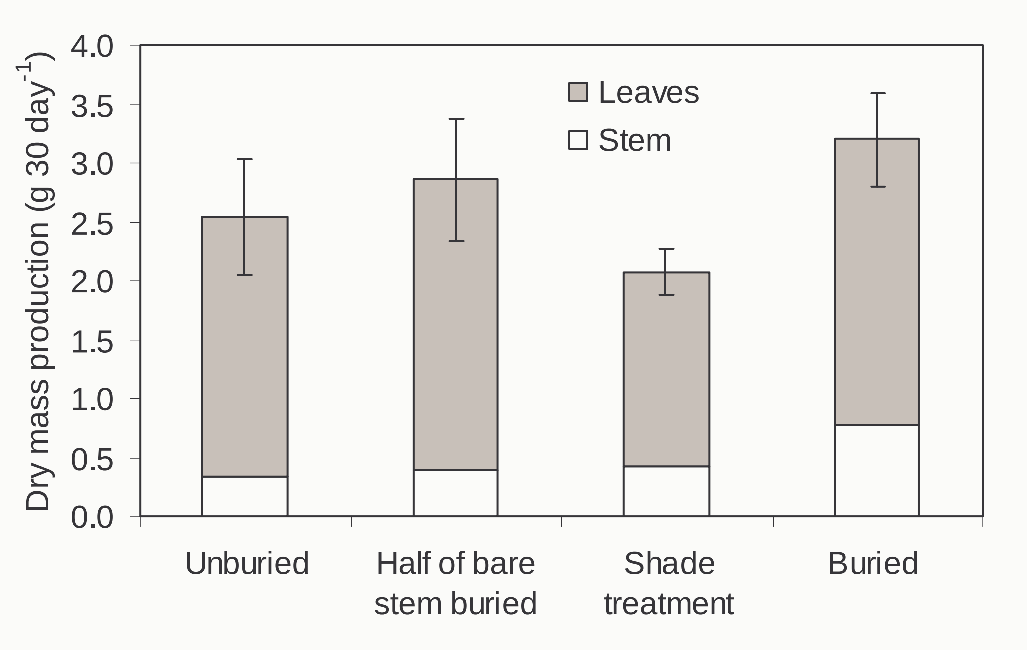


Figure 7.18: Leaf and stem mass production for unburied, buried, the shade-burial simulation treatment and for shoots which had half of the bare stem buried, but no leaf area. Standard errors of the mean are shown for each treatment.

DISCUSSION

Description of the growth of *S. plumieri* shoots after burial

The shoots of buried treatment recovered the buried leaf area within 81 days of burial (Figure 7.8). Comparisons of the vegetative growth of buried and unburied shoots indicated that growth increased after burial (e.g. Figure 7.10e). However, when reproductive tissue was taken into account the buried and unburied shoots no longer had significantly higher mass production, although a small increase in production remained (Figure 7.10e and f). The most marked stimulation of growth occurred in stem production, with a large increase in the lengths of internodes (Figure 7.17), net stem production (Figure 7.9b), and stem mass (Figure 7.10b). The production of leaf area and mass was less (Figure 7.9a and Figure 7.10c), or not affected by burial (Figure 7.12). Thus, even though the burial response of *S. plumieri* is clearly nutrient limited (Figure 7.11), this species is able to replace buried leaf area without compromising increases in stem production. How does *S. plumieri* access the internal and external resources to achieve this response?

A physiological model of the factors influencing the burial response of *S. plumieri*?

The results presented in this section allow the physiological model of burial response presented in Figure 7.1 to be re-evaluated and the relative magnitude of each factor incorporated into the model (Figure 7.19). This diagram is referred to as each mechanism is discussed.

New resources, remobilisation or allocation?

Does the growth stimulation of *S. plumieri* after burial derive from access to new resources, remobilisation from other organs, or changes in allocation? The data presented here, for two sets of experiments, indicate that the burial response of *S. plumieri* derives from a large variety of influences. Of primary importance is remobilisation of resources from buried (non-photosynthetic) leaves (Figure 7.12), allocation from reproductive sinks to stem production (Figure 7.9), and reduction in the carbon and nitrogen costs of stem and leaf production (Table 7.2; see summary in Figure 7.19). A reduction in reproductive allocation with burial is not universal, with some annual species showing the opposite trend e.g. *Cakile edentula* (Zhang and Maun, 1992; Zhang, 1996). The soil surrounding the buried stem, potentially could provide sufficient nitrogen to account for the entire burial response, although removal of available soil nitrogen did not have a large effect on the growth of the buried shoot. As shoots of this species are nitrogen limited, and do access the new soil volume after burial; this may be of some importance to the burial response (Figure 7.11). However, the freshly blown beach sand used to bury *S. plumieri* did not have a five times increased nitrogen content in comparison to stable-dune sand, as found by Fay and Jeffrey (1992).

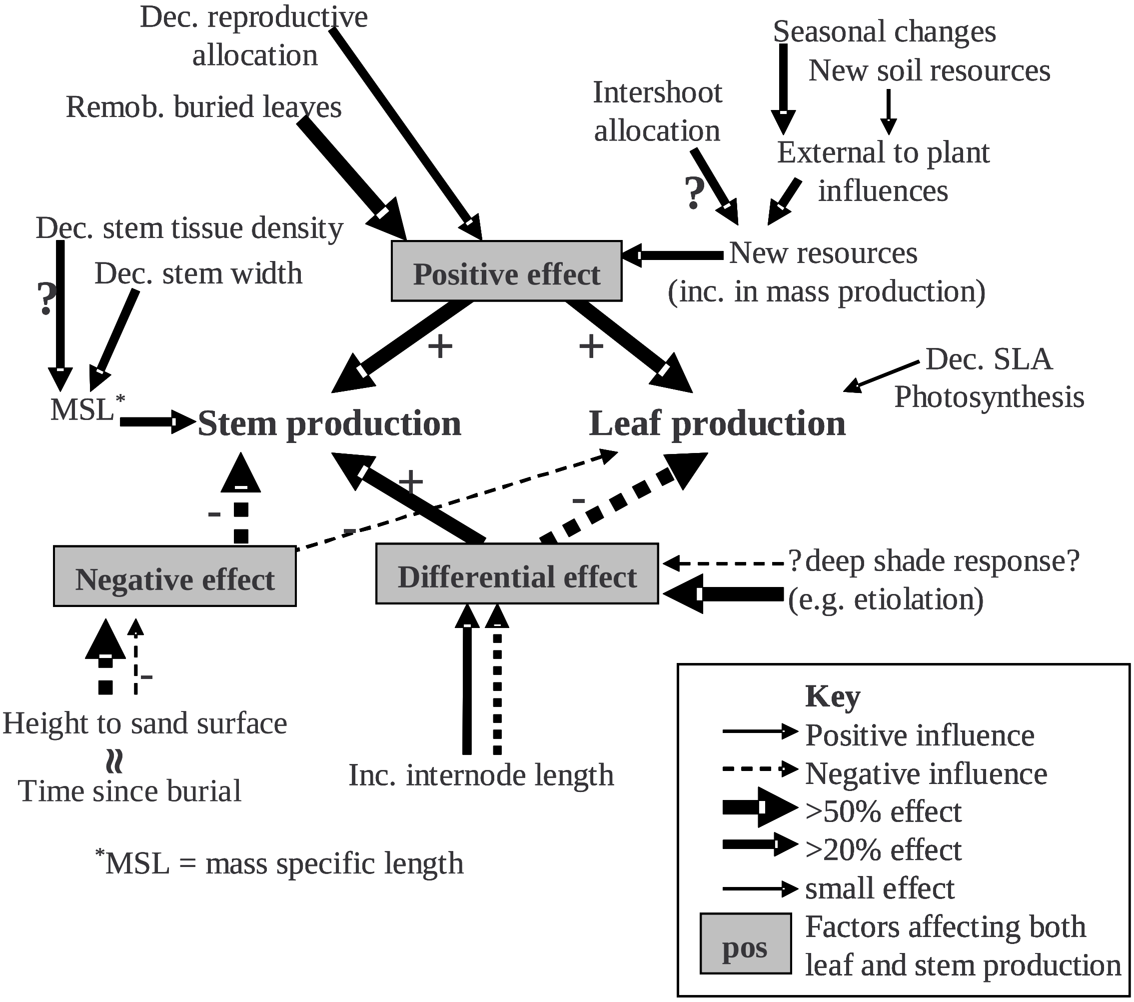


Figure 7.19: Physiological model of the response of *S. plumieri* to partial burial**.** The size and solidity of the arrows indicate the magnitude of the effect of each factor on the growth of stem and leaf production in response to partial burial. Three types of factors affected both stem and leaf production: a dual positive effect on both stem and leaf production, a differential effect where stem production was enhanced while leaf production was reduced, and a dual negative effect on both factors.

No study, including this one, takes size into account when considering plant response to burial. For example, the contrasting burial responses measured by Disraeli (1984) and Yuan *et al*. (1993) for *Ammophila breviligulata* may simply be a size related effect. Disraeli's field experiment was done on large individuals and burial resulted in a 1000% increase in biomass, while Yuan's greenhouse experiment was done on seedlings and a 230% increase in total plant mass was measured. Larger plants are capable of a number of physiological shifts that seedlings are not. Large underground stores of carbohydrates and reallocation from unburied ramets may allow large plants to have a greater response to burial. Alternatively, the differences between the experimental protocols may account for the differences between these studies, rather than plant size.

Buried leaves represented 66-80% of the total shoot nitrogen pool, and when removed buried shoots had reduced production of mass (Figure 7.12), decreased stem and leaf carbon and nitrogen costs (Table 7.4), and took longer to recover buried leaf area (Figure 7.8). Thus, remobilisation of resources from buried leaves is a vital aspect of the physiology of burial that has not been explicitly considered in the dune literature. These results indicate that the small increase in total biomass production after burial can mostly be accounted for without requiring that the shoot access new external resources (Figure 7.19).

The results presented here for *S. plumieri*, confirm those for other specialist dune species, that burial is required in order to maintain high growth rates (Eldred and Maun, 1982; *Ammophila arenaria* and *A. breviligulata*). In this case, burial induces higher net growth, while growth declines slowly after burial stops (Figure 7.5e and f).

Costs of producing tissue

The requirements for survival of a partially buried shoot are to replace lost photosynthetic leaf area, and in order to achieve this, the shoot must increase the rate of stem length production. Thus, the response to burial must be measured in units of photosynthetic capacity and stem length, while the resources required to achieve this are expressed in units of mass. The connection between these different units is the production costs of leaves and stems. Thus, a plant could achieve a response to burial via an increase in mass allocated to leaves and stem, or a change in allocation between leaves and stem, or an reduction in the mass cost of producing the leaves or stem. Clearly, the shoot changes allocation (Figure 7.10 and 7.14) and slightly increases mass production in response to burial (Figure 7.10), but does the buried shoot produce cheaper tissue?

The carbon and nitrogen costs of producing leaf area and stem length were reduced in the burial treatments, particularly under conditions were the resources available for growth were limited (Table 7.2). Greater leaf area per unit leaf mass, greater stem length per unit stem mass, and greater stem mass per unit mass of nitrogen contributed to the increase in stem length and leaf area production of the burial treatment (Figure 7.14). The increase in length per unit stem mass in buried treatments could be partially attributed to a decrease in stem width (Figure 7.15), while reduced stem tissue density, may or may not play a role (e.g. see Chapter 4). Contrary to the burial responses of the *Ammophila breviligulata, Calamovilfa longifolia* (Yuan *et al.*, 1993), and a number of dicot species (Perumal and Maun, 2006), *S. plumieri* shows a small, but consistent decrease in leaf photosynthetic rate and *SLA* with burial (Table 7.4 and Figure 7.16). Thus, increases in the photosynthetic capacity of the remaining leaves after burial cannot account for the changes in the growth of buried *S. plumieri*. This species consistently showed no effect of increased nutrient supply on photosynthetic rate (Figure 7.16). These results indicate that *S. plumieri* preferentially increases leaf production rather than change the quality of leaves. This type of response may not be general to most plants. The literature review suggests that many species respond to burial with and increase in photosynthetic rate. Other leaf characteristics are also positively affected by burial: *SLA* (Martinez and Moreno-Casasola 1996), as are related parameters such as chlorophyll content (Disraeli 1984, Perumal and Maun 2006; but see Zhang, 1996), photosynthetic rate and leaf thickness (Yuan et al. 1993, Permual and Maun, 2006). The ability of dune species to change leaf characteristics is further discussed in Chapter 11, relative to the findings of Vos *et al*. (2005).

A shift in the ratio of root mass to leaf area due to burial, does not result in higher transpiration rates in buried shoots (Figure 7.16b). Thus, there is no indication that higher mass flow would occur in buried shoots, resulting in greater nitrogen supply in the transpiration stream. However, this does not discount other consequences of this shift. Namely, a greater root to leaf area ratio would also allow greater active root absorption of soil nutrients relative to the aboveground biomass, even if transpiration did not increase. This idea was explored, but not included in this thesis.

What causes stimulation of growth under burial conditions?

The growth of buried shoots was similar to the growth of shoots which were placed in complete darkness, apart from the apical bud. This simulated burial demonstrated that a burial response could be induced in shoots, simply through the action of a treatment of darkness/shade. Thus, the increased internode length of buried shoots and increased allocation to stem mass is plausibly physiologically similar to etiolation of the species in darkness (e.g. Figure 7.16). However, the shade treatment did not result in an increase in biomass, as did burial (Figure 7.17). Thus the stimulated growth of buried shoots is not entirely a physiological consequence of the buried tissue being subjected to darkness. A soil medium may be required for the species to have a normal response to simulated burial. These results differ from those of Voesenek *et al.,* (1998), where *Ammophila breviligulata* was shown to have induced allocation shifts due to darkness treatments, but no increase in internode length. By comparison, *S. plumieri* displayed a clear increase in internode length in response to the darkness treatment (Figure 7.17). Thus, darkness induced more of the typical characteristics of the burial response of *S. plumieri* than for *A. breviligulata*, and is a good candidate for the induction mechanism for this species burial response (Figure 7.19).

Loss of photosynthetic capacity after burial

The burial of leafless bare stem was initially hypothesised to result in a burial response, as this mechanism would result in the shoot responding to burial prior to a reduction in photosynthetic capacity. This was not the case (Figure 7.16), as burial of the bare section of stem did not result in any detectable difference in growth relative to the unburied shoots. Thus, this bare stem, characteristic of the species (0-50cm) can be viewed as a buffer against burial of the leaves. This is demonstrated by Figure 7.18, where the entire leaf area of typical shoots is buried by 6 cm of sand accumulation. However the presence of the bare stem buffer segment allows the shoot to avoid much larger burial events (Figure 7.19).

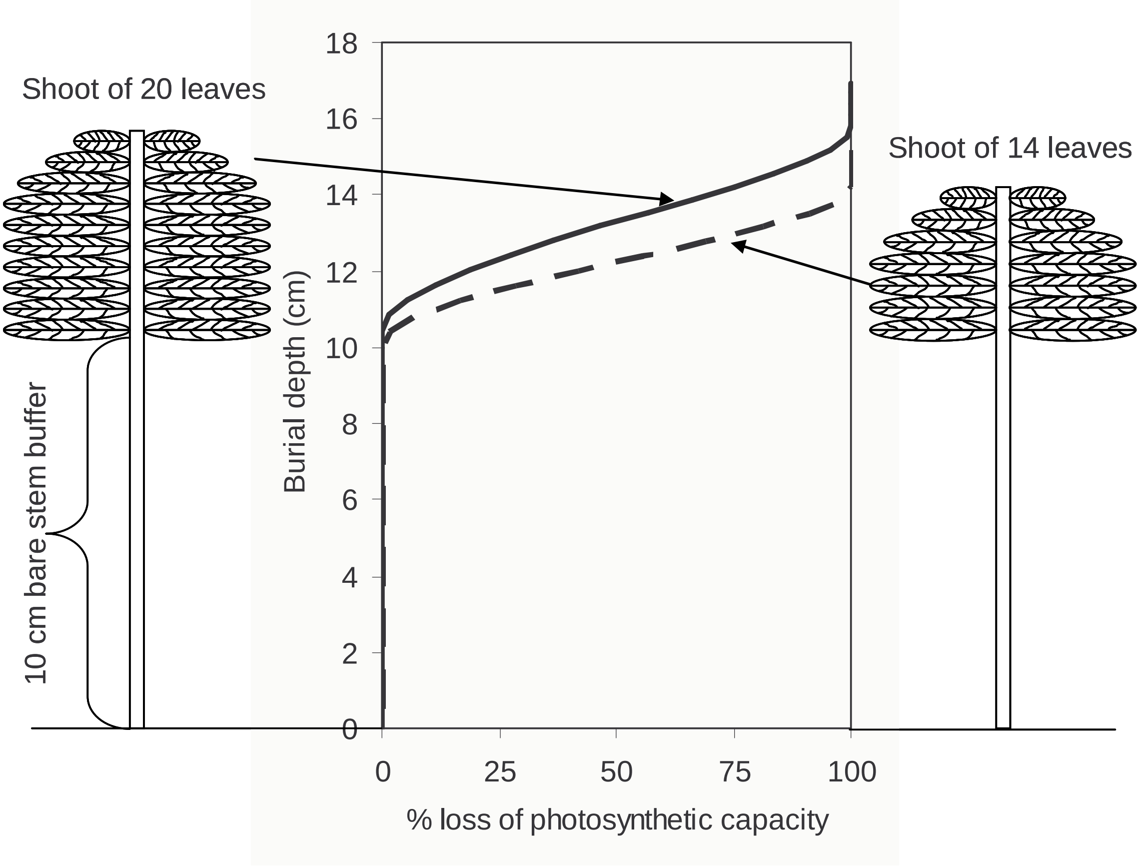


Figure 7.20: Loss of photosynthetic capacity for two modelled shoots of different size. The loss of photosynthetic capacity was modelled using the photosynthetic capacity curves shown in Figure 7.7b, and the changes in the internode lengths of unburied shoots in Figure 7.16, and a leafless (bare) stem segment of 10 cm.

The large increase in internode length after burial (Figure 7.16), could be viewed as advantageous in two different manners: firstly the leaves above the expanding internodes would be pushed away from the sand surface, and secondly this results in a longer length of stem bearing leaves (Figure 7.5b) and thus a less quick loss of leaf area upon subsequent burial (unlike the shoots in Figure 7.20).

Seasonality of growth and burial

The observed seasonality of the burial response corresponds to the high rates of summer burial that occurred at OWR (Figure 7.9). Thus, shoots of *S. plumieri* grow the fastest during the period of highest burial, at least for this particular site. In areas of higher winter burial the reduced growth during this season may be a limiting factor to the distribution of species. The lack vegetation in the extensive Alexandria dune field may derive from a high proportion (41%) of the strong sand transporting winds occurring during winter (Figure 2.1.13), combined with reduced growth of dune species during this season.

Summary

This section tested the relative contribution to the growth of a buried shoot of a large number of mechanisms of burial response. Early work in this field focussed on the contribution of single factors e.g. root pathogens (van der Putten *et al.*, 1988), soil nutrients (Willis, 1965; Fay and Jeffrey, 1992) to the stimulation of growth of partially buried plants. These external stress or resource based factors have been augmented with studies of internal (physiological) mechanisms of growth response e.g. allocation (Harris and Davy, 1988), changes in photosynthesis (Yuan *et al.*, 1993). The results of a quantitative analysis of the literature, and experiments in this study indicate that both external and physiological mechanisms contribute to the stimulation of plant growth after burial (see Figure 7.19 for a summary of these mechanisms). A number of new hypotheses were also developed, and were shown to account for a large portion of the burial response of *S. plumieri* (e.g. remobilisation of resources from buried leaves). This study highlights the need for further research into the physiological mechanisms accounting for the burial response of dune plants.

The following section further analyses the physiology of a number of dune species, with regard to the optimisation of nitrogen use in the production of carbon (Chapter 8). Chapter 9 proposes a model of the effect of varying plant biomass on burial responses of dune species. Finally, Chapter 10 demonstrates that some dune plants grow with a geometry that allows greater survival of burial, whilst requiring less stem production.

Chapter 8

The nitrogen cost of carbon fixation

Elaboration on concepts of photosynthetic- and whole-plant nitrogen use efficiency — Measurements of components of *NUE* — Calculation of optimality of nitrogen use for photosynthesis of dune plants — Strategies of nitrogen use in dune plants

INTRODUCTION

The dry mass production of dune plants is limited by the amount of soil nitrogen and the internally recycled nutrients that are available for growth (Chapter 5, 6 and 7). Concurrently dune plants must survive burial and these plants achieve this by having high rates of total dry mass production (Chapter 5 and 7). Thus, the efficiency of whole plant carbon fixation relative to the nitrogen used (*NUE*) is an important aspect of mobile-dune plant ecology. A large component of *NUE* is the relationship between leaf level photosynthesis and leaf nitrogen (photosynthetic nitrogen use efficiency; *PNUE*). For these reasons, this section attempts to review and synthesise concepts of *NUE* and *PNUE*, generating a mechanistic framework whereby the efficiency of dune plant *NUE* can be determined.

BACKGROUND AND THEORY

Production and nitrogen use occur at two scales in dune plants: 1) whole plant allocation and production characteristics of biomass and nitrogen, and 2) photosynthetic fixation of carbon and allocation of nitrogen between different components of the photosynthesis system. Each scale is dealt with separately here.

Whole plant nitrogen use: theoretical components and calculation

Whole plant nitrogen use efficiency (*NUE*) is comprised of two components, the rate of biomass production using a specific mass of nitrogen (nitrogen productivity), and the length of time that the nitrogen is present in the plant (mean retention time). Nitrogen productivity (P_N, g biomass g^{-1} N yr^{-1}) is defined as the biomass produced relative to the total nitrogen present in a plant (Berendse and Aerts, 1987). Thus,

$$NUE=P_N*MRT \qquad \text{eqn 8.1}$$

Where, *NUE* is the nitrogen use efficiency (g biomass g^{-1} N) and *MRT* the mean retention time (yr) of a molecule of nitrogen in plant tissue. Using this equation it can be seen that the *NUE* of a plant can be substantially increased by decreasing the nitrogen needed to produce plant tissue (i.e. increasing P_N), OR by enhancing the length of time that nitrogen stays in the plant, through remobilisation of nitrogen from senescing tissue, or having long tissue life spans (i.e. increasing *MRT*). As the roots, stems, leaves and reproductive structures have different life spans and nitrogen costs, the concepts of *MRT*, P_N and *NUE* vary depending on the organ under consideration. For instance, growth of stem represents near permanent sequestration of low quantities of nitrogen, while leaf senescence represents a considerable loss of nitrogen. Thus, whole plant *NUE* can be divided into leaf, stem, root and reproductive components, each of which has different significance with regard to fitness, accessing new nitrogen and fixing carbon.

As the majority of the biomass of large perennial dune plants is relatively inaccessible belowground, the *in situ* root components of *NUE* are difficult to measure. Thus, parameterising the equation for *NUE* for all tissue components is difficult. As a result various indicators of *NUE* have been suggested. Chapin (1980) suggested that *NUE* could be estimated as the inverse of plant nutrient concentration. Vitousek (1982) considered this to be an inadequate definition for perennial plants due to long term retention of a molecule of nitrogen due to remobilisation from senescing tissue (also see Berendse and Aerts, 1987). Vitousek (1982) suggested that aboveground *NUE* is most easily measured as the inverse of the nutrient concentration of litterfall, a definition that takes into account remobilisation of nutrients from senescing tissue. Most authors focus on leaf or litterfall nutrient dynamics as representative of plant *NUE*. This study attempts to parameterize the *NUE* equation specifically for the leaves as this component is: 1) most closely related to whole plant productivity, 2) easier to measure, 3) is comparable to much of the literature, 4) stem and root tissue components contain lower nutrient concentrations (Chapin, 1989), and 5) stem nutrients are not generally lost to litterfall in coastal dune plants, while leaf nutrients represent a quantitatively large loss. It is noted that seeds often contain the highest amounts of nutrients, and these are lost to the plant upon dispersal. This is a clear reduction the *NUE* of the plant (although an increase in fitness), and would reduce the nutrient pool available for the plant to respond to burial. The results of Chapter 7 demonstrated that buried plants had a reduced amount of reproductive mass (Figure

7.10d). Thus, dune plants appear to be able to increase *NUE* under burial conditions by reducing reproductive growth.

Biomass allocation/distributions

The nitrogen dynamics *within* the stem, leaf, root and reproductive tissue are partly responsible for determining whole plant *NUE*, while the allocation of nitrogen and biomass *between* each of these components is also an important aspect of *NUE*. In particular, allocation of carbon and nitrogen between the productive leaves or nutrient accessing roots is of great importance to growth. These allocational strategies were not further investigated due to the difficulty of working on deep rooted dune plant roots.

Leaf biomass *NUE*

The *NUE* of leaves has the most direct influence on the interaction between carbon gain and the use of nitrogen. In Appendix 3 a proxy measure of leaf *NUE* (NUE_{leaf}) is derived. The derived equation allows the relatively easy and useful parameterisation of leaf *NUE*:

$$NUE_{leaf} \approx \frac{P_{Nleaf} * L_{leaf}}{1 - R} \qquad \text{eqn 8.2}$$

Where P_{Nleaf} is the leaf nitrogen productivity (g biomass g^{-1} N yr^{-1}), L_{leaf} is the leaf longevity (yr), and R the proportion of the maximum leaf nitrogen lost in senescing leaves. This equation has the advantage of estimating the mean retention time of nitrogen in leaves by using the components: leaf longevity and nitrogen remobilisation. It also allows the calculation of NUE_{leaf} without using stable nitrogen isotopes or other cumbersome techniques.

Many studies have focussed on between species variation in leaf nutrient remobilisation, resorbtion proficiency and leaf longevity (Killingbeck, 1996, and studies cited in Aerts, 1996). Fewer studies use this data to estimate mean retention times and leaf P_N (Berendse and Aerts, 1987), although these are vital components of leaf and whole plant *NUE*. The remobilisation of nutrients from senescing leaves is an important component of *NUE*, due to the large potential loss of nitrogen incorporated in the nitrogen-rich leaf photosynthetic apparatus. Two measures of this mechanism have been suggested: leaf nitrogen remobilisation *efficiency* and leaf nitrogen remobilisation *proficiency* (Killingbeck, 1996). The efficiency of remobilisation (*R*) refers to the proportion of nitrogen in fully formed young leaves remobilised by senescing leaves. The proficiency of remobilisation is defined as the minimum level to which leaf nitrogen can be reduced in senescing leaves. Thus, a plant is said to be nitrogen efficient if the leaf nitrogen is considerably reduced upon senescence (>50% reduction) and proficient if the leaf nitrogen is reduced to levels lower than 0.7% (concentration by mass; Killingbeck, 1996).

Subsequently, other authors have pointed out that the calculation of remobilisation efficiency of nitrogen can be affected by reductions in the dry mass of the leaves upon senescence (van Heerwaarden *et al.*, 2003). In general, this effect results in an underestimate of leaf remobilisation efficiency, and thus this parameter may have greater importance than the data from the older literature may indicate (van Heerwaarden *et al.*, 2003). However, leaf remobilisation efficiency may be influenced by both reductions in dry mass of senescing leaves (concentrating the residual nitrogen) and transport of other compounds into these leaves (diluting the remaining nitrogen). While this may not be quantitatively important for most plants, this may be very important for dune species which accumulate salts in senescing leaves (e.g. Ripley, 2002). In the context of dune plants it is thus not possible to account for changes in leaf mass relative to leaf nitrogen content without budgeting the change in components of leaf biomass with senescence. Thus, the advice of van Heerwaarden *et al.*, (2003) is important to consider, but may prove difficult to implement for coastal dune plants.

Photosynthesis: the most basic formulation

The version of Fick's law that applies to photosynthesis is one of the most basic and insightful formulations of photosynthesis:

$$Flux = conductance * pressure.differential = A = g_{CO2} * (c_a - c_i) \quad \text{eqn. 8.3}$$

where A is the photosynthetic rate (flux), g_{CO2} the conductance of the stomata to diffusion by CO_2, c_a the ambient CO_2 concentration, and c_i the CO_2 concentration within the intercellular airspaces. At the most simple level, it can be seen from this equation that photosynthetic rate is determined by the diffusion of CO_2 into the leaf and the amount of photosynthetic apparatus present (responsible for the drawdown of CO_2 from c_a to c_i). Wright *et al.* (2003) elegantly demonstrated that plants maximise photosynthetic rates by investing in water use (g_{CO2}) and/or in nitrogen use (the enzymes needed to draw c_i down from c_a), and these investments corresponded to the major limiting factors present in the environments studied. As the photosynthetic apparatus uses a large amount of nitrogen it may be expected that plants growing in the water-available and nitrogen-limiting dune environment would maximise photosynthesis by: 1) increasing the g_{CO2} part of the photosynthesis equation, and 2) maximising the efficiency of nitrogen use. What follows is an in depth analysis of mechanisms whereby these two strategies may be realised.

The equation that described Fick's law (equation 8.3) has greater significance when analysing the stomatal and non-stomatal limitations to photosynthesis. Equation 8.3 describes CO_2 supply function of the CO_2 response curve: the concentration gradient from intercellular airspaces to the CO_2 supply of the atmosphere. By assuming that A and c_i are variables, this formula defines a diagonal line intersecting an experimentally determined relationship of A to c_i. The relationship of A to c_i represents the demand (concentration gradient) that the photosynthetic apparatus has for CO_2 at different A's, thus it is called a demand function. The supply function intercepts the x-axis at c_a (380 ppm) and has a slope of g_{CO2}. The intersection of the demand function and supply function define the photosynthetic operating point, usually determined by a photosynthetic spot measurement made under ambient conditions (it's not quite that simple, see Pammenter, 1989). The photosynthesis supply (diagonal lines) and demand functions (curved relationships) are illustrated in many figures through out this chapter (e.g. Figure 8.2, 8.3 and 8.6).

The equations of the form of equation 8.3 are generally applicable to many other situations, particularly the diffusion of CO_2 from the intercellular airspaces to the sites of photosynthesis (in the chloroplast). Resistance to diffusion within cells further limits photosynthesis and is termed internal (or mesophyll) resistance (=1/conductance, the symbol for conductance is g_i). Thus, photosynthesis operates most directly with c_c the chloroplastic CO_2 concentraion, and via g_i relates to c_i. The measurement of g_i (and thus c_c) is generally difficult, resulting in most studies reporting the next best thing, c_i.

Table 8.1: Definitions of variables and constants used in this section. The style, format and units of the photosynthetic parameters were used as given in von Caemmerer (2000), or alternatively Long and Bernacchi (2003).

Parameter	Value and unit	Description
Variables:		
NUE	g g^{-1}N	whole plant production (dry mass) per unit N present
P_N	g g^{-1}N yr^{-1}	biomass produced relative to the total nitrogen present in a plant
MRT	yr^{-1}	mean retention time of a molecule of N in plant tissue
NUE_{leaf}	g g^{-1}N	nitrogen use efficiency of the leaf tissue
P_{Nleaf}	g g^{-1} N yr^{-1}	leaf nitrogen productivity
L_{leaf}	yr	leaf longevity
R	dim'less	proportion of the maximum leaf N lost in senescent leaves
$PNUE$	µmolCO$_2$ mol^{-1} Ns^{-1}	photosynthetic nitrogen used efficiency
A	µmolCO$_2$m^{-2}s^{-1}	photosynthetic rate
N_{total}	gN m^{-2}	total nitrogen in a leaf expressed on a leaf area basis
N_{other}	gN m^{-2}	nitrogen in non-photosynthetic capacity
N_{PC}	gN m^{-2}	nitrogen in photosynthetic capacity
N_{PCR}	gN m^{-2}	nitrogen used directly in PCR cycle
N_{ETR}	gN m^{-2}	nitrogen used directly in electron transport
N_{LHC}	gN m^{-2}	nitrogen used directly in light harvesting
g_{st}	molH$_2$O m^{-2} s^{-1}	stomatal conductance to water vapour
g_{CO2}	molCO$_2$ m^{-2} s^{-1}	stomatal conductance to CO$_2$ (=g_{st}/1.6)
g_i	molm^{-2}s^{-1}bar^{-1}	conductance of CO$_2$ from intercellular airspaces to Rubisco
c_i	µmol mol^{-1}	CO$_2$ concentration in intercellular airspaces
c_c	µmol mol^{-1}	CO$_2$ concentration at sites of photosynthesis
$L_{stomatal}$	%	stomatal limitation of photosynthesis
$L_{internal}$	%	limitation of photosynthesis due to internal conductance
A_{max}	µmolCO$_2$m^{-2}s^{-1}	maximum rate of photosynthesis under normal conditions
A_{sat}	µmolCO$_2$m^{-2}s^{-1}	light saturated photosynthetic rate
A_c	µmolCO$_2$m^{-2}s^{-1}	Rubisco limited photosynthetic rate ($c_i < 200$)
A_j	µmolCO$_2$m^{-2}s^{-1}	RUBP regeneration limited photosynthetic rate ($c_i > 400$)
$PNUE_c$	µmolCO$_2$g^{-1}Ns^{-1}	*PNUE* of Rubisco limited photosynthetic rate
$PNUE_j$	µmolCO$_2$g^{-1}Ns^{-1}	*PNUE* of RUBP regeneration limited photosynthetic rate
V_{cmax}	µmolCO$_2$m^{-2}s^{-1}	Maximal rate of Rubisco carbon fixation
J	µmol e$^-$m^{-2}s^{-1}	Rate of electron transport from the light reactions (PSII: 4e$^-$~1CO$_2$)
J_{max}	µmol e$^-$m^{-2}s^{-1}	Maximal rate of electron transport
J_F	µmol e$^-$m^{-2}s^{-1}	Rate of electron transport measured using calibrated fluorescence
Γ_*	µmol mol^{-1}	CO$_2$ compensation point in the absence of R_d
R_d	µmolCO$_2$m^{-2}s^{-1}	Day respiration
Chl	µmol Chl m^{-2}	Chlorophyll *a* + *b* concentration
Constants:		
X	359molNmmol CO$_2^{-1}$ s	N used by Rubisco to fix a mole of CO$_2$ s^{-1} (Pons& Westbeek, 2004)
Y	0.316 µmol e$^-$m^{-2}s^{-1}	conversion of J to N_{ETR} (Evans, 1989)
Z	33.1 mmol m^{-2}	N conversion of J to N_{ETR} (Evans, 1989)
W	molN(mol Chl)$^{-1}$	conversion of Chl to N_{LHC} (Evans, 1989)
K_c	mbar	Maximal rate of Rubisco carboxylation
O	210 mmol mol^{-1}	atmospheric oxygen concentration
K_o	mbar	maximal rate of Rubisco oxygenation

Photosynthetic nitrogen use efficiency: theoretical components and calculation

The fixation of carbon derives from leaf photosynthesis, and as a result the productivity component of P_N relates to a number of leaf characteristics. These include leaf mass fraction of total biomass, specific leaf area, and the photosynthetic nitrogen use efficiency (*PNUE*; μmol CO_2 s^{-1} mol N^{-1}). The importance of photosynthetic nitrogen use efficiency is illustrated by the similar relationship that all C_3 plants share between maximum photosynthetic rate and leaf nitrogen expressed on a mass basis (e.g. Figure 1.1 of Field and Mooney, 1986). However, the nature of this relationship is affected by a large number of factors: light, temperature, VPD, CO_2, soil water content, nitrogen availability, enzyme kinetics, stomatal conductance, mesophyll conductance, within leaf anatomy, and within chloroplast nitrogen partitioning (Evans, 1989). Thus, optimisation of *PNUE* can vary between leaves, environments, species, and taxa with different phylogeny. The nitrogen-limited coastal dune plants may be able to maximise *NUE* through many different mechanisms including *PNUE*, adjustments to whole plant nitrogen allocation and use, and nitrogen productivity.

PNUE is defined as:

$PNUE = A/N_{total}$ eqn. 8.4

Where A is the photosynthetic rate, and N_{total} the total nitrogen in a leaf, both expressed in the similar units relative to leaf area or mass (Field and Mooney, 1986).

The carbon fixed by a given total leaf nitrogen investment is determined by the partitioning of nitrogen in the leaf. The innumerable leaf enzymes, proteins and nitrogenous compounds can be divided into two functional groups: nitrogen containing compounds that determine the photosynthetic capacity (N_{PC}: mmol m^{-2}), and the compounds that are not directly involved in carbon fixation (N_{other}: mmol m^{-2}). The nitrogen directly determining the photosynthetic capacity can be further divided into the functional photosynthetic units of: light harvesting proteins (N_{LHC}: mmol m^{-2}), electron transport (N_{ETR}: mmol m^{-2}), and photosynthetic carbon reduction cycle enzymes (N_{PCR}: mmol m^{-2}; Figure 8.1). The allocation of nitrogen to these four divisions of leaf nitrogen has been estimated and studied by a range of authors including Evans (1989), Poorter and Evans (1998), Pons and Westbeek (2004), Westbeek *et al.* (1999), and Pons *et al.* (1994). The investment in these nitrogen fractions determines the maximum rate of Rubisco carboxylation (V_{cmax}), and the light saturated rate of electron transport (J_{max} or J under non saturating conditions), these in turn determine the optimal photosynthetic operating point maximising A relative to nitrogen use. Other factors such as: k_{cat}, g_{st}, g_i, R_d, also influence the realisation and position of the photosynthetic operating point and thus *PNUE* (see Figure 8.1; some of these factors are described below).

Decreases in the nitrogen invested in non-photosynthetic capacity (N_{other}), and the optimal apportioning of nitrogen between the enzyme components of photosynthetic capacity would result in increases in *PNUE*. Similarly, minimisation of the nitrogen invested in photosynthesis related enzymes, such as carbonic anhydrase (related to internal CO_2 transfer conductance; Bernacchi *et al.*, 2002), would result in maximisation of *PNUE*. Alternatively, leaves with higher photosynthetic rates attained by increasing stomatal conductance, optimising light saturation, and increasing photosynthetic capacity nitrogen (N_{PC}) relative to N_{total} would result in higher *PNUE*. A literature and theoretical review of these mechanisms of increasing *PNUE* follows, and is outlined in Figure 8.1.

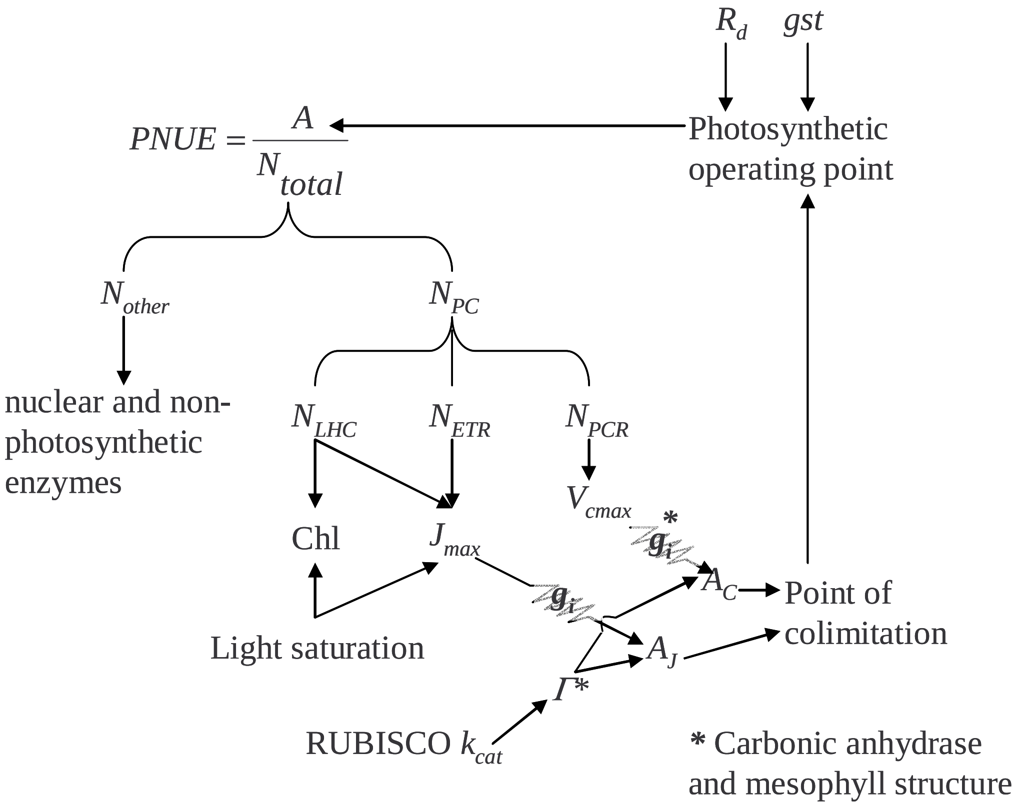


Figure 8.1: Components of leaf photosynthetic nitrogen use efficiency, for a C_3 plant. See text for explanation. A similar diagram was developed by Poorter and Evans (1998; Figure 8).

Mechanisms of increasing *PNUE*

Six mechanisms of increasing *PNUE* are outlined in detail below:

1) Colimitation point optimisation

The photosynthetic rate of a leaf is determined by the Rubisco and RuBP regeneration limitations to photosynthesis at low (<200 μmol mol^{-1}) and high c_i concentrations, respectively. The point at which these two major photosynthesis limiting processes are of equal importance is called the point of colimitation. This point of colimitation is defined by the ratio of V_{cmax} to J, the major determinants of Rubisco (A_c) and RuBP (A_j) limited photosynthetic rates. As V_{cmax} and J relate to nitrogen invested in photosynthetic carbon reduction and electron transport enzymes and proteins, the point of colimitation is determined by the division of nitrogen within the photosynthetic apparatus. The photosynthetic operating point is then determined by the c_i, as this determines which factor is most limiting given a set nitrogen distribution.

Further insight into the optimal partitioning between the photosynthetic carbon reduction cycle (PCR) and electron transport limited photosynthetic rates can be attained by considering the biochemical equations of Rubisco (A_c) and RuBP (A_j) limited photosynthesis (von Caemmerer, 2000):

$$A_c = \frac{V_{c\max} * (c_i - \Gamma *)}{c_i + K_c * (1 + O / K_o)} - R_d \qquad \text{eqn. 8.5}$$

and,

$$A_j = \frac{J * (c_i - \Gamma *)}{4 * c_i + 8 * \Gamma *} - R_d \qquad \text{eqn. 8.6}$$

From these equations, the ratio of V_{cmax} to J that results in colimitation of A by both Rubisco and RuBP regeneration for a specific c_i (or stomatal conductance) is found by setting A_j and A_c equal to each other and solving for the ratio:

$$\frac{V_{c\max}}{J} = \frac{c_i + K_c * (1 + \frac{O}{K_o})}{4 * c_i + 8 * \Gamma *} \qquad \text{eqn. 8.7}$$

Similarly, the point of colimitation can be found by setting A_c=A_j and solving for c_i.

Two solutions are possible, as the A_c and A_j to c_i relationships intersect at two points (at roughly ambient values of c_i and at c_i values below the CO_2 compensation point). The point of interest is:

$$c_i = \frac{J * K_c * (1 + \frac{O}{K_O}) - 8 * V_{c\max} * \Gamma *}{4 * V_{c\max} - J} \qquad \text{eqn. 8.8}$$

(a similar equation is found in von Caemmerer, 2000). Thus, the c_i under normal photosynthetic conditions determines how close to the colimitation point the plant operates and therefore determines the *PNUE*. However, the nitrogen costs of electron transport and PCR differ, and may be non-proportional (allometric) in nature. Therefore, further analysis was attempted by parameterising the nitrogen costs of these two processes using widely used empirical constants (see Appendix 4 for derivation of these).

PNUE is related to photosynthetic rate and leaf nitrogen components by:

$$PNUE = \frac{A}{N_{total}} = \frac{\min(A_c, A_j)}{N_{other} + N_{PCR} + N_{ETR} + N_{LHC}} \qquad \text{eqn. 8.9}$$

where $\min(A_c, A_j)$ represents the most limiting process for the operating c_i value. As V_{cmax} and J relate linearly to N_{PCR} and N_{ETR}, respectively, these relationships can be used to determine the *PNUE* for all c_i values on a CO_2 response curve. The nitrogen components can be estimated by the following equations:

N_{other} = constant = assumed to be 20% of N_{total},
$N_{PCR} = x*V_{cmax}$ (see Table 12.1 of Appendix 4),
$N_{ETR} = y*J/4 + z*\mathrm{Chl} - N_{LHC}$ ($N_{ETR} = 0.316*J/4+33.1*\mathrm{Chl} - N_{LHC}$; Evans, 1989), and
$N_{LHC} = w*\mathrm{Chl}$ (w = 41 mol N (mol Chl)$^{-1}$; Evans, 1989).

By substitution of these equations into equation 8.9 it can be shown that *PNUE* is the minimum of:

$$PNUE_c = \frac{[V_{c\max} * (c_i - \Gamma*)] / [c_i + K_c * (1 + O / K_o)] - R_d}{N_{other} + x * V_{c\max} + y * J + z * Chl} \qquad \text{eqn. 8.10}$$

and

$$PNUE_j = \frac{[J * (c_i - \Gamma*)] / [4 * c_i + 8 * \Gamma*] - R_d}{N_{other} + x * V_{c\max} + y * J + z * Chl} \qquad \text{eqn. 8.11}$$

Thus, *PNUE* is largely dependent on the operating c_i and the invested nitrogen that determines V_{cmax} and J.

These calculations and assumed values for V_{cmax}, J and Chl were used to generate the illustrative Figure 8.2. From Figure 8.2 it is clear that:

1) for a given nitrogen investment in V_{cmax} and J, *PNUE* is maximised by a infinite stomatal conductance,
2) *PNUE* is less sensitive to changes in stomatal conductance (and c_i) at values of c_i above the point of colimitation,
3) if the photosynthetic operating point is not positioned at the colimitation point of Rubisco and RuBP regeneration activity, then one of these two processes has an excess allocation of nitrogen to that which is used.

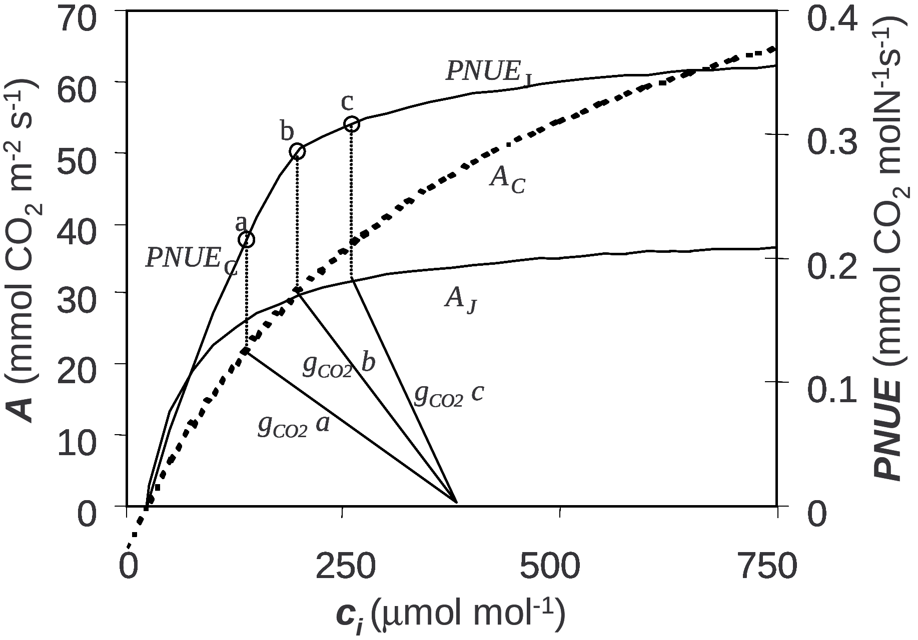


Figure 8.2: Illustration of the relationship between *PNUE* and c_i for a set ratio of V_{cmax} to J. The lower two curves represent the Rubisco (A_C) and RuBP regeneration (A_J) limited portions of the CO_2 response curve. The upper curve indicates the *PNUE* measured as a function of c_i for the CO_2 response curve using assumed nitrogen allocations (see above for an explanation). The diagonal lines represent the photosynthesis supply functions at three different stomatal conductances. Thus, the three stomatal conductances result in different *PNUE*'s for Rubisco limited (a), colimited (b), and RuBP regeneration limited (c) portions of the CO_2 response curve. *PNUE* was determined as the minimum of A_c/N_{total} and A_j/N_{total}. A_c and A_j were determined from values for V_{cmax} and J, and the assumed biochemical constants of von Caemmerer (2000). N_{total} ($=N_{LHC}+N_{PCR}+N_{ETR}+N_{other}$) was estimated from the relationships of Pons and Westbeek (2004) and Evans (1989) relating V_{cmax} to N_{PCR} and J to N_{ETR}, respectively. N_{other} was given an arbitrary value of 20% of N_{total}.

The CO_2 assimilation rates of V_{cmax} and J_{max} can be calculated relative to the approximate cost of nitrogen invested in N_{PCR} and N_{ETR}, by using the constants given in Appendix 4 (Table 12.1). Rubisco carboxylation (V_{cmax}) has a constant nitrogen cost of 0.313 mol N_{PCR} $mmol^{-1}$ CO_2 s for all values of V_{cmax}. The CO_2 equivalent of RuBP regeneration ($J_{max}/4$) has an allometric cost of nitrogen, decreasing from low costs towards 0.316 mol N_{ETR} mmol^{-1} CO_2 s at high values of J_{max}. Thus, investment in N_{ETR} can be considerably more cost effective for leaves with low J_{max}'s. This would suggest that leaves of low electron transport capacity may benefit from a relative overinvestment in electron transport capacity, as this component is relatively cheaper than N_{PCR}. As electron transport capacity increases the leaf may invest in both photosynthetic compartments and tend toward colimitation as the costs of the two processes become similar.

These considerations demonstrate important points about the optimisation of carbon fixation at the expense of both nitrogen and water, and are partially defined by the light intensity incident on the leaf. However, these considerations are simplified and not mathematically rigorous: the reader is referred to Buckley *et al.* (2002; pp. 648) for an excellent, but complex, demonstration of the mathematics of joint nitrogen and water use optimisation of photosynthesis. The interactive effects of light intensity, transpiration, leaf temperature, and more broadly water and nitrogen use must all be taken into account before suggesting reasonable *PNUE* optimisation strategies (Buckley *et al.*, 2002), and thus the role of intuition may be limited.

2) Rubisco characteristics

In many plants the photosynthetic operating point falls on the initial V_{cmax} (Rubisco) limited portion of the CO_2 response curve, and thus modifications of the components V_{cmax} would influence photosynthetic rate and *PNUE*. V_{cmax} is largely determined by the concentration and activation state of Rubisco, and the influence of the kinetic constants of Rubisco on V_{cmax} are thought to be relatively unimportant when modelling photosynthesis (von Caemmerer, 2000). Hence, the majority of photosynthesis modelling studies use a standard kinetic constant from tobacco plants measured by von Caemmerer *et al.* (1994) or Bernacchi *et al.* (2003). The kinetic properties of the carboxylation activity of Rubisco are relatively conserved in many C_3 plant species (a coefficient of variation of 16%; Yeoh *et al.*, 1981). Increasing the carboxylation kinetic properties of Rubisco, and especially reducing the oxygenation properties, would result in increased V_{cmax}, and *A,* or *PNUE* (Long *et al.*, 2006). While this mechanism is plausible for the species studied here, it was not further investigated, and similar to many studies forms an initial assumption of the investigation.

3) Stomatal and internal CO_2 transfer conductance limitations

The diffusion of CO_2 from the atmosphere to Rubisco is restricted by two major resistances: stomatal and internal (mesophyll) resistance. Stomatal and internal resistances are more conveniently expressed as in units of conductance (1/resistance; g_{CO2} and g_i, respectively) as these are more linear predictors of the quantities of diffusion. The diffusion of water vapour out of the leaf is similarly limited by the stomata and is used to determine the conductance of the stomata to CO_2 (g_{CO2}) as these two conductance's differ by a proportion of 1.6 ($g_{CO2} = g_{st}/1.6$; assuming the influence of boundary layer conductance is negligible). In many places within this section only g_{st} is reported, but is strictly proportional to g_{CO2}. Interestingly, the value for g_{CO2} calculated from g_{st} technically represents the conductance of CO_2 to the sites of evaporation, and not the sites of CO_2 uptake in the mesophyll (Farquhar and Sharkey, 1982). Fortunately for ecophysiologists, the difference between this estimated g_{CO2} and the actual g_{CO2} is thought to be minor (thus CO_2 differs by less than 10 ppm between these two sites; Farquhar and Sharkey, 1982).

Clearly, an increased stomatal or internal conductance to CO_2 would result in greater CO_2 concentrations at the site of photosynthesis enhancing the photosynthetic rate, and thus *PNUE*. However, the degree to which the photosynthetic rate increases is dependent on the slope of the CO_2 response curve, and the nitrogen partitioning between the two limiting processes represented by V_{cmax} and J_{max}. A further influence of nitrogen partitioning on internal conductance limitation may be the allocation of nitrogen to carbonic anhydrase, a factor determining the internal conductance (Bernacchi *et al.*, 2002). Thus, photosynthetic nitrogen use efficient plants may have high stomatal conductances (and low stomatal limitations), effectively fixing carbon at the expense of the freely available water, but minimising nitrogen use.

The stomatal limitations to photosynthesis are estimated by the standard techniques of Farquhar and Sharkey (1982) illustrated in Figure 8.3. In a similar manner, photosynthetic limitation imposed by low internal conductances can also be calculated.

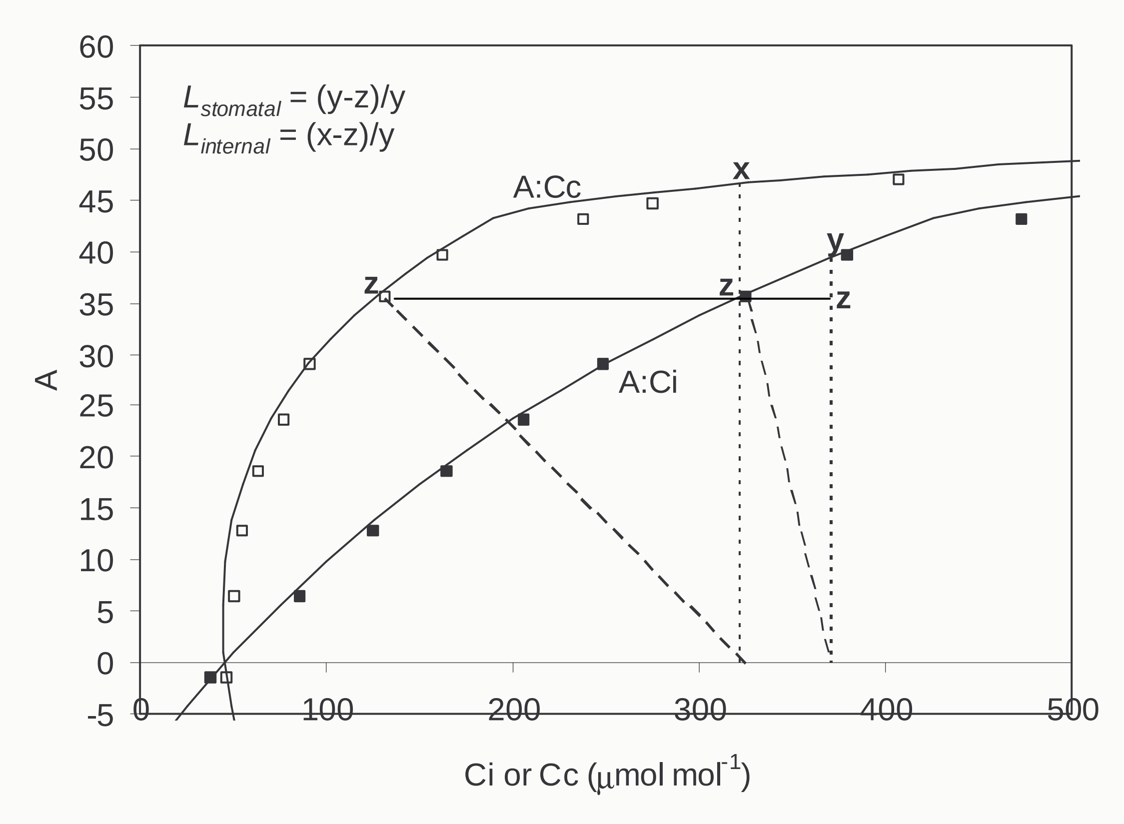


Figure 8.3: The calculation of the limitations to photosynthesis that result from stomatal and internal (mesophyll) conductance. Shown is a typical CO_2 response curve relating *A* to c_i (solid symbols), including the supply function of CO_2 (dashed lines), connecting the ambient CO_2 concentration (c_a) with the measured photosynthesis and c_i values of a leaf at ambient conditions. The slope of this line is the stomatal conductance (but see Pammenter, 1989). Stomatal limitation is calculated as the difference in photosynthetic rates measured under ambient conditions, and the predicted photosynthetic rate at infinite stomatal conductance (y) relative to y. The value for y was predicted using a von Caemmerer-type fit to the presented data and using a c_i of 380 ppm (ambient c_a). The response of *A* to c_c (open symbols) includes the calculation of the internal conductance limitation to photosynthesis (dotted line) and was calculated as the difference between the photosynthetic rate at ambient conditions and the photosynthetic rate where c_c is equal to c_i (x). In order to plot the A to c_c curve, a g_i value was calculated using the variable J method of Harley *et al.* (1992a), and using this a c_c value estimated for each point ($c_c = c_i - A/g_i$). Actual photosynthetic rates and the interpolated curves were then plotted against each estimated c_c value. See methods for details of the calculation of c_c and g_i.

4) Distribution of nitrogen between non-photosynthetic and photosynthetic processes

The relationship between N_{total} and photosynthetic rate has a clear intercept on the N_{total} axis (Field and Mooney, 1986). The intercept has been interpreted as nitrogen invested in non-photosynthetic capacity (N_{other}; Field and Mooney, 1989). The presence of this intercept means that *PNUE* does not scale directly proportionately to A/N_{PC}, but is maximised at higher ratios of N_{PC} to N_{other} (see example in Figure 8.4).

Possible mechanisms that maximise *PNUE* through a high N_{PC} to N_{other} ratio are suggested here:

1) Larger mesophyll cells, with more or larger chloroplasts would result in higher cellular *PNUE*'s due to a shift from N_{other} to N_{PC} (although a small non-photosynthetic cellular machinery may set limits on the amount of associated photosynthetic machinery),
2) Greater amounts of photosynthetic mesophyll cells relative to epidermal and other non-photosynthetic tissue would result in higher leaf *PNUE*'s, particularly thicker leaves (although there are tradeoffs' between photosynthesis and water supply by vasculature or water loss by reduced amounts of epidermis),
3) Leaves with lower levels of polyploidy would have reduced amounts of nuclear nitrogen relative to photosynthetic nitrogen,
4) Leaves with fewer nitrogen-rich defence compounds (Craine *et al.,* 2003), osmoticants (Hasegawa *et al.,* 2000), cell walls (Onoda *et al.,* 2004) and non-photosynthetic nitrogen containing metabolites,

Unlimited increases in *PNUE* by increasing N_{PC} are prevented by a number of factors. One of these may be shading limitation of the light reaching chloroplasts at high allocations to photosynthetic capacity. In an analogous manner to the whole plant models of Hirose (1984), leaf level *PNUE* may become non-linear at high N_{total}'s, resulting in clear optimal *PNUE*'s, above which the plant becomes light limited (see Figure 8.4b for a graphical explanation).

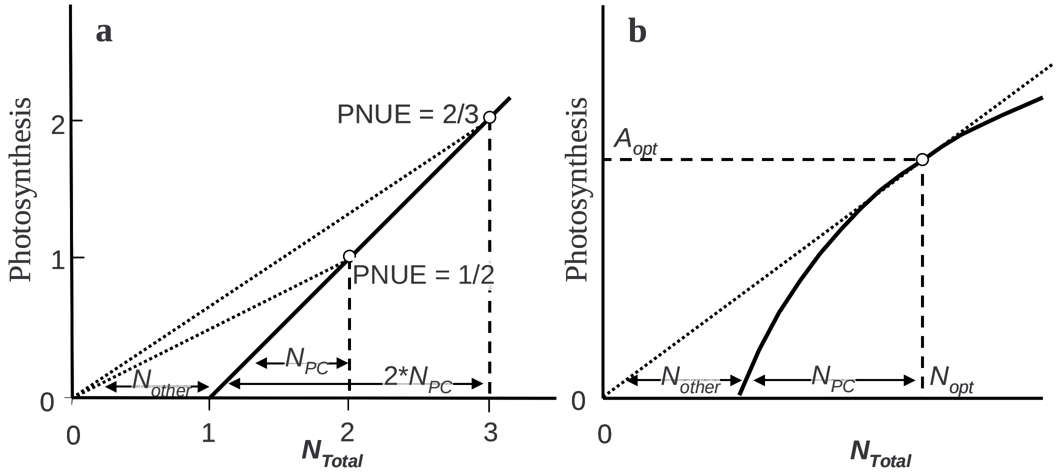


Figure 8.4: Illustrations of the calculation of *PNUE* (dotted lines) as the slope of the line from the origin to the measured photosynthetic rate (A) and total leaf nitrogen (N_{total}), where the relationship between A and N_{total} is linear (a) or non-linear (b) (bold lines). As a result of the allometric trajectories of A to N_{total}, for both cases, increases in N_{total} result in greater *PNUE*, although for the non-linear relationship *PNUE* peaks at N_{opt}. For the first figure *PNUE* is evaluated for two points, the second with twice the amount of N_{PC}, and consequently higher *PNUE*.

5) Leaf nitrogen allocation, light intensity and saturation

The integrated photosynthetic rate for a day (or leaf lifespan) is a better ecological measure of leaf carbon gain than A_{max} (Field and Mooney, 1986). Thus, *PNUE* measured as A_{max}/N_{total} is a measure of the maximum potential *PNUE* of a leaf under specific light, temperature, VPD, and hydraulic conditions. In particular, the light intensity and saturation of a leaf plays a large role in determining *PNUE*. Typically, *PNUE* is measured at full sunlight, and assumed to be light saturated. Greater capacity for photosynthesis above full sunlight implies that the leaf is either: 1) functioning in an ecological setting with additional sources of light (e.g. reflection off sand, Greaver and Herbert, 2004), 2) true light saturation is not a normal occurrence, but leaf maximises the daily integrated photosynthesis by having a special distribution of light capture pigments, or 3) that leaf nitrogen is not optimally invested in the light harvesting apparatus.

6) Respiration

Respiration on a whole plant level occurs through out the day and night, and uses large amounts of photosynthate to provide the energy for growth and active metabolism. Thus, whole plant *NUE* is maximised when plants have reduced respiration rates of all organs. *PNUE* is affected by leaf respiration in the light (R_d). This parameter is typically small relative to the photosynthetic rate (<10%), however it increases at a higher rate than any other photosynthetic parameter with temperature (von Caemmerer, 2000). Thus, the high light intensities that dune plants are exposed to may result in higher leaf temperatures and R_d's relative to many other species. Reduction of R_d would have a small positive effect *PNUE* (see equations 8.10 and 8.11). While leaf respiration may be low to reduce costs of maintaining tissue, the respiration of all tissues would be high under conditions of fast growth in response to burial.

Other mechanisms of increasing *PNUE*

A number of other mechanisms of increasing *PNUE* are important to note. CO_2 concentrating mechanisms along with increased Rubisco specificity to CO_2 correspond to high photosynthetic rates with low nitrogen investment in C_4 plants (e.g. Field and Mooney, 1989). Few of the species present on South African dunes have C_4 physiology. Thus, while C_4 photosynthesis may confer obvious advantages to dune plants, it appears that there are other reasons why species with this physiology are not more common along the Eastern Cape coast.

AIMS

The broad aim of this chapter was to assess the optimality of all levels of dune plant *NUE*. Due to practical constraints (time and limitations of IRGA's) only three of the study species were selected for this chapter. The following reasoning was used to select *Arctotheca populifolia*, *Scaevola plumieri* and *Myrica cordifolia* as potentially the most interesting species. *A. populifolia* and *S. plumieri* share an environment with high light, available water, but are nitrogen limited whilst requiring a high productivity to survive burial (Chapter 2, 4, 5, 6 and 7). In such circumstances it would be beneficial for these species to optimise whole plant *NUE* by increasing growth at the expense of light and water use, whilst minimising the use of nitrogen. At the other extreme, *M. cordifolia* shares space with a higher biomass of competitors, possibly limiting light and water more than the two mobile-dune species (Ripley and Pammenter, 2004b). This species may also have a reduced need to optimise nitrogen use due to an actinorhizal nitrogen-fixing symbiosis, which may contribute to this species displaying no response to nutrient addition (Chapter 5). Thus, *M. cordifolia* may show a relative lack of optimisation with regard to productivity and nitrogen, but may show a greater optimisation of light, phosphorus and water use. Data for *M. muricata* was included for whole plant *NUE*, but not photosynthetic measurements.

The aims of this chapter were to measure:

1) whole plant, leaf level and photosynthetic nitrogen use of two nitrogen-limited mobile-dune species and the nitrogen-fixing *M. cordifolia*,
2) the efficiency of these processes comparing the contrasting species, and
3) the degree to which mechanisms are used that enhance productivity or photosynthesis, but minimise nitrogen use.

METHODS

Leaf senescence, longevity and nitrogen remobilisation

Shoots of seven individuals of each species were harvested at the start of a year-long monitoring experiment at OWR (427 days from 7 February 2004 to 9 April 2005). Leaf nutrient contents were measured on the youngest fully expanded leaves (nitrogen: Kjeldahl analysis; phosphorus: Bray extraction and molybdenum blue colorimetric analysis; potassium: ammonium acetate extraction with atomic absorption spectroscopic analysis; Matrocast Laboratories, Capetown). By marking cohorts of leaves on adjacent shoots with coloured wire, leaf production and leaf longevity (= leaf number per stem * 1/production rate of leaves) were measured for all species on 8 occasions during the 427 day measurement period. Leaf nitrogen resorption efficiency (*R*; Killingbeck, 1996) was measured as the percentage reduction in leaf nitrogen content from the youngest fully expanded leaf to the last (senescing) leaf on a shoot. Resorption proficiency (Killingbeck, 1996) was measured as the minimum level to which nitrogen content was reduced in a senescing leaf. These two parameters were measured for five shoots on the youngest fully expanded leaf and the last senescent leaf on shoots of *A. populifolia* and *S. plumieri*. Numerous young or senescent leaves were harvested from five individuals of the two small-leafed species, *M. cordifolia* and *M. muricata*. The resorption efficiency estimated from these leaves was used to calculate the mean retention time (*MRT*) of nitrogen in the shoot (Berendse & Aerts, 1987) by the formula: *MRT* = leaf longevity/(1-*R*) (adapted from Poorter & Garnier, 1999, see Appendix 3). This assumes that resorption of nitrogen from senescing leaves supplies only the production of new leaves. This is likely to be a satisfactory simplification as stems do not senesce and have a considerably lower proportion of the total shoot nitrogen relative to the leaves (Chapin, 1989; and this study, data not shown). Prior to harvesting, light saturated photosynthetic rates and photosynthetic nitrogen use efficiency were measured on the youngest fully expanded leaves of *A. populifolia* and *S. plumieri* and young shoots of *M. cordifolia* and *M. muricata*, using the same gas exchange apparatus as mentioned below (pp. 237) and under similar environmental conditions.

Study Site for leaf nitrogen partitioning measurements

Gas exchange and *PNUE* measurements were made from the 20 to 22 March 2007, on the dune succession at the mouth of the Buluga river, Glengariff, Eastern Cape Province, South Africa (see Chapter 1 for more details). This dune system is comparable in species composition and exposure to the OWR and Cintsa West study sites. The reason this site was chosen was the convenient road access needed to transport the large quantities of equipment.

CO_2 response curves

The CO_2 response curves of young fully expanded leaves of *A. populifolia* and *S. plumieri* were measured using a pulse amplitude modulated fluorescence cuvette attached to a LI-6400 photosynthesis system (LI-COR, Inc., Lincoln, NB, USA). To allow measurement of the small (< 1 cm^2) leafed *M. cordifolia*, shoots of approximately 15 cm^2 leaf area, were measured using a transparent LICOR 6400-05 conifer chamber. Once the leaf was acclimated to the leaf chamber a gas exchange measurement was taken under ambient conditions. This measurement was intended as an indication of the operating photosynthetic rate of the species *in situ* for comparison with the CO$_2$ response curve data. The CO$_2$ response curve measurement protocol was adapted from Long and Bernacchi (2003). The CO$_2$ reference was reduced from the ambient equivalent at 390 μmol mol^{-1} to 325, 250, 200, 150, 100 and 50 μmol mol^{-1}, in a short period, only allowing for the CO$_2$ sample to stabilise between matching and measuring the photosynthetic rate (Long and Bernacchi, 2003). The CO$_2$ reference was returned to ambient and increased to 450, 550, 600, 750, 900, 1100, 1300 and 1550 μmol mol^{-1}. Fluorescence measurements were made simultaneously to the photosynthesis measurements, for *A. populifolia* and *S. plumieri*, with the multiple saturation flash setting enabled. Full sunlight intensity (2000 μmol m^{-2} s^{-1} of PAR) was used for all fluorescence cuvette measurements, while shoots of *M. cordifolia* were illuminated by natural light, ranging between 1891 to 2114 μmol m^{-2} s^{-1} of PAR. The light intensities used for all species were near to saturation (Figure 8.11), and were necessary for practical reasons. These intensities represent the maximum possible direct light encountered by leaves, excluding all diffuse and reflected light. The leaf temperatures for these measurements were 25.9±0.5, 28.1±0.4 and 34.4±0.5^{o}C for *A. populifolia*, *S. plumieri* and *M. cordifolia*, respectively. Due to practical constraints of cooling the IRGA in full sunlight, the leaf temperatures of *A. populifolia* and *S. plumieri* (fluorescence chamber) were lower than those of *M. cordifolia* (conifer chamber). The photosynthetic rates of *M. cordifolia* in this experiment were consistent with rates measured on four other occasions (e.g. Chapter 5). To account for the differences in leaf temperature the derived photosynthetic parameters were converted to a uniform temperature of 25 ^{o}C, as described below.

Light response curves and light saturation

To illustrate the impact the different anatomies of the leaves of *A. populifolia* and *S. plumieri* have on photosynthesis, single light response curves were made on plants growing in the greenhouse. The standard 3x2cm LICOR 6400 cuvette, with built-in light source, was attached to a LICOR 6400 in the normal manner, allowing a light response curve to be made by illuminating the upper surface of the leaf. The lower half of the cuvette was then removed and replaced by an upside-down clear cuvette with a port for a fluorescence probe. The port for the fluorescence probe was used to fit a thermocouple to the lower surface of the leaf, and was sealed during measurements. Light response curves of the lower surface of the leaf could be made using a pin spot (Euro EcoEXN 12V 50W) and a calibrated LICOR quantum sensor. By varying either the distance of the pin spot from the lower leaf surface or the intensity of the LICOR light source, light response curves could be made for either side of the leaf, or both sides simultaneously.

The light response curves done in the laboratory were measured by reducing the light from full sunlight intensities on the upper surface in a stepwise manner until dark. At this point an abaxial light response curve was made by illuminating the lower surface. Following an acclimation period of full sunlight intensities on the upper surface, the lower surface was sequentially increased to measure a combined surface light response curve, allowing an assessment of the additional contribution of abaxial illumination to the photosynthesis of a well lit leaf.

Field light saturation measurements were made on the same leaves as measured in the field CO_2 response curves, however at a later stage (within 48 hours). The same apparatus as was used in the greenhouse was used for these field measurements. Photosynthesis measurements were made at full sunlight intensities after an acclimation period, for the upper surface, upper and lower surface simultaneously illuminated, and lower surface only illuminated. These measurements were expressed as a percentage of the photosynthetic rate measured with the upper surface illuminated.

Chlorophyll contents

The chlorophyll *a* and *b* concentration of leaves used in the field gas exchange measurements were measured according to the method of Lichtenthaler and Wellburn (1983). A 3 cm^2 longitudinal section of leaf was excised from *A. populifolia* and *S. plumieri* leaves, and 4 randomly chosen leaves from the measured shoot were measured for *M. cordifolia*. Fifteen millilitres of chilled freshly made up 80% aqueous solution of acetone was used to extract leaves with an Ultra-Turrax blender (Ika Work, Germany). The extract was centrifuged at 3000g for 3 minutes. When not being worked on the samples were kept on ice, in the dark. The supernatant was diluted such that absorbance readings were less than 0.8. The absorbance of the diluted extract was measured relative to an 80% acetone blank at 663, 646 and 710 nm using a PU8670 Vis/NIR spectrophotometer (Phillips, U.K.). The 710 nm absorbance was removed from the other absorbance's to account for non-chlorophyll differences between extracts (Porra, 2002). The formulae of Lichtenthaler and Wellburn (1983) were used to calculate chlorophyll contents:

Chl *a* (μg ml^{-1}) = $12.21*(A_{663} – A_{710}) – 2.81*(A_{646}–A_{710})$

Chl *b* (μg ml^{-1}) = $20.13*(A_{646} – A_{710}) – 5.03*(A_{663}-A_{710})$

Photosynthesis calculations

R_d, V_{cmax} and J were estimated from the CO_2 response measurements for each leaf used in the *PNUE* analysis. The sample CO_2 concentration was leak corrected using an empirically determined function of the size of CO_2 leaks across the chamber gaskets. The leak correction function was determined for each of the chambers and accounted for flow rate and the CO_2 differential between the sample and the atmosphere. R_d was estimated by fitting equation 8.5, the Rubisco limited photosynthesis function of Von Caemmerer (2000), to data points with c_i values below 200 μmol mol^{-1}. Using the estimated value of R_d, the Rubisco and RuBP limited photosynthesis functions (equations 8.5 and 8.6) were fitted simultaneously by a least squares non-linear fitting algorithm (Statistica 8, Statsoft Inc. Tulsa OK, USA). Equations 8.5 and 8.6 were combined using the min(A_c,A_j) function of Statistica, and included recent temperature and pressure corrected kinetic constants taken from Bernacchi *et al.* (2001 and 2003). The A_j function was fit without including a light response function or reference to the measurement light intensity, thus the output of this equation represents *in situ* electron transport rate, J, and not the calculated light saturated rate, J_{max}. This avoided the use of the empirical model of light saturation, with its assumed constants (von Caemmerer, 2000), and allowed the use of a normal cuvette with a single illumination source to be used for CO_2 response curves in the field (a practical constraint). Further work demonstrated that the photosynthetic rates of the plants in the field were close to light saturation, and thus J is likely to be representative of J_{max}.

Internal CO_2 transfer conductance

The internal conductance to CO_2 transfer (g_i) for *A. populifolia* and *S. plumieri* was calculated using the variable J method of Harley *et al*. (1992a). This method involves assuming a direct relationship between electron transport rates measured by fluorescence and actual transport rates in the absence of photorespiration (at low O_2 concentrations). The electron transport rate measured using fluorescence (J_F) is estimated by the electron transport rate implied from CO_2 measurements (4 e^- per CO_2), but needs a correction for internal conductance (g_i). Harley *et al.* (1992a) derived the following formula calculating g_i as the proportional difference between the two estimates of electron transport:

$$g_i = \frac{A}{c_i - \frac{\Gamma^*(J + 8^*(A + R_d))}{J - 4^*(A + R_d)}} \qquad \text{eqn. 8.12}$$

It is noted that for thick leaved species fluorescence may probe fewer of the PSII than those contributing to photosynthesis. This was partially evident in *A. populifolia* in laboratory conditions, where electron transport rates calculated from fluorescence measurements were *under* estimates of photosynthetic rates measured under non-photorespiratory conditions, at non-light saturating intensities. Thus, for laboratory plants, at light saturation, the assumption of the variable J method appeared to hold for *A. populifolia*. It was not possible to further validate this assumption under field conditions. The g_i of *M. cordifolia* could not reliably be calculated using this method, or the constant J method (Harley *et al.*, 1992a) due to the lack of fluorescence measurements made for this species.

δ^{13}C measurements

δ^{13}C measurements were made for three young fully expanded leaves of *A. populifolia* collected at OWR. The samples were oven dried and subsequently ground in a ball mill (800M mixer/mill; Glen Creston, Middlesex, UK). These measurements were performed courtesy of Doug Ibrahim and Colin Osborne using a mass spectrometer (PDZ Europa 20-20; Cheshire, UK) at the University of Sheffield.

Temperature corrections

The *in vivo* temperature corrections of Bernacchi *et al.* (2003) were used to convert K_C, K_O and Γ_* from values at 25°C to the temperature at which the CO_2 response curves were measured. These values were used to fit the Rubisco and RuBP limited photosynthesis equations to the data, to estimate R_d, V_{cmax} and J. The estimates of R_d, V_{cmax} and J for the ambient conditions were corrected to a standard 25°C using the equations of Bernacchi *et al.* (2003).

Nitrogen partitioning between components of photosynthetic machinery and non-photosynthetic machinery

Leaf nitrogen was partitioned into a number of components largely based upon the empirical work of Evans (1989):

N_{PC} – nitrogen contained in the enzymes and proteins needed for photosynthetic capacity,

N_{other} – nitrogen invested in non-photosynthetic capacity such as nuclear DNA and RNA, osmotic components and herbivory defence compounds,

N_{ETR} – electron transport related enzymes and proteins,

N_{LHC} – light harvesting components,

N_{PCR} – photosynthetic carbon reduction associated enzymes and proteins.

All of these components were estimated from established relationships between chlorophyll content, photosynthetic parameters and leaf nitrogen measurements. The derivation of these estimates is discussed in detail in Appendix 4.

Literature survey

A literature survey was conducted to allow a comparison between the photosynthetic parameter values of the three species studied here, with parameters from as broad a range of species as possible. The literature was surveyed for tables containing data relating to parameters measured in this study. These included: 1) chlorophyll or leaf anatomy measurements: Baltzer and Thomas (2005), Cao (2000), Craine and Reich (2001), Poorter and Evans (1998), 2) photosynthetic parameters: Bunce (2005), Mediavilla and Escudero (2003), Oguchi *et al*. (2003), Wong *et al*. (1985), 3) internal conductance measurements: Gillon and Yakir (2000), Harley *et al*. (1992a), Manter and Kerrigan (2004), 4) CO_2 response curve parameters: Ellsworth *et al*. (2005), Wullschleger (1993; a mine of information), 5) delta ^{13}C values from O'Leary (1988), 6) a broad range of these parameters: Araus *et al*. (1986), Cruz *et al*. (2003), Garnier *et al*. (1999), He *et al*. (2006), Pons and Westbeek (2004), Warren and Adams (2004), Westbeek *et al*. (1999), and Wohlfarht *et al*. (1999). The studies surveyed included about 500 species from desert, savannah, grassland, rainforest and high altitude regions. As far as possible, only data from high-light or field experiments were used; exceptions were rainforest species, and a number of high-light greenhouse studies. Known C_4 and CAM species were excluded from this analysis. The results of the analysis were presented as the absolute range of values for all species, and as the rank of the species studied here within the literature survey (as a percentage of 0 to 100;

low to high values). It was not considered possible to correct all photosynthetic parameters for differences in leaf temperature.

Analysis of allocation between J_{max} and V_{cmax}

The allocation of leaf nitrogen between the J_{max} and V_{cmax} components of leaf biochemistry can potentially limit photosynthesis. As this allocation was determined for the three dune species it was of interest to ask whether the allocation of dune species differed from other species. To answer this question a detailed analysis of data from the literature was conducted. The large data set of Wullschleger (1993) was used as a basis for testing whether dune plants had unusual ratios of J_{max} to V_{cmax}. As the ratio of J_{max} to V_{cmax} and the leaf temperature determines the point of colimitation of Rubisco and RuBP regeneration limited photosynthesis, equation 8.8 could be used to calculate the c_i at which this occurs. The enzyme kinetic constants K_C, K_O and Γ_* were temperature corrected using the formulae of Harley *et al*. (1992b) as these were used in the original paper (Wullschleger, 1993). J (rather than J_{max}) was calculated using the constants given by Wullschleger (1993) and the light intensity under which the CO_2 response curves were made. Thus the colimitation point could be estimated for each CO_2 response curve presented by Wullschleger at the measured temperature and cuvette light conditions. From this information the proportion of plants in Wullshleger's dataset that were likely to be Rubisco or RuBP regeneration limited could be estimated.

Due to the large amount of variation caused by the wide range of temperatures included in Wullschleger's data set, as demonstrated by Leuning (1997), it was necessary to plot the data at a common temperature of 20^{O}C, calculated using the J_{max} and V_{cmax} temperature corrections of Harley *et al*. (1992b). Unlike Leuning (1997), the data was presented using J at the measured light intensity, rather than J_{max}. Thus the calculations and presented data best represent the actual conditions under which the CO_2 response curves were made. It was noted that without performing these corrections a much broader range of colimitation point values of c_i were obtained.

RESULTS

Whole plant *NUE*

The efficiency of nitrogen resorption, measured as the percentage reduction in nitrogen from green to senesced leaves, differed greatly between species (Table 8.2).

The mobile-dune species were more efficient at resorbing nitrogen from senescing leaves than the stable-dune species. Apart from the nitrogen-fixing *M. cordifolia*, the other species were able to reduce nitrogen in senesced leaves to extremely low levels (< 7 mg N g^{-1} DW; Killingbeck, 1996).

Leaf longevity changed seasonally in the three species for which this parameter was measured, with higher rates of leaf turnover during periods of maximum growth and lower rates of leaf turnover during the winter (Table 8.2). The average leaf longevity for a year period showed that the fast growing mobile-dune species had shorter-lived leaves. However, due to the higher efficiency of nitrogen resorption from senescing leaves these species had longer shoot nitrogen retention times than the stable-dune nitrogen fixing species, *M. cordifolia* (Table 8.2).

Table 8.2: Characteristics relating to leaf and stem production for plots of four species of coastal dune plants measured under neutral sand accumulation conditions.

	PNUE	% N resorption efficiency	N resorption proficiency	Leaf longevity			Mean retention time of nitrogen in leaves
				over year	maximal growth period	winter	
	μmol CO_2 mol^{-1} N s^{-1}	%	mg g^{-1}	days	days	days	days
A. populifolia	183± 7.5a	73.1±2.0a	5.1±0.4b	127± 2.1b	54.6±19.8b	195± 30.6b	433± 39ab
S. plumieri	268± 5.2a	52.1±5.3b	3.2±0.2c	254± 2.0a	168± 10.7a	337± 23a	554± 60a
M. cordifolia	53.5± 5.3b	-1.8±3.0d	14.9±0.1a	352± 10.8a	172± 17.4a	452± 67a	341± 8b
M. muricata	na	25.9±5.9c	6.2±0.4b	na	na	na	na
ANOVA F-statistics, df	$F_{2,16} = 329$	$F_{3,13} = 46.3$	$F_{3,13}=203$	$F_{2,91} =39.7$	$F_{2,11} =15.9$	$F_{2,15} = 12.2$	$F_{2,11} = 6.9$
P<	0.001	0.001	0.001	0.001	0.001	0.001	0.011

Means are shown with standard errors (n ranges from 3 to 49). Different letters in each column represent significant differences at $P < 0.05$, for one-way ANOVA.

Photosynthetic nitrogen use efficiency

The definition of photosynthetic nitrogen use efficiency implies that this parameter is equal to the slope of the line connecting the photosynthetic measurement and nitrogen content with the origin (Figure 8.5). Relative to the distribution of observations of 112 other C_3 species, *A. populifolia* and *S. plumieri* have high photosynthetic rates relative to the leaf nitrogen content (Figure 8.5). Although these species have very different leaf nitrogen contents and photosynthetic rates, the *PNUE* of each is considerably higher than any other species from the literature survey (Figure 8.5).

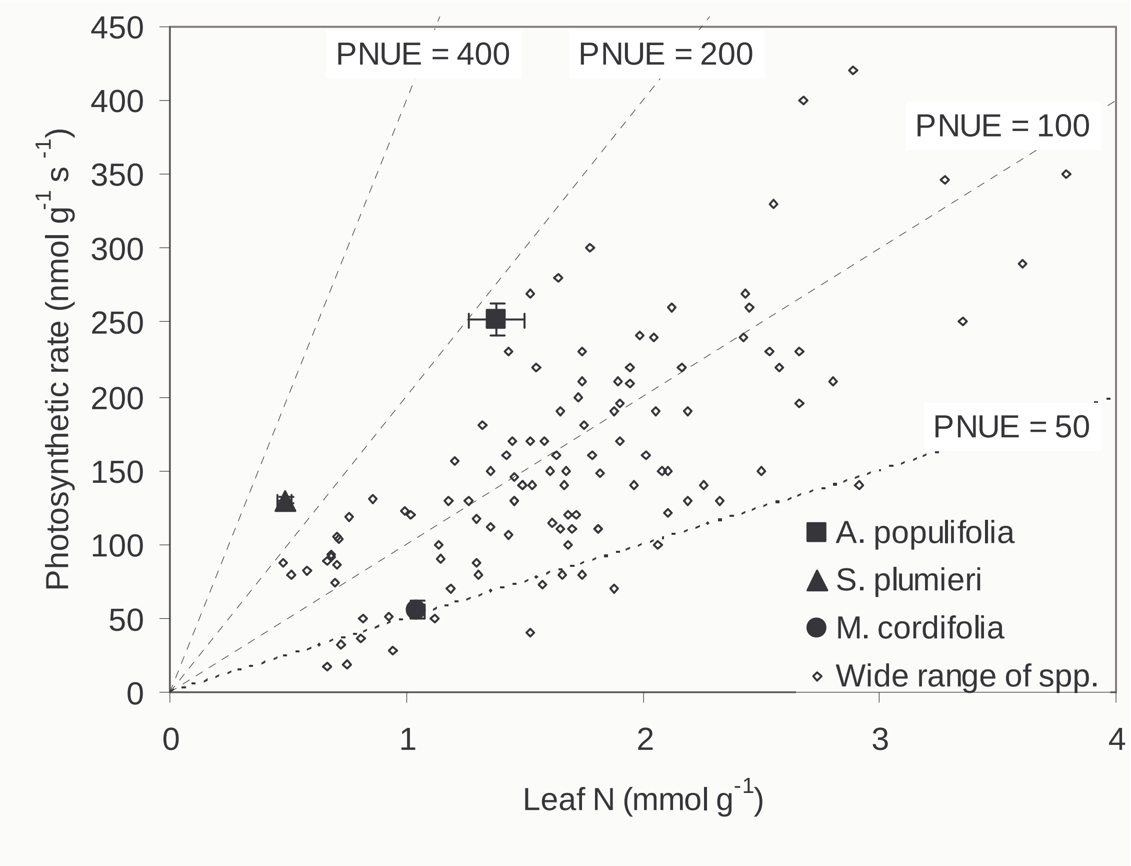


Figure 8.5: The relationship between leaf nitrogen content and photosynthetic rate both expressed per unit leaf mass. The three filled symbols represent the average measurements made for the three species measured in this study, and the open symbols the average values measured for 112 species from various studies. The dashed lines represent isoclines along which all points have equal *PNUE* (nmol CO_2 mmol N^{-1} s^{-1}).

Operating point optimisation

The location of the spot measurements on the CO_2 response curve were considerably lower down the Rubisco limited region of the curve than the colimitation point of the curves for all three species (Figure 8.6). The unused electron transport capacity under ambient conditions was between 16% and 80% greater than the ambient spot measurement (Table 8.3). However this large excess electron transport capacity does not result in the three species studied here having different ratios of J to V_{cmax} to a large variety of species taken from the literature (Figure 8.7).

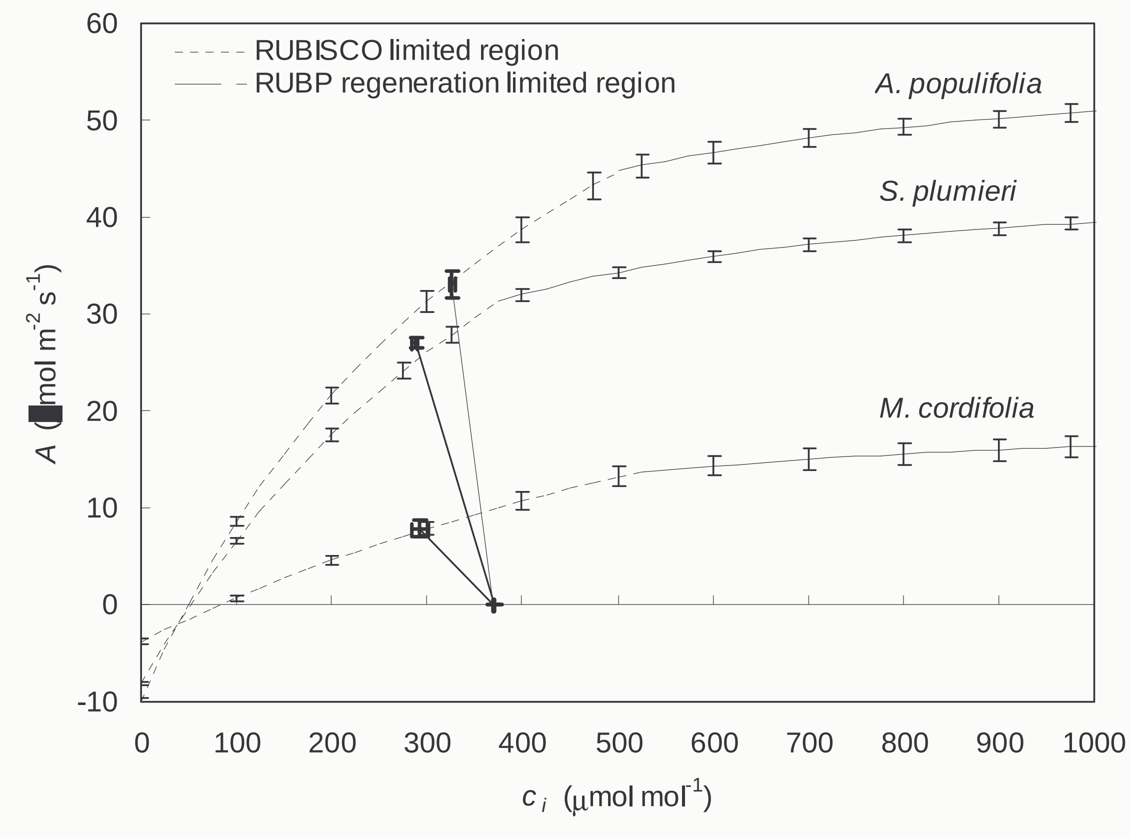


Figure 8.6: Operating points and the points of colimitation of Rubisco and RuBP regeneration limited photosynthesis for three species**.** Error bars are standard errors of the mean for spot measurements under ambient conditions or for interpolated points from fitted curves (n=6-7).

Stomatal and internal conductance to CO_2 diffusion

A. populifolia and *S. plumieri* had extremely high stomatal conductance values relative to *M. cordifolia* and a literature survey of 26 other species (g_{st} in Table 8.3, and see the slopes of the supply functions in Figure 8.8a). In part, high stomatal conductance determines the c_i/c_a ratio, with high conductance resulting in high c_i/c_a and therefore more negative δ^{13}C values. δ^{13}C values provide an estimate of the c_i/c_a values integrated over the lifespan of the leaf carbon (O'Leary, 1988). δ^{13}C was measured on three leaves of *A. populifolia* as a independent verification of the high c_i/c_a values of this species (Table 8.3). The observed δ^{13}C values corresponded to a c_i/c_a of 0.75, an estimate of the integrated c_i/c_a of the leaf over the leaf lifespan, and were considerably lower than the measured 0.89 (Table 8.3). These measurements were done on different leaves, from different sites. This δ^{13}C value is consistent with the observed g_{st} being on the larger half of the range for C_3 plants (O'Leary, 1988).

The high stomatal conductances of the two mobile-dune species result in a low stomatal limitation of photosynthetic rate (Table 8.4 and Figure 8.8a). *M. cordifolia* had a greater stomatal limitation.

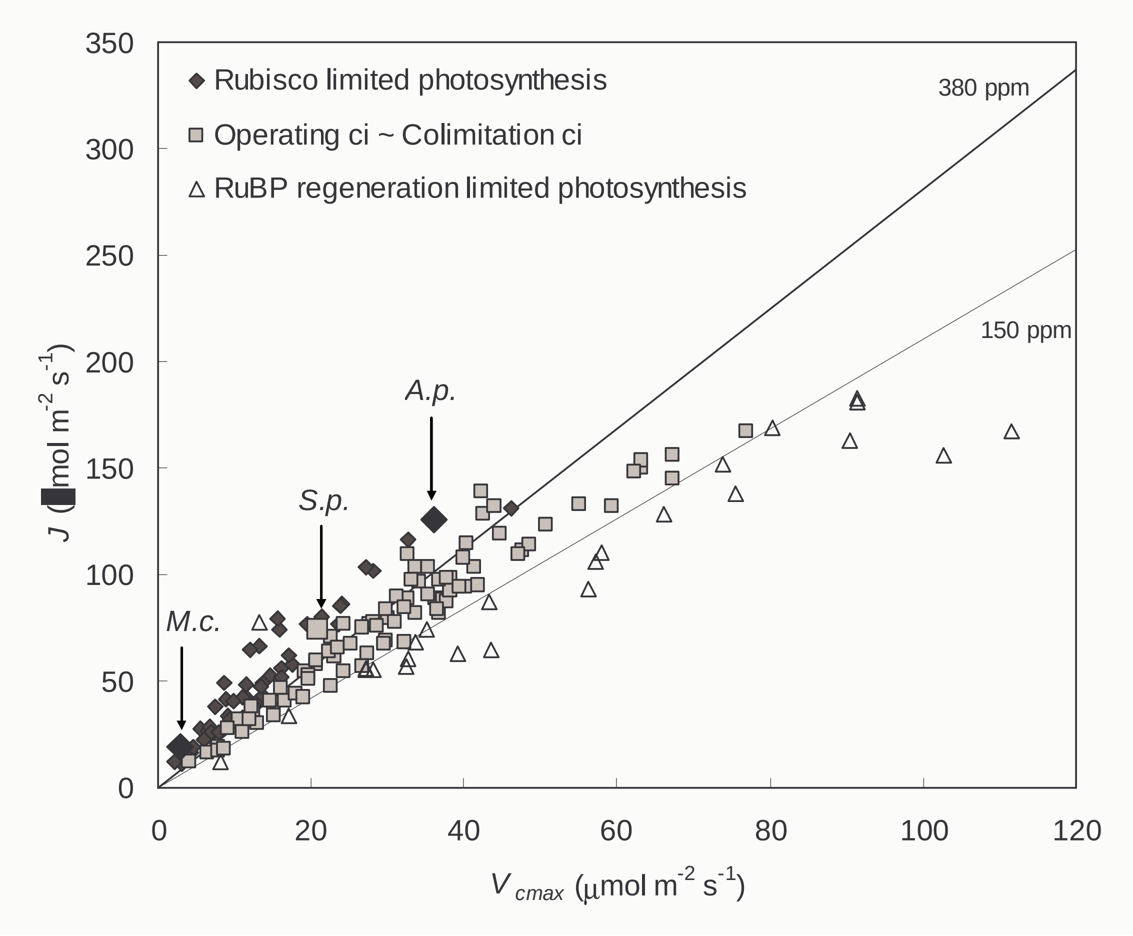


Figure 8.7: Coordination of amount of enzymes allocated to the PCR cycle or the light reactions as indicated by V_{cmax} and *J*. The different shaped and shaded points represent data from CO_2 response curves measured on 154 plants of 107 species presented by Wullschleger (1993). These points were corrected for different measurement temperatures by the equations of Harley *et al*. (1992b) as detailed in Leuning (1997). As the colimitation point should be calculated for the measurement conditions, *J* was calculated for the light intensities given by Wullschleger (1993), rather than use J_{max}. The ratio J/ V_{cmax} determines the c_i at the photosynthetic colimitation point (for a specific temperature; equation 8.8) thus each point presented here is shaded according to whether photosynthesis is likely to be limited by Rubisco or RuBP regeneration, assuming a range of normal c_i values of 150 to 380 ppm. *A.p.* = *A. populifolia*, *S.p.* = *S. plumieri*, and *M.c.* = *M. cordifolia.*

Table 8.3: Photosynthetic and related leaf characteristics of *A. populifolia*, *S. plumieri* and *M. cordifolia*. Shown are the average values for 6-7 leaves of each species for which CO_2 response curves were made for field plants. Data from a literature survey of a wide range of herbaceous and woody species is also presented, and includes the number of species surveyed, the range of values, and the rank of the species studied here relative to the species in the literature survey (from 0 to 100; low to high, as this study's species were not included in the literature survey, these three species may have the same rank).

	Parameter	Units	*A. populifolia*	*S. plumieri*	*M. cordifolia*	Literature survey	Rank of spp. out of 100			
CO_2 response curve data	# curves		6	6	7	# spp	*A.p*	*S.p.*	*M.c*	Range
	V_{cmax} @25°C	µmol m^{-2} s^{-1}	90.3±2.8a	65.0±8.1b	23.4±1.7c	221	79	57	14	6-194
	J @25°C	µmol m^{-2} s^{-1}	222.3±6.4a	153.5±4.3b	48.4±3.8c	207	89	63	12	17-372
	R_d @25°C	µmol m^{-2} s^{-1}	0.79±0.39a	0.26±0.22ab	0.36±0.10b	10	-	-	-	0.17-2.15
	g_i	mol m^{-2} s^{-1}	0.197±0.005a	0.13±0.004b	-	40	67	41	-	0.005-0.638
	$\delta^{13}C$	$^{o}/_{oo}$	-28.96±0.51	-	-	>100	~13	-	-	-21 to -35
Spot measurement for CO_2 response curve leaf	*A*	µmol m^{-2} s^{-1}	33.1±1.4a	27.1±0.5b	7.8±0.8c	153	99	97	12	2.2-35.4
	c_i	µmol mol^{-1}	326.6±2.5a	287.5±2.7b	292.8±8.7ab	-	-	-	-	-
	g_{st}	mol m^{-2} s^{-1}	1.37±0.11a	0.537±0.022 b	0.181±0.024 c	26	100	98	49	0.014-0.635
	c_i/c_a		0.886±0.007a	0.745±0.019 b	0.791±0.024 ab	35	100	58	85	0.61-0.82
	c_c	µmol mol^{-1}	157.2±9.4a	98.0±17.4b	-	-	-	-	-	-
Limitations of spot measurement	$L_{stomatal}$	%	11.1±1.2	11.3±1.5	29.1±7.2	-	-	-	-	-
	$L_{internal}$	%	26.7±1.6	23.0±2.0	-	-	-	-	-	-
	Extra J	%	37.0±8.1	16.0±5.4	79.4±17.5	-	-	-	-	-
Leaf characteristics	Chl content	µmol m^{-2}	220±51ab	275±13a	130±10b	44	8	14	0	190-920
	Chl a:b		3.3±0.2b	3.4±0.2b	4.7±0.3a	35	87	88	97	0.91-5.6
	A/Chl	mmol CO_2 mol^{-1} Chl s^{-1}	189±39	99.3±4.0	61.2±5.6	17	100	100	55	7-94
	SLA	cm^2 g^{-1}	76.2±1.8a	48.0±1.2b	71.5±1.6ab	270	8	3	6	9.3-616
	Leaf thickness	µm	2010±180b	2585±200a	725±45c	121	100	100	100	96-640

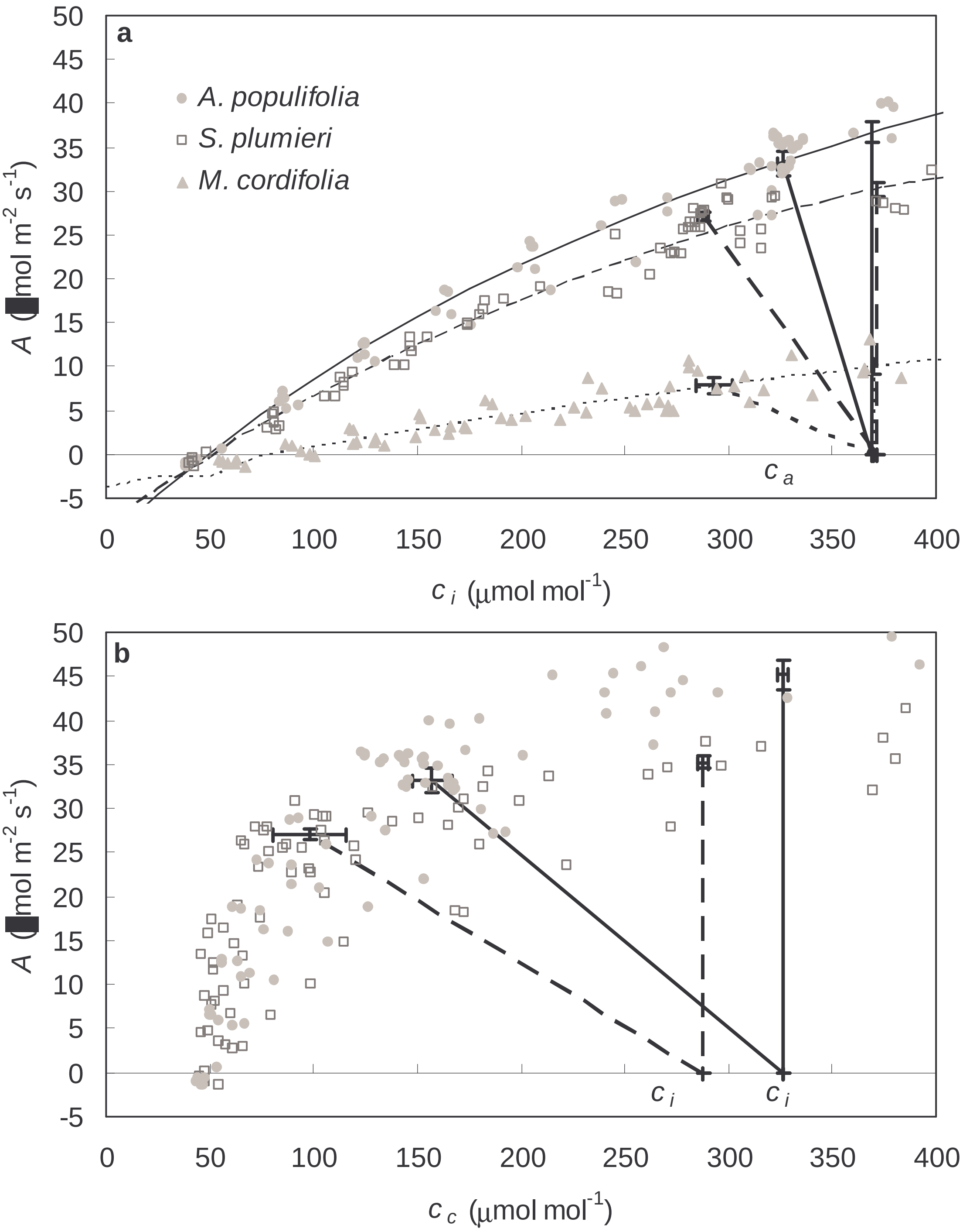


Figure 8.8: The photosynthesis supply functions and CO_2 response curves of three species, shown for the intercellular (c_i; a) and mesophyll (c_c; b) CO_2 concentrations. Vertical lines represent the CO_2 supply function calculated for an infinite stomatal (a) or mesophyll conductance (b) to CO_2. The diagonal lines represent the stomatal and mesophyll supply functions measured under ambient conditions. The error bars are standard errors of the mean (n = 6-7 leaves). The relative difference in photosynthetic rate between the infinite and ambient supply functions is an estimate of amount by which stomatal ($L_{stomatal}$) and internal conductance ($L_{internal}$) limit photosynthesis.

Light saturation

S. plumieri had the capacity to develop thick leaves for which photosynthesis was saturated only when both sides of the leaf were simultaneously illuminated with relatively high light intensities (Figure 8.9b). *A. populifolia* did not demonstrate this capacity (Figure 8.9a). Thus, the capacity to produce leaves that have saturated photosynthesis only under conditions of lower surface illumination corresponds to the observed angles the leaves of each species maintain under field conditions (Figure 8.10). However, the light response curves shown in Figure 8.9 were made on greenhouse plants. In the field, the *A. populifolia* leaves used for the *PNUE* analysis showed only slightly increased photosynthetic rates when both the upper and lower surface were illuminated (Figure 8.11). Illumination of the lower surface of *S. plumieri* leaves resulted in considerably higher photosynthetic rates than *A. populifolia* (Figure 8.11). Thus, the high light reflected off the sand surface (Figure 8.10), the acute angle of *S. plumieri* leaves, and the greater capacity for abaxial photosynthesis (Figure 8.9 and Figure 8.11), indicate that this species reaches light saturation of photosynthesis under different light conditions to *A. populifolia*.

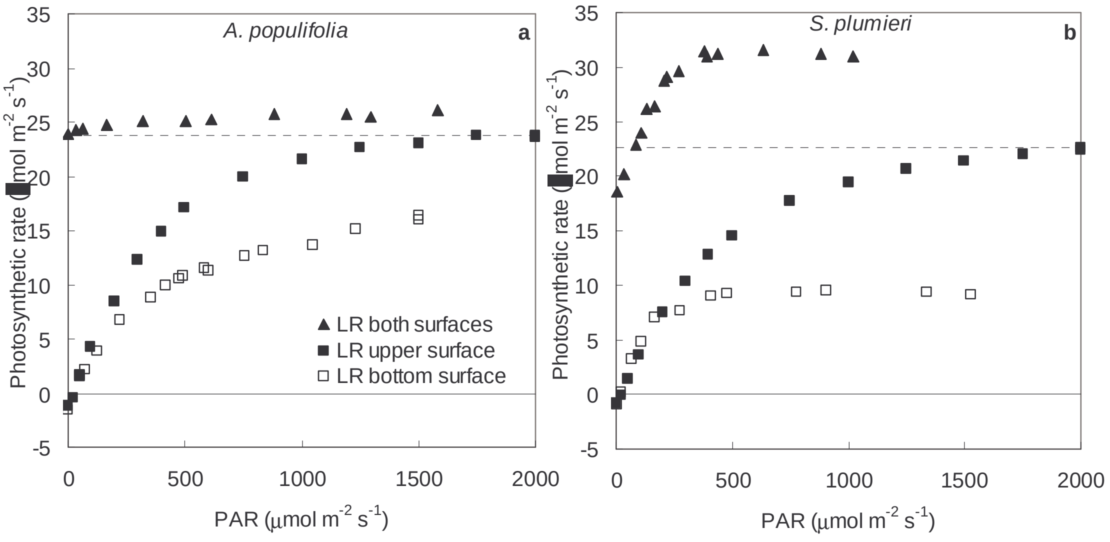


Figure 8.9: Light response curves measuring leaf gas exchange under three combinations of illumination direction, for greenhouse grown *A. populifolia* (a) and *S. plumieri* (b). PAR represents photosynthetically active radiation. The dashed line represents the maximum photosynthetic rate when the only upper surface was illuminated.

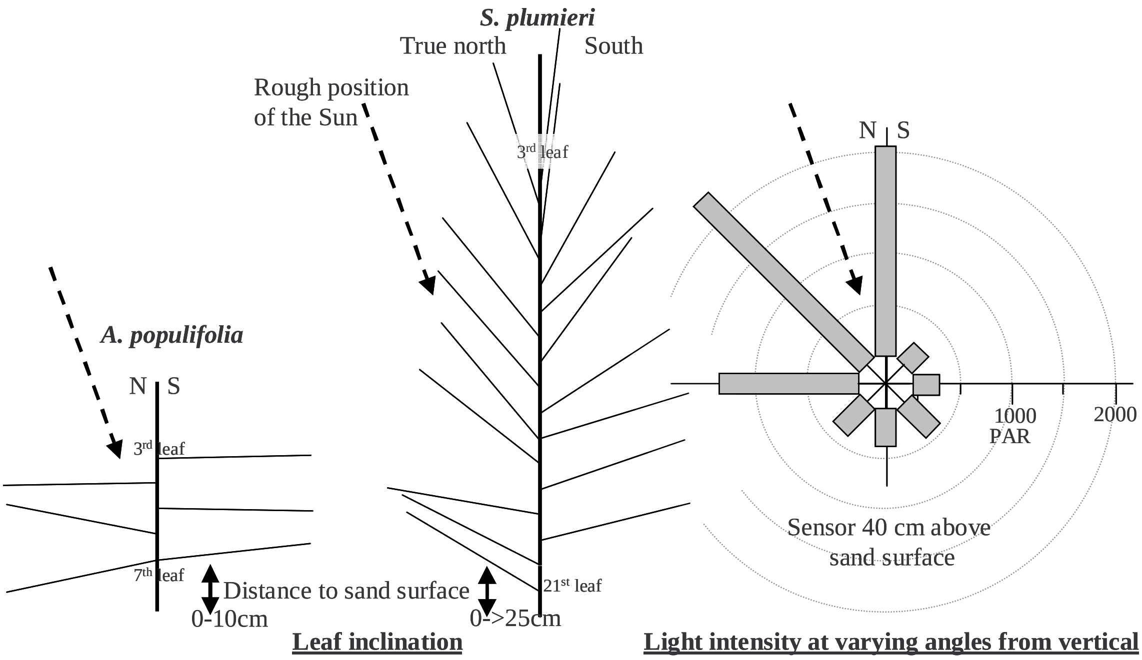


Figure 8.10: Typical inclinations of leaves growing north or south, for two species of dune plants, and the photosynthetically active radiation measured at midday for varying angles from vertical. The leaf inclinations are the average of three shoots, for leaves facing within 45° of true north and south. Light reflected off the sand surface was 450 µmol m^{-2} s^{-1} at the sand surface, or 20% of the incoming PAR (2290 µmol m^{-2} s^{-1}). The attenuation of reflected light with distance from the sand surface was measured as 2 µmol m^{-2} s^{-1} per cm.

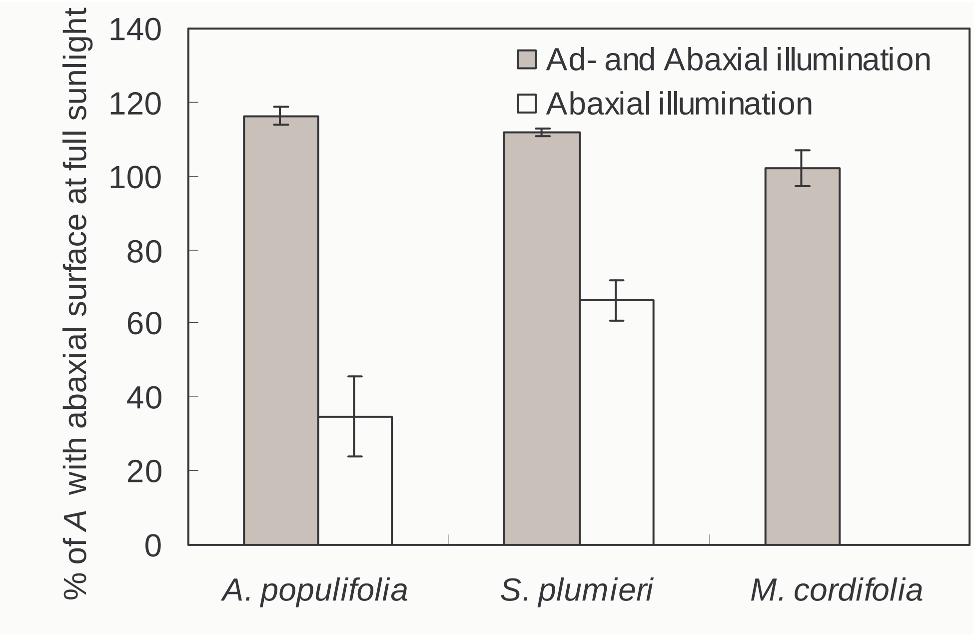


Figure 8.11: The light saturation of field photosynthetic rate (A) as a percentage of the photosynthetic rate measured with the adaxial surface illuminated by 2000 µmol m^{-2} s^{-1} PAR, and under two other illumination conditions for three species. Adaxial illumination (top surface of the leaf) was 2000 µmol m^{-2} s^{-1} PAR, while abaxial illumination (bottom surface of the leaf) was 0 or greater than 2000 µmol m^{-2} s^{-1} PAR. Error bars are standard errors of the mean (n = 4 for the first two species and 7 for *M. cordifolia*).

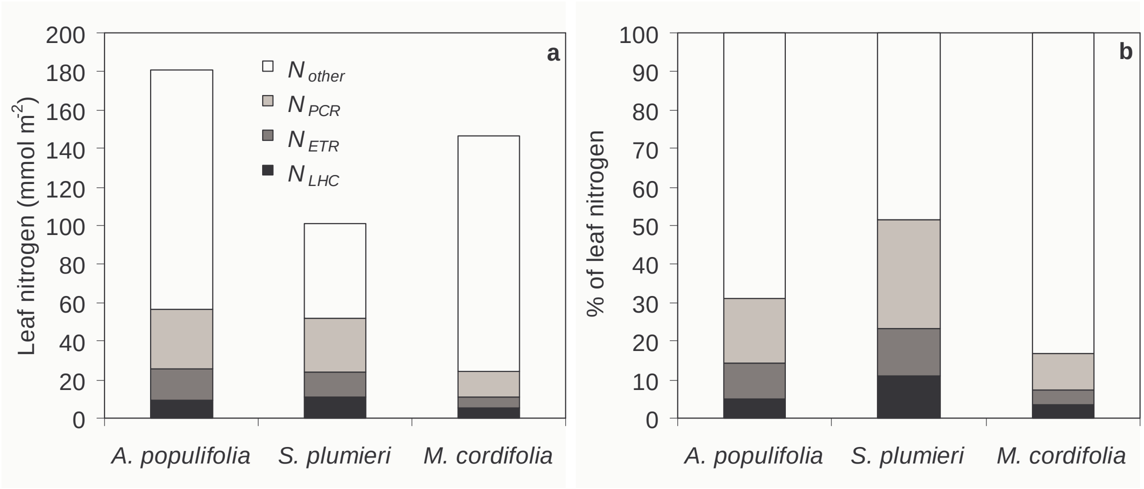


Figure 8.12: The leaf nitrogen content of three species expressed per unit leaf area (a) or as a percentage of leaf nitrogen (b), and partitioned into four components: nitrogen incorporated in photosynthetic carbon reduction metabolism (N_{PCR}), nitrogen used in proteins and enzymes of the electron transport metabolism (N_{ETR}), nitrogen in reaction centres and light harvesting complexes (N_{LHC}), and other (non-photosynthetic) nitrogen. Standard errors of the means are given in Table 8.3.

Distribution of nitrogen within leaf components

The total leaf nitrogen content of the three species differed considerably (Figure 8.12 and Table 8.4). The leaf nitrogen invested in the functional photosynthetic capacity of each species was correlated with the observed photosynthetic rates, and closely related to *PNUE* (Figure 8.12, 8.13 and Table 8.4). The PCR (incl. Rubisco) portion of the photosynthetic nitrogen was the largest fraction followed by the N_{ETR} and then N_{LHC} (Figure 8.12 and Table 8.4). A variable proportion of N_{ETR} was unused under ambient CO_2 conditions in all species, but made up little of the total leaf nitrogen (Table 8.4).

The three species studied here had low, but not extreme, N_{PC} relative to photosynthetic rates (Figure 8.13a). The leaf nitrogen incorporated in the photosynthetic apparatus related negatively to the measured *PNUE* of the species taken from the literature survey (Figure 8.13b). *M. cordifolia* was an outlier for this relationship, while the two mobile-dune species were similar to other species, but did not have as high *PNUE* as some of the other species (unlike the data presented in Figure 8.5 which included other species). This unexpected relationship was investigated further by plotting N_{PC} versus N_{other} (Figure 8.13c). For this relationship there was a clear positive N_{PC} intercept ($P < 0.001$), signifying that this relationship is allometric. The implication of this relationship is that the percentage of N_{PC} decreases with increasing N_{total}, and should result in a negative relationship of *PNUE* with N_{total}, as is demonstrated in Figure 8.13d.

Table 8.4: Partitioning of leaf nitrogen and estimations of the contribution of various factors to the measured and optimal PNUE of three species. The *PNUE* of leaves under various "ideal" conditions was calculated as observed A_{max} relative to N_{total}, N_{PC} or N_{total} minus unused N_{ETR}, or as the estimated A at infinite g_{st} or g_i relative to N_{total}. The sensitivity of *PNUE* to shifts from observed conditions to "ideal" optimal conditions was calculated as the percentage increase in *PNUE* from the observed to ideal conditions, as reported in the **Methods**

	Parameter	Units	*A. populifolia*	*S. plumieri*	*M. cordifolia*
Leaf N partitioning	N_{PCR}	%	15.6±0.5	22.3±0.7	5.0±0.3
	N_{ETR}	%	8.7±5.0	9.8±0.3	1.9±0.2
	N_{LHC}	%	5.0±1.2	11.2±0.5	3.6±0.3
	N_{PC}	%	29.3±1.1	43.3±1.1	10.5±0.7
	N_{other} - N budget	%	70.7±1.1	56.7±1.1	89.5±0.7
	N_{other}- Senescing leaves	%	26.5	46.8	-
	N_{total}	mmol N m^{-2}	181±16	101±6	147±4
Optimal partitioning of leaf N	Operating point (Rubisco or RuBP regeneration limiting region)		Rubisco limited	Rubisco limited	Rubisco limited
	Unused N_{ETR} (% of N_{total})	%	2.1±0.3	1.3±0.4	1.2±0.2
	Unused N_{ETR} (% of N_{ETR})	%	23.6±3.7	13.2±4.5	64.8±8.4
PNUE calculations	*PNUE* (A_{max}/N_{total})	mmol CO_2 mol N^{-1} s^{-1}	182.9±7.5	267.6±5.2	53.6±5.3
	PNUE (A_{max}/N_{PC})	mmol CO_2 mol N^{-1} s^{-1}	624.9±23.6	618.4±9.8	504.0±29.8
	PNUE (A_{max}/N_{total} at colimitation point)	mmol CO_2 mol N^{-1} s^{-1}	186.6±7.1	271.1±4.5	54.2±5.4
	PNUE (A@infinite g_{st}/N_{total})	mmol CO_2 mol N^{-1} s^{-1}	202.8±6.7	297.9±7.5	67.7±5.6
	PNUE (A@infinite g_i/N_{total})	mmol CO_2 mol N^{-1} s^{-1}	249.4±8.9	348.0±7.1	-
PNUE sensitivity analysis	*PNUE* @ N_{PC} (% of PNUE)	%	243±13	132±6	873±63
	PNUE @ colimitation (% of PNUE)	%	2.1±0.4	1.4±0.4	1.3±0.2
	PNUE @ infinite g_{st} (% of PNUE)	%	11±1	11±2	29±7
	PNUE @ infinite g_i (% of PNUE)	%	37±3	30±4	-

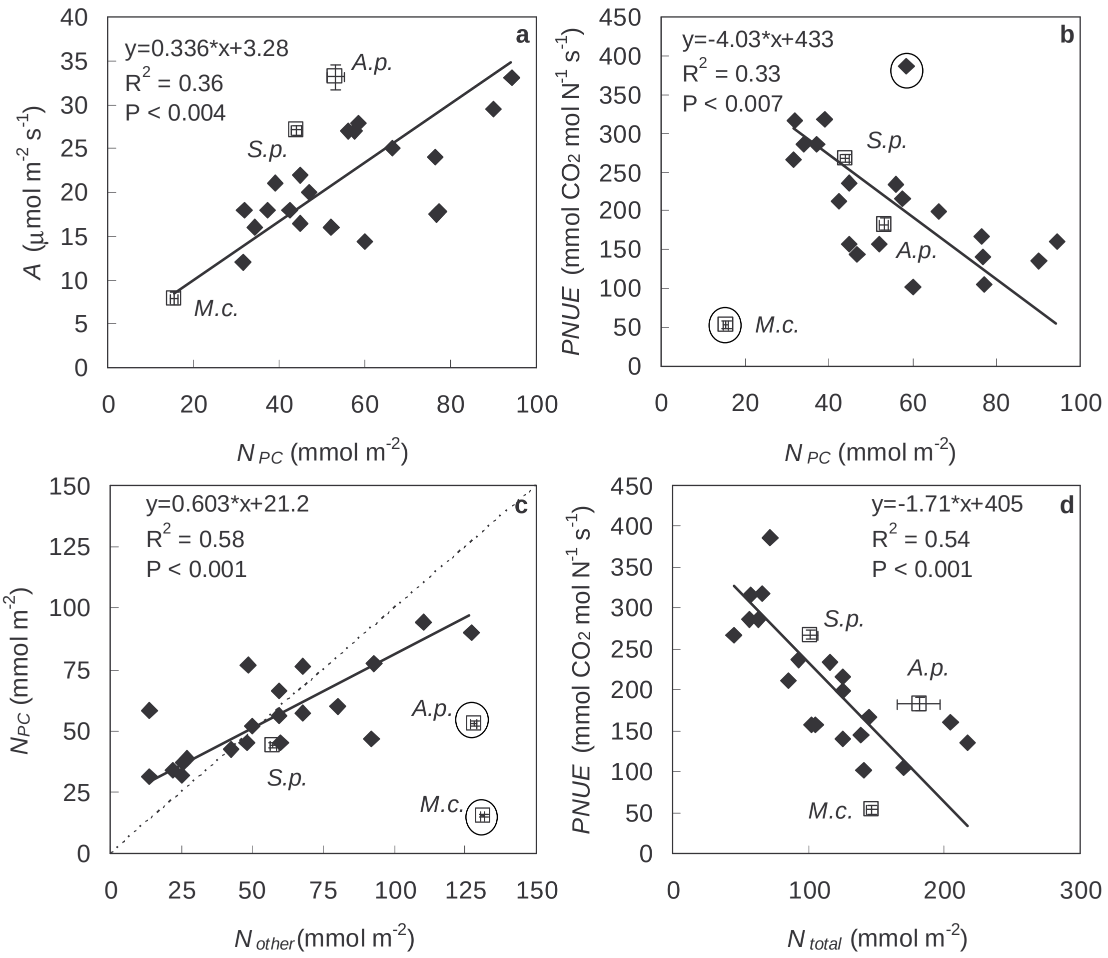


Figure 8.13: Partitioning of nitrogen between N_{PC} and N_{other} and the effect of this on A and *PNUE* for the three species studied here (open symbols with error bars) and 20 species from the literature (closed symbols). Circled points were excluded from the fitted regression lines. The dashed line in panel c is a one to one line for equal amounts of N_{PC} and N_{other}. *A.p. A. populifolia*; *S.p. S. plumieri*; *M.c. M. cordifolia.*

Impact of altered allocation on *PNUE*

The *PNUE* of the leaves studied here can be theoretically manipulated to allow an estimate of the impact of an altered allocation of nitrogen between leaf components, or changes in CO_2 conductances. The observed *PNUE* was calculated for each species and recalculated with altered leaf nitrogen components and photosynthesis components (Table 8.4). The percentage change, or sensitivity, of *PNUE* with each manipulation was also calculated (Table 8.4). The greatest limitation to *PNUE*, for all species, was the allocation of nitrogen to the non-photosynthetic apparatus (>>100% increase in *PNUE* when N_{other} was removed; Table 8.4). The large amount of unused electron transport capacity (Table 8.3) did not have a great impact on *PNUE* (Table

8.4), as this represented only a small percentage of the total leaf nitrogen content (Table 8.3). Removal of internal resistance to CO_2 diffusion was a more important limitation to *PNUE* than stomatal resistance for the two mobile-dune species (Table 8.4). *M. cordifolia* had a higher stomatal limitation to *PNUE* than the other species (Table 8.4), and internal limitation could not be calculated.

DISCUSSION

Optimisation of *PNUE*

The two mobile-dune species (*A. populifolia* and *S. plumieri*) had extremely high rates of photosynthesis and high *PNUE* relative to other species or *M. cordifolia* (Table 8.3 and Table 8.4). Given that this is a vital attribute for these nitrogen limited plants, the following question is of great interest: What factors contribute to these plants having an extremely high *PNUE*? As *A. populifolia* and *S. plumieri* have considerably different leaf nitrogen contents, this may indicate that these species have different physiologies, or employ different methods of achieving a high *PNUE*.

Limitations to photosynthesis and the allocation of nitrogen

The large amount of excess capacity for electron transport in all three species is of interest: Why do these plants invest excess nitrogen in photosynthetic capacity that is unused under optimal and saturating light conditions? A number of possible explanations could be suggested. Firstly, the large excess capacity for electron transport is not strictly proportional to the amount of nitrogen invested in the electron transport apparatus (Evans, 1989). As a result *M. cordifolia* has an electron transport cost of about 0.23 mol N_{ETR} mmol CO_2^{-1} s in comparison with a Rubisco carboxylation cost of 0.313 mol N_{PCR} mmol CO_2^{-1} s (calculated for the *Chl*, V_{cmax} and *J* values in Table 8.3 using the formulae in Appendix 4, Table 12.1). The other two species have higher electron transport costs relative to N_{ETR} (0.28 and 0.26 mol N_{ETR} mmol CO_2^{-1} s for *A. populifolia* and *S. plumieri*, respectively; the Rubisco carboxylation cost is the same for all species). Thus, the estimated nitrogen cost of electron transport differs between species of different electron transport capacity, and is lowest in the species with the greatest excess investment in electron transport. The improvement in *PNUE* after removing the excess electron transport-related nitrogen was smaller than any of the other manipulations made in Table 8.4, suggesting that this was of relatively little importance in determining *PNUE*.

Many other species show a pattern similar to the plants studied here: that of operating below the photosynthetic colimitation point (Figure 8.7). This may be a general pattern for many plants, as most plants fall on a single relationship of J to V_{cmax}, suggesting that allocation to these two processes is coordinated (Figure 8.7; Wullschleger, 1993 and Leuning, 1997). The implication of this allocation pattern is that the total amount of nitrogen is conserved at the expense of plants operating on the steeper portion of the CO_2 response curve (Figure 8.3). Consequently, these plants will have greater sensitivity of photosynthesis to changes in stomatal conductance. Thus, it seems interesting to compare dune plants to plants from high nitrogen environments with scarce water. Such plants may operate above the colimitation point, thus maximising photosynthesis relative to water and not nitrogen. Grassi *et al*. (2002) present data for *Eucalyptus grandis* where the ratio of J to V_{cmax} increases with nitrogen availability and the stomatal conductance remains the same, such that there is a larger amount of Rubisco than can be used at higher nitrogen availabilities. This is clearly the opposite pattern to that found for dune species. But this pattern is consistent with the observation that the nitrogen used for N_{ETR} (J) is incorporated at a lower cost than that in N_{PCR} (V_{cmax}). Consequently it may be expected that a shift from greater nitrogen allocation to V_{cmax} capacity to preferential allocation to J capacity would occur with decreasing nitrogen availability. A consequence of this hypothesis is that plants from environments with differing nitrogen availabilities may have different limitations on photosynthesis due to shifts in investment between the two biochemical compartments. Although some studies (e.g. Tan and Hogan, 1995) present limited data supporting this hypothesis, the hypothesis remains to be adequately tested empirically. How does the allocation between J and V_{cmax} of dune species compare with other species?

The broad range of J to V_{cmax} ratios found in the large data set of Wullschleger (1993) corresponds to a large range of c_i values estimated for the photosynthetic rates required for Rubisco and RuBP regeneration to colimit photosynthesis (Figure 8.14). Since these ratios were measured for plants growing under ambient conditions the highest possible c_i value at which these plants could be operating is between 350 to

380 ppm (depending on the year of measurement). A more reasonable range of operating c_i values was obtained from data for 35 species where operating c_i was reported (also from the literature survey). Thus, the photosynthesis of all plants with colimitation point c_i values greater than 380 ppm, are necessarily limited by Rubisco, as the operating point must be below 380 ppm, the current atmospheric CO_2 concentration. Similarly, no C_3 species were operating at c_i values below 200 ppm (inset Figure 8.14), although 150 ppm was used as a conservative estimate of the lower limit of possible values of c_i for C_3 species. Thus, any colimitation point below 150 ppm must indicate that photosynthesis is limited by RuBP regeneration rates (Figure 8.14). From this model it was estimated that Rubisco is likely to limit the photosynthesis of 29% of species measured, RuBP regeneration 16% of all species, and 54% of all species would be in of the range of J to V_{cmax} ratios where colimitation was likely to occur. Finally, two of the dune species are within the range of values of c_i where Rubisco limits photosynthesis, but are by no means extreme (Figure 8.14). Thus, there is little evidence of a large trend towards excess allocation to electron transport, as would be evidenced by the majority of species having a Rubsico limitation. However the majority of species were Rubisco or colimited, suggesting that many species have an overinvestment in relatively inexpensive electron transport capacity.

Hikosaka (1997) demonstrated that the ratio of J to V_{cmax} is largely temperature dependent. As V_{cmax} is more temperature responsive than J this means that at high temperatures there will be a shift towards a lower ratio of J to V_{cmax}. Therefore, a shift in temperature above that which results in colimitation will result in increasing RuBP regeneration limitation of photosynthesis. Since all three dune species had considerable Rubisco limitation of photosynthesis, and not RuBP regeneration limitation, this indicates: 1) that the leaf temperatures used in this study are below those that the leaves experience in the field and for which colimitation occurs, or 2) colimitation does not occur in these plants due to some other reason.

More evidence is needed to sufficiently test these hypotheses, consequently it is hoped that this discussion may serve as a starting point. For instance, it is vital that corrections for temperature are included in future work as was done here and in

Leuning (1997). The reader is also referred to Franks and Farquhar (1999; pp. 1344-1345) for a partially complementary viewpoint to the views presented here.

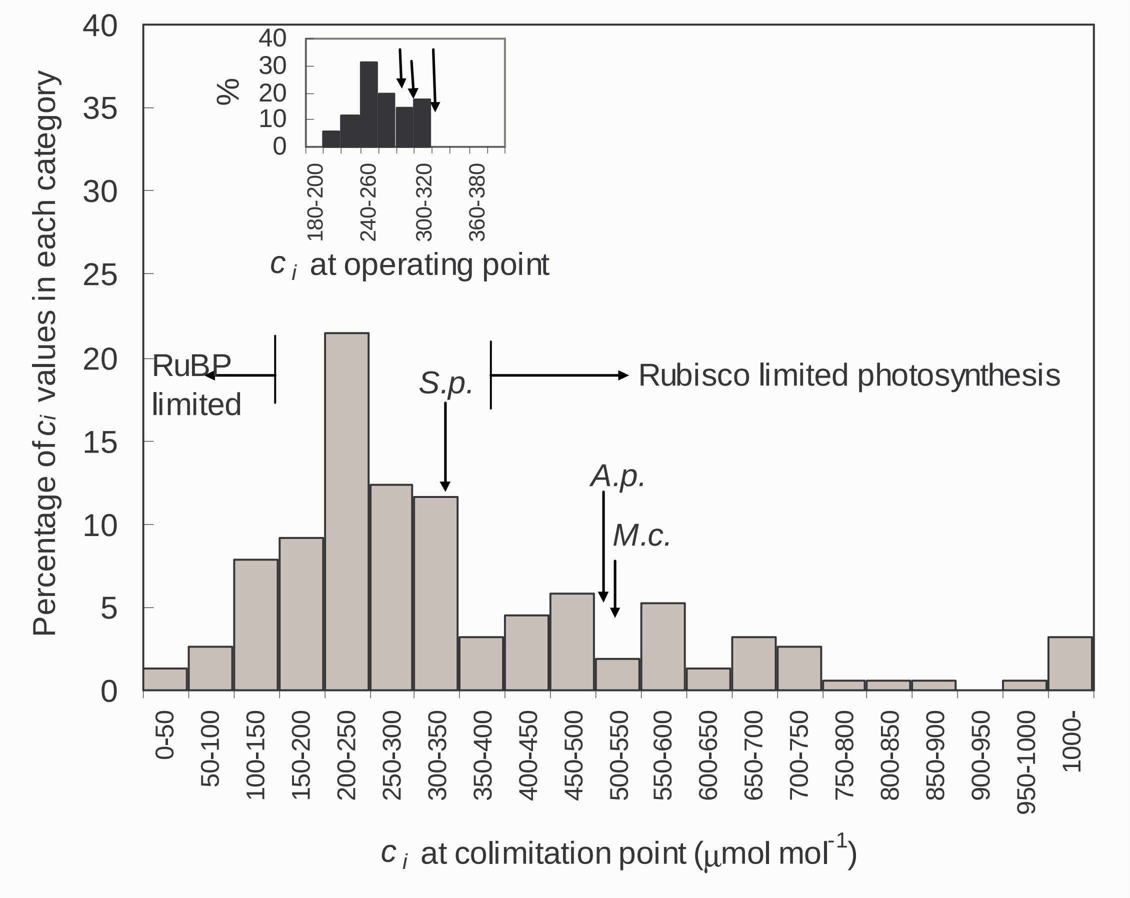


Figure 8.14: Theoretical evaluation of limitation of photosynthesis by Rubisco and RuBP regeneration across more than 150 species, from Wullschleger (1993). The c_i at the ***colimitation point*** of Rubisco and RuBP limited photosynthesis was calculated for 154 plants for which J_{max} and V_{cmax} were measured (literature review see **Methods** and Figure 8.7, using equation 8.8). The inset shows the distribution of c_i values for the ***operating point.*** Leaves are not likely to operate at above ambient c_i (380 μmol mol^{-1}), and no C_3 leaves are likely to operate below 150 μmol mol^{-1} (see inset), therefore, plants with colimitation point c_i values of less than or greater than these two thresholds are likely to be Rubisco or RuBP regeneration limited, respectively. The positions of the operating and colimitation point are indicated for the three species studied here.

Light harvesting optimisation

The different components of the photosystems contain different proportions of chlorophyll *a* and *b* (the reaction centre of PSII has greater amounts of chlorophyll *a* than the light harvesting complexes, while the latter incorporate roughly similar amounts of chlorophyll *a* and *b*). Thus, chlorophyll *a* to *b* ratios can be taken as an indication of the amount of reaction centres relative to associated light harvesting complexes (Kitajima and Hogan, 2003). The three dune species studied here had extremely high chlorophyll *a* to *b* ratios relative to a range of other plants (Table 8.3). The dunes are an exceedingly high light environment, therefore, a reduced light harvesting capacity relative to reaction centres would decrease the amount of nitrogen used while the photosynthetic rate would remain unaffected. As a result of this the dune species should have high rates of photosynthesis per unit chlorophyll. When expressed in this manner the photosynthetic rates of these plants are high (Table 8.3).

The ability of the thick leaves of *S. plumieri* to have considerable photosynthetic rates upon abaxial illumination indicates that the acute leaf angles of this species may allow greater light capture during specific periods of the day (Figure 8.10 and 8.11). However, work by Pammenter (pers. comm. 2007) indicates that these acute leaf angles may not contribute to the leaf having considerably greater daily carbon gain. A similar phenomenon was found for *I. pes-caprae*, for which reflected light, and non-horizontal leaf inclinations resulted in greater total light capture (Greaver and Herbert, 2003). In contrast, *A. populifolia* had lower photosynthetic rates under abaxial illumination and horizontal leaves (Figure 8.10 and 8.11).

Allocation between photosynthetic and non-photosynthetic nitrogen

Nitrogen used for non-photosynthetic metabolism made up between 57 and 90% of the nitrogen in leaves of all species (Table 8.4). Such a large proportion of non-photosynthetic nitrogen was unexpected in the light of the large increase in *PNUE* if this portion was reduced (Table 8.4). Thus, dune plants incorporate a relatively large amount of leaf nitrogen in other processes to photosynthesis. What makes up this non-photosynthetic nitrogen pool? Storage of nitrogen in the leaves is plausible, and would be beneficial under burial conditions. Rubisco has been suggested as a storage compound for excess nitrogen (Warren *et al.*, 2003). As not all Rubisco is necessarily active, this pool of Rubisco may not contribute to V_{cmax} and thus would not be included in the value of N_{PC} calculated by the methods used here.

The dune species appear to have little N_{PC} relative to N_{total} (Table 8.3). Are these values representative of other studies? When plotted as the relationship between A and N_{PC} (percentage of N_{total} or absolute) the three species studied here are marginal to other species, but not outliers (Figure 8.13a). The models demonstrated in Figure 8.4 suggest, on a theoretical basis, that the highest *PNUE* should be found in species with the greatest leaf nitrogen content. This is not the case for the three dune species studied here (Figure 8.13d), or the twenty other species from Westbeek *et al*. (1999), Pons and Westbeek (2004) and Warren and Adams (2004). The relationship of N_{PC} to N_{other} has a significant positive intercept and shallow slope (Figure 8.13c) such that leaves with high N_{total} have disproportionately larger amounts of N_{other}, reducing the potential *PNUE* of these leaves. Thus, leaves with higher nitrogen levels are allocating considerably more nitrogen to non-photosynthetic compartments. In general this result indicates that leaves with low total leaf nitrogen contents are more nitrogen use efficient, unless N_{PC} increases at the same rate as N_{total}. Field and Mooney (1986) found that leaves with high *PNUE* had high leaf nitrogen contents (>2 mmol g^{-1}), possibly reflecting a low percentage allocation to non-photosynthetic nitrogen. Thus, the data presented in Figure 8.5 and Figure 8.13 is contrary to this trend. The most extreme example in Figure 8.5 was *S. plumieri* which had extremely low leaf nitrogen contents, but the highest *PNUE*. If this is a general trend, this result has implications for the breeding of efficient crop plants and the nature of the photosynthetic apparatus of plants growing in severely nitrogen limited ecosystems.

The leaves of *S. plumieri* and *A. populifolia* are able to reduce leaf nitrogen to low levels during senescence, and effectively metabolise the photosynthetic apparatus. Therefore, it seems likely that storage of nitrogen within the photosynthetic apparatus is a better strategy than in less directly productive non-photosynthetic compounds. It is possible that the thick succulent leaves of these two species have greater amounts of nitrogen invested within cell wall's (Onoda *et al.*, 2004) and osmotic compounds (Rozema *et al.*, 1985) than the thin mesic leaves of species to which they are compared. A third pool of non-photosynthetic nitrogen compounds are anti-herbivory compounds (Craine *et al.*, 2003). The leaves of all three species encounter high amounts of herbivory (see Chapter 3). A final possibility is that the calculations of the components of photosynthetic leaf nitrogen are underestimates of the real concentrations (see Appendix 4 for a discussion of this).

What other factors result in a plant having a high *PNUE*?

The following factors are suggested to account for the observed high efficiency with which leaf nitrogen was used to fix carbon in *S. plumieri*: 1) an average R_d (Table 8.2), 2) a high photosynthetic rate (Table 8.3), 3) a low stomatal limitation to photosynthesis (Table 8.3), 4) the least excess electron transport capacity of the three species studied (Table 8.3), and low increase in photosynthetic rate at above ambient light (Figure 8.11), 5) a high chlorophyll *a* to *b* ratio reflecting a lowered investment in light harvesting relative to electron transport capacity (Kitajima and Hogan, 2003), and 6) this species had the lowest allocation to non-photosynthetic nitrogen compounds of the three species studied (Figure 8.12b and Figure 8.13). As a consequence of the relative efficiency of the photosynthetic apparatus of *S. plumieri*, this species had the lowest proportional increases in *PNUE* in response to simulated optimisations of photosynthesis or leaf nitrogen components (see the last section of Table 8.4).

Finally, the long-term *NUE* of this species may be increased due to the high amounts of reflected light in this environment. This corresponds to the capacity of this species to absorb reflected light, both in terms of the distribution of the leaf angles (Figure 8.10), and abaxial photosynthetic light responses of this species (Figure 8.11).

A. populifolia also had a high *PNUE* relative to species included in the literature survey (Figure 8.5). While the high *PNUE* of *S. plumieri* could be attributed to a large number of factors, the high *PNUE* of *A. populifolia* appears to be related to only a few factors. For instance, the extremely high photosynthetic rate of *A. populifolia* was likely to be due to a high stomatal conductance (Table 8.3), and not the relatively inefficient allocation of leaf nitrogen to photosynthetic components (Table 8.4).

Does *A. populifolia* really have such a high stomatal conductance?

Are the high photosynthetic rates and exceedingly high stomatal conductances measured here representative of *A. populifolia*, in general? And, are such high values found in the literature? As a part of a separate investigation, Mostert, Ripley and Gilbert (Mostert, 2006) measured photosynthetic rates of *A. populifolia* at OWR at midday. These measurements confirmed the high (33.1±1.3 μmol m^{-2} s^{-1}; n = 6) photosynthetic rates of this study, but the plants at OWR had *higher* stomatal conductances than those measured at Glengariff. These plants were operating at leaf water potentials of -0.55±0.02 MPa at midday. In comparison with pre-dawn water potentials measured on the same day (-0.2 to -0.3 MPa), these plants clearly did not develop large water deficits as a result of the high stomatal conductances. The data of Ripley (2002) also confirm, using a different IRGA, that *A. populifolia* is capable of these high photosynthetic rates. The stomatal conductances measured in that study were high, but lower than those measured here. A seasonal influence was also evident in the data of Ripley (2002). The extremely high gas exchange parameters measured here may not be entirely representative of all *A. populifolia* plants, as was indicated by the $\delta^{13}C$ values for this species. However, this species clearly operates at this extreme for sufficient lengths of time for this phenomenon to be of physiological importance.

Figure 1.6 of Field and Mooney (1986) includes photosynthesis and stomatal conductance values for a number of annuals. This data set includes annual plants operating at photosynthetic rates of ~35 μmol m^{-2} s^{-1} and extreme stomatal conductances of up to 1 mol m^{-2} s^{-1}. Hogan *et al.* (1995) consistently measured stomatal conductances of up to 2 mol m^{-2} s^{-1} for a range of tropical trees. These values are comparable to those of *A. populifolia* (Table 8.3).

The nitrogen use efficiency of nitrogen-fixing plants

The ability to fix nitrogen on nitrogen limited coastal dunes appears to have large implications for the ecology and physiology of *Myrica cordifolia*. This species was not responsive to additions of full nutrient fertilisers (Chapter 5). Senescing leaves of this species displayed no ability to remobilise nitrogen, in contrast to the efficient remobilisation of the two nutrient limited mobile-dune species (Table 8.2). This finding is consistent with other nitrogen fixing species studied elsewhere (e.g. *Myrica faya*, Vitousek and Walker, 1989; Tateno, 2003). An important consequence of this lack of conservation of nitrogen is that other plants growing adjacent to the nitrogen fixer are able to benefit from high litter nitrogen contents (Witkowski and Mitchell, 1987; and Chapter 6).

The photosynthetic apparatus of *M. cordifolia* leaves was poorly allocated relative to the mobile-dune species, and had a very large component of the leaf nitrogen incorporated in non-photosynthetic compounds (Table 8.4). While this species was clearly not conservative with regard to nitrogen, the stomatal conductance was considerably lower than the mobile-dune species and the stomatal limitation of photosynthesis correspondingly larger (Table 8.3). Thus, the growth and photosynthetic rate of *M. cordifolia* was limited by other factors to the two mobile-dune species, for which water is abundant, and nitrogen limiting.

Dune plant photosynthetic strategies

The three species studied here appear to have photosynthetic strategies widely separated, and some on extreme ends of a complex photosynthesis-nitrogen use-water use continuum (Figure 8.15). *A. populifolia* had an extremely high photosynthetic rate as a consequence of high leaf nitrogen (and photosynthetic nitrogen) and an extremely high stomatal conductance. *S. plumieri* appears to be operating at the low leaf nitrogen extreme of the continuum, but with a high proportion of leaf nitrogen invested in the photosynthetic apparatus. This optimisation, along with a high stomatal conductance results in this species having the highest *PNUE* of the species studied here. The leaf characteristics of *M. cordifolia* were at the low extreme of the A-N_{PC}-g_{st} continuum relative to the species included in the literature survey (Table 8.3), and in comparison to the two mobile-dune species.

The surface of the A-N_{total}-g_{st} continuum depicted in Figure 8.15 is a useful tool to demonstrate the operating position of each species with regard to changes in N_{PC} and g_{st}. Thus, *A. populifolia* and *S. plumieri* operate at the extreme right of the stomatal conductance axis, and due to the saturation of the A to g_{st} relationship, these species will have little increase in A relative to increases in g_{st} due to small stomatal limitations of A (Table 8.3). The operating position of *M. cordifolia* and *S. plumieri* on the N_{PC} axis is such that both of these species would have considerably greater A with an increase in N_{total}. *S. plumieri* operates at the low extreme of the N_{total} axis, but due to a high allocation to photosynthetic capacity, this species has a moderate N_{PC} and achieves high photosynthetic rates as well as *PNUE*.

A striking characteristic of all the dune species, but particularly *A. populifolia* and *S. plumieri*, was the presence of unusually thick leaves (Table 8.3).The high rates of photosynthesis per unit leaf area are to some degree determined by the low *SLA*'s of these two species (Table 8.3). Thus, these plants have a high amount of leaf mass per unit area, due to having thick leaves (Table 8.3), allowing greater photosynthetic capacities (Long *et al.*, 2006), particularly in this extremely high light environment. The heliophytic leaf anatomy of many other mobile-dune species (Barbour *et al.*, 1985), suggests that these characteristics are widely spread amongst dune plants worldwide (e.g. see the leaf cross sections of Purer, 1936)

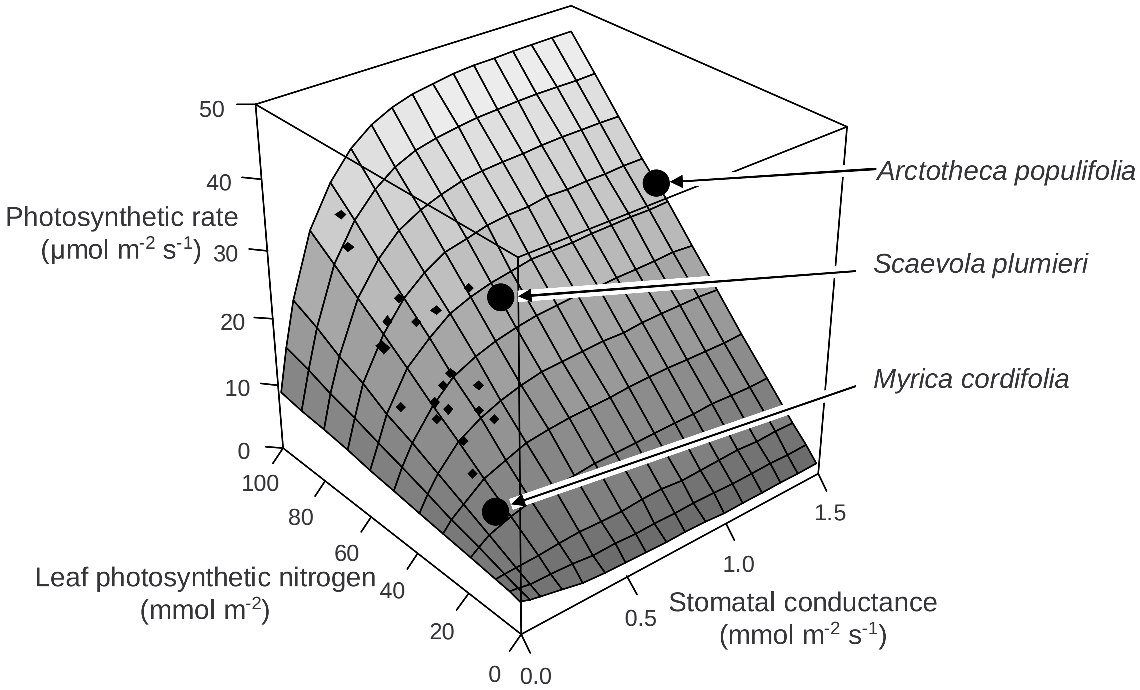


Figure 8.15: An illustration of the position of the three dune species on a hypothetical photosynthesis-leaf photosynthetic nitrogen-stomatal conductance continuum (A-N_{PC}-g_{st} continuum). The surface of the A-N_{PC}-g_{st} continuum was estimated as a linear multiple regression model of A as a function of N_{PC}, a linearised g_{st} and N_{PC} * linearised g_{st}, where A was forced to be zero for a photosynthetic nitrogen allocation of zero. The basic A to g_{st} relationship was assumed to be a saturated exponential function (similar to that of Causton and Dale, 1990). This function was fitted to the dataset and then used to linearise g_{st}. A dataset of 23 species was compiled from this study (large circles represent this study, the other studies are represented by small circles) and Pons and Westbeek (2004), Westbeek *et al.* (1999), and Warren and Adams (2004). While this surface has many of the properties expected from this set of theoretical relationships, a plot with a greater sample of actual data is unlikely to fit this surface well (see Wright *et al.*, 2004 for an example). Thus, it must be emphasised that this diagram is for illustrative purposes only.

Dune plant leaf and whole plant *NUE* strategies

The two mobile-dune species had leaf *NUE* characteristics that allowed a large proportion of leaf nitrogen to be resorbed prior to leaf senescence. This resulted in these species having long shoot mean retention times for leaf nitrogen (Table 8.2). *M. cordifolia* had no ability to resorb nitrogen in senescing leaves relative to photosynthesis, a clear consequence of the ability of this species to fix nitrogen.

Dune plant nitrogen use

The results presented in this chapter demonstrate that mobile-dune plants have high *NUE*, high photosynthetic rates and *PNUE*'s, but achieve these via different strategies. Water does not seem to be limiting to the photosynthesis of the two mobile-dune species, and as a consequence these plants use a large amount of water to achieve high photosynthetic rates and *PNUE*. Since nitrogen is not limiting to the nitrogen-fixer, *M. cordifolia*, nitrogen is not remobilised from senescing leaves, or allocated in an optimal manner, but this higher biomass species is more water conservative than the two mobile-dune species.

Further discussion of the relevance of these characteristics of whole plant and photosynthetic nitrogen use is given in the **Synthesis** in the context of a disturbed nitrogen-limited dune environment (Chapter 11, pp. 328).

Chapter 9

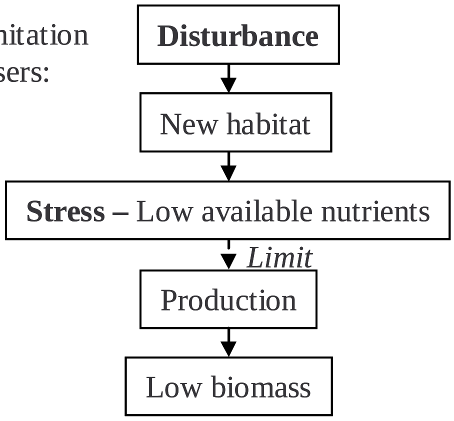
Could low aboveground biomass allow coastal dune plants to achieve the high growth rates needed to outgrow disturbance?

Limitations of plant growth on coastal dunes: disturbance and stress; burial and low nutrients — Competition model of low biomass — Experimental tests of these models — Discussion of the validity of the competition model

INTRODUCTION

Succession is commonly described in terms of species turnover, but it can also be defined as changes in biomass and production (e.g. Odum, 1969). One of the most universal observations of primary succession is that biomass increases over time (e.g. Glacier Bay: Chapin *et al.*, 1994; Lake Michigan: Lichter, 2000). One explanation of this gradient is that low nutrients in early successional soils limit the amount of production and thus the biomass present (Figure 9.1a). This feed-forward limitation of biomass does not take into account the recurrence of disturbance and, therefore, is missing an important aspect of dune ecology. A feed-back model was developed in this chapter, and differs considerably from the feed-forward limitation model, by suggesting that dune colonisers maintain a low aboveground biomass in order to maximise production and, thus, survive recurrent burial disturbance (Figure 9.1b). This model stems from the work of Ripley and Pammenter (2004b), who found that coastal dune species from the Eastern Cape Province do not show signs of nutrient stress in the nutrient limiting dune environment. Similarly, a low leaf area index was shown to result in dune plants having sufficient soil water reserves to avoid drought during dry years (Ripley and Pammenter, 2004a). A low aboveground biomass may also result in lower disturbance rates due to lower trapping of sand as this is proportional to cover (e.g. Buckley, 1987).

a) Conventional feed-forward limitation of the biomass of primary colonisers:



b) Feed-back limitation of the biomass of primary colonisers:

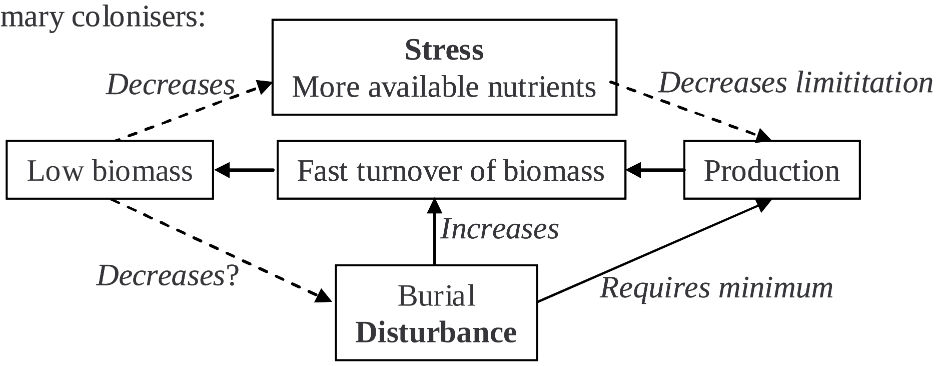


Figure 9.1: Schematic diagrams of feed-forward (a) and feed-back (b) limitations on biomass in coastal dune succession. The feed-forward model suggests that a low biomass is a consequence of the low nutrients limiting production and biomass accumulation. The feed-back model incorporates a disturbance factor (burial) into the scheme. Disturbance requires a minimum high production rate in order for plants to survive burial. As a result, the plant can only survive by maintaining a low biomass, which allows greater nutrient availability to the existing biomass, and fast production rates. At higher biomasses, where competitors may be present, no plants can achieve high enough growth rates to survive burial and are excluded from the mobile-dunes. The influence of the second factor (burial) results in the high turnover of buried biomass, and acts as the feed-back mechanism.

In this chapter a competition model was derived, relating the low aboveground biomass in coastal areas to the growth of dune plants. This competition model relates to the growth of an individual shoot growing with low densities or low total biomasses of competing plants *or* shoots of the same individual. It is suggested that at a low aboveground biomass the action of this model would allow increased available nutrients to aboveground biomass, and greater production, under circumstances of high burial. Graphically, the action of this model gives further insight into the potential growth benefit for dune plants (Figure 9.2a and b). A succession gradient is depicted where burial disturbance and nutrient availability vary in an opposite manner. An adaptation that allows greater nutrient availability on a per gram of biomass basis, results in greater growth rates, survival of greater burial, and an increase in the area in which the plant can potentially grow (Figure 9.2b). The maintenance of low biomass potentially allows plants to occupy a new, more extreme niche, on the dune environmental gradient – a seaward range expansion. Burial and nutrient availability can be defined in terms of disturbance and stress, and covary such that low biomass plants may survive high levels of both factors. Low biomass is thus a potential mechanism whereby dune plants can occupy the “no viable strategy” quadrant of Grime’s CSR strategy scheme (Grime, 1977 and 2001). In this case, the attributes of plants may modulate the levels of stress or disturbance that they encounter.

Chapters 2, 5, 6, 7 and 8 indicate that nutrient dynamics (especially nitrogen) and burial play important roles in determining growth of the mobile-dune colonisers, *A. populifolia* and *S. plumieri*. Thus, low biomass-related adaptations that allow these two species to grow in the highly disturbed areas of the foredunes, are of great interest. The model is derived in the following pages and is followed by three sets of experiments testing the model, and finally an overall discussion of the validity of the model.

AIMS

The aim of this chapter was to derive and test the competition model of low biomass. Specifically, this study attempted to:

a. Formulate a mathematical model of this hypothesis,
b. Validate a number of assumptions made by this model,
c. Test the model with a variety of greenhouse and *in situ* experiments, and
d. Test whether this mechanism allows greater growth under burial conditions.

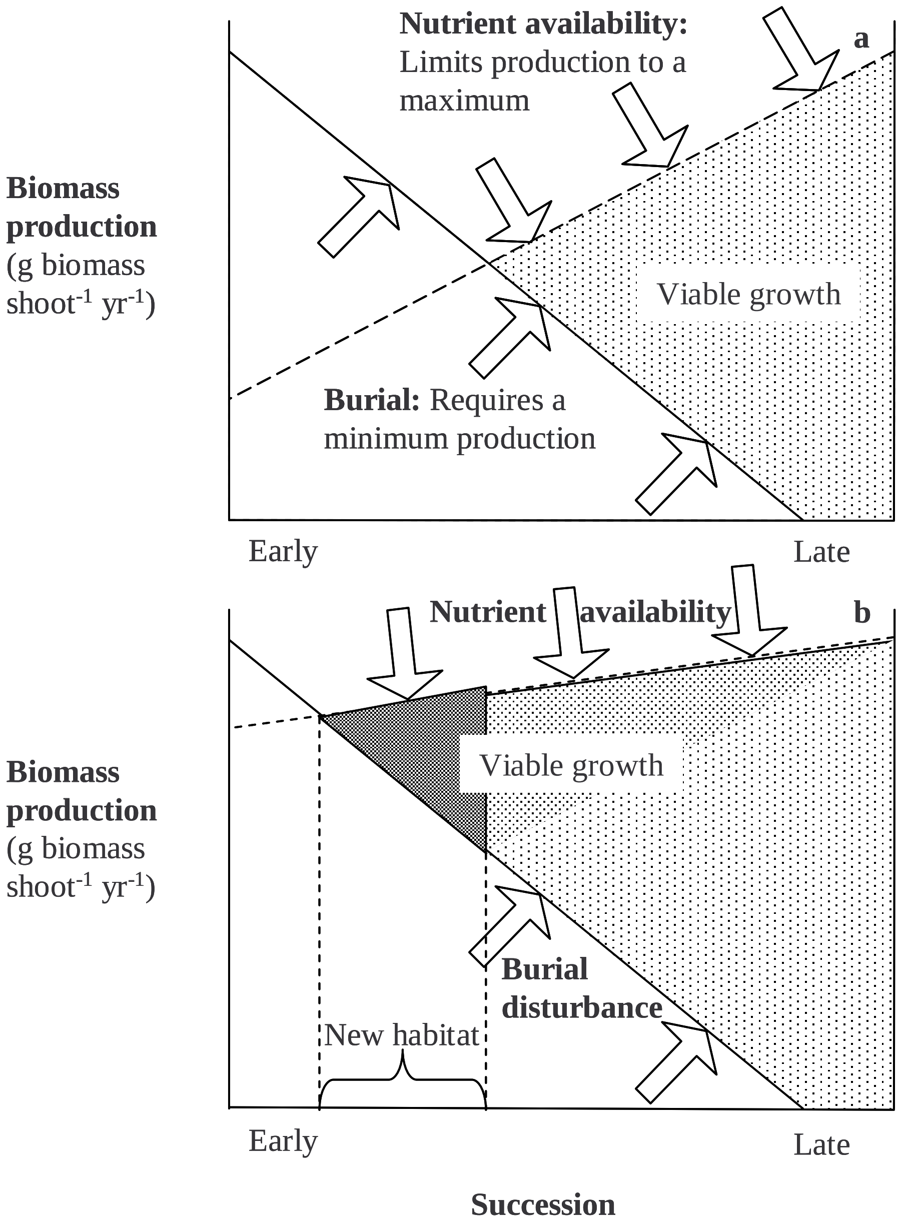


Figure 9.2: Schematic diagram depicting the production limits to coastal dune plants (a), and a seaward range expansion for coloniser plants that maintain a low biomass (b). Specifically, burial (disturbance) requires single shoots to produce a minimum amount of production to maintain leaf area, while nutrient availability per unit biomass (stress) in conjunction with the total density/biomass per dune area which competes for the available nutrients limits the growth rate (a). Thus, a shoot surrounded by low densities of competing shoots, has more available nutrients and is able to grow faster (b). Thus, the increase in the biomass *production rate* is seen in the second panel, and this effect is due to an upwards shift in the nutrient availability as a consequence of a low standing biomass The maintenance of this low density, by burial and growth form would then allow these plants to expand their distributions to more extreme areas of the dune succession.

BACKGROUND

In an oligotrophic environment, competition for the most limiting belowground resource has been suggested to play a large role in determining which species are dominant (Tilman, 1988). However, there is much debate on whether competition is important in environments that have low belowground resources (summarised in Grace, 1991). The importance of competition in unfertile environments differs between two of the most cited models of plant growth and succession (Goldberg and Novoplansky, 1997). Grime’s CSR model requires that in unproductive environments competition is of little importance (Grime, 1977), while for Tilman’s resource ratio model competition is equivalently important at low and high soil resources, but differs from belowground to aboveground competition (Tilman, 1988). Goldberg and Novoplansky (1997) suggest that this difference is due to each model being appropriate in environments with different characteristics. In particular, in an environment where belowground resources occur in pulses (e.g. rain causes pulses in water availability) and stress between pulses is extreme, stress-tolerance is a better determinant of survival than competition (Goldberg and Novoplansky, 1997). Thus, the unfertile dune ecosystem, where nutrients are mineralised relatively constantly, may represent the opposite type of environment, where competition is likely to be important. Two exceptions should be noted, for dune plants with roots near the tidemark, and for plants in highly seasonal temperate dune systems nutrients may be released in pulses, and for these plants competition may be less important.

Grace (1991) offers a slightly different view to that of Goldberg and Novoplansky (1997). Grace suggests that Grime and Tilman’s models refer to different aspects of competition, and so are not necessarily contradictory. For instance, Grime focuses on the importance of competition relative to stress and disturbance factors, while Tilman predicts changes in intensity of competition, or net growth reduction (Grace, 1991). A further alternative manner of looking at this debate is to view the productivity of stressful environments as dependent on the characteristics of the plants in the environment. The competition model of low biomass potentially offers plants a loophole, resulting in the same environment having greater productivity on a per shoot basis. In other environments such a mechanism can not occur as the presence of competitors would result in an unproductive environment. In the dune environment burial disturbance allows plants to utilise this loophole, effectively filtering out plants that do not have a low biomass of competing plants. In other environments the long-term net uptake of nutrients may lead to increases in standing biomass. Burial or specific growth strategies may act as a feedback maintaining a low biomass on these dunes (Figure 9.1b).

Neighbourhood density and biomass have been shown to negatively affect the growth of individual plants monitored in old agricultural fields (Goldberg, 1987), forests (Weiner, 1984), and dune ecosystems (Silander and Antonovics, 1982, competition not necessarily density in the latter case). The relative importance of belowground or aboveground competition determines the nature of the competition effect. Belowground competition differs from that of aboveground competition, as the belowground competitive ability of plants may be unrelated to size, while aboveground competition is size asymmetric (Schwinning and Weiner, 1998; Berntson and Wayne, 2000). Thus, smaller plants have no competitive disadvantage when competing for belowground resources with larger plants in relatively homogenous environments (Schwinning and Weiner, 1998). Rather, the growth of focal plants has been shown to relate to: the cumulative size of all neighbours and distance to neighbours squared (Weiner, 1984). Belowground competition may be more important than aboveground competition for limiting growth of plants growing on the short stature and low leaf area index mobile-dunes (0.2 to 1 m^2 leaf area m^{-2} dune surface area; Ripley, 2002). The high cover found on the stable-dunes may shift this limitation so that the ability to grow tall and compete for light is important later in the dune succession (Chapter 4).

A concise statement of this hypothesis is that: a focal plant that maintains within-individual low biomass or occurs in areas of low total biomass of neighbouring plants has greater amounts of nutrients available per unit biomass. As the mobile-dune plants are nutrient limited a low biomass may result in higher growth rates. It is suggested that plants which have a high biomass or grow in areas of high biomass are unable to outgrow burial as they lack the resources to respond sufficiently. Thus, burial could result in removal of plants, causing the formation of a zone of individual plants and neighbours with a low total biomass, but with sufficient resources to respond to burial successfully. This competition model does not require increases in the total pool of nutrients, rather the greater availability of nutrients at low biomass is due to there being less biomass using the same nutrient pool.

THEORY

Basic competition model

The relative rate of growth of biomass (*R*) is related to the amount of the biomass (*Z*), the rate of supply of the most limiting soil resource (*X*), and the physiological conversion rate of resource to biomass (*P*). By assuming that the biomass accesses all of the available soil resource, then the absolute growth is the physiological conversion rate times the supply rate of the resource (*P***X*). When growth is expressed relative to the amount of biomass, *R* is:

$$R = \frac{P * X}{Z}$$

Where, *X* is the amount of limiting resource available as a constant rate (g resource m^{-2} of dune surface day^{-1}), *Z* is the total biomass (g dry weight m^{-2} of dune surface), *P* is the unit of biomass produced per unit resource (g dry weight g^{-1} resource), and *R* is the rate of growth per gram of biomass (g dry weight g^{-1} dry weight day^{-1}).

Therefore, growth relates directly to the rate of nutrient availability, resource needed to form a unit of biomass, and inversely to the sum of the biomass competing for the resource. This competition model, while rudimentary, should be applicable to the growth of competing plants, or to the within-individual growth of shoots of species where shoots compete for resources. In particular this model may be applicable to the growth of large individuals of *S. plumieri*. The shoots of this species are connected belowground, but to some degree are independent of each other due to adventitious rooting from each shoot. Thus, high densities of shoots may affect each shoot’s resource acquisition, while the plant is still a single entity.

For the sake of simplicity, it is assumed that individuals of a single species are present and thus, *P* is constant across individuals. In this model no differential competition occurs, beyond each plant growing at the same relative rate determined by the resource supply rate. This aspect of the model is consistent with symmetric belowground competition (Schwinning and Weiner, 1998). The implications of relaxing these assumptions are discussed in Appendix 5.

This formulation is similar to the model of Weiner (1984), however the current model lacks a correction for the distance of the competitor. Instead it was thought that the influence of a competitor is likely to be limited to a zone of influence and not to the biomass of all surrounding plants by the square of the distance to the competitor.

Inclusion and significance of an asymptote

The competition model specifies an inversely proportional relationship, which lacks an asymptote term (when linearised an asymptote would be estimated as the y-intercept). However, the standing biomass of a shoot contains some reusable resource, which is not factored into the competition model. Thus, the resources used in the production of a shoot have two components, some of the resources derive from a resource pool competed for by other shoots or plants (X_{comp}), and some of the resources are not competed for by plants and most of these could derive from remobilisation from senescing biomass (X_{sen}). The relationship between resource use (or production) relative to shoot density (or competing biomass) may require such an asymptote term (e.g. Figure 9.3). The following equation includes an asymptote term:

$$R = \frac{P * X}{Z} + C$$

were C has units of g of biomass produced g^{-1} biomass present day^{-1}. This asymptote is necessary to fit a reasonable model to the data, but only represents the amount of resource that is remobilised from the standing biomass (X_{sen}), *at high biomasses*. If this term is related to biomass, then X_{sen} will change with biomass, as indicated in Figure 9.3. In order to parameterise this aspect of resource dependency it is necessary to know the rate of turnover of the biomass (T; g g^{-1} yr^{-1}), and the proportion of the resource remobilised from senesced biomass (S). Thus an estimate of the rate of resource remobilisation from senescing biomass (X_{sen}) is:

$$X_{sen} = S * T$$

If it is assumed that biomass is maintained at a constant level by the plant, then the turnover rate is equal to the growth rate, and thus:

$$X_{sen} = S * R$$

This equation allows an estimate of the maximum rate of resource remobilisation that a plant can possibly achieve. For a shoot:

$$R = P * X_{sen} + P * X_{comp}$$

Therefore, an estimate of X_{comp} can be found by:

$$X_{comp} = \frac{R}{P} - X_{sen} = \frac{R}{P} - S*T \approx \frac{R}{P} - S*R$$

Since X_{sen} is a maximum estimate of the rate of resource supply from senescing tissue, then X_{comp} is a minimum estimate of the rate of resource supply from belowground competitive processes. Although these estimates are highly derived, they allow a rudimentary test of whether an observed pattern of increasing growth with lower biomass, could derive from higher nutrient use efficiencies or the competition model *per se*.

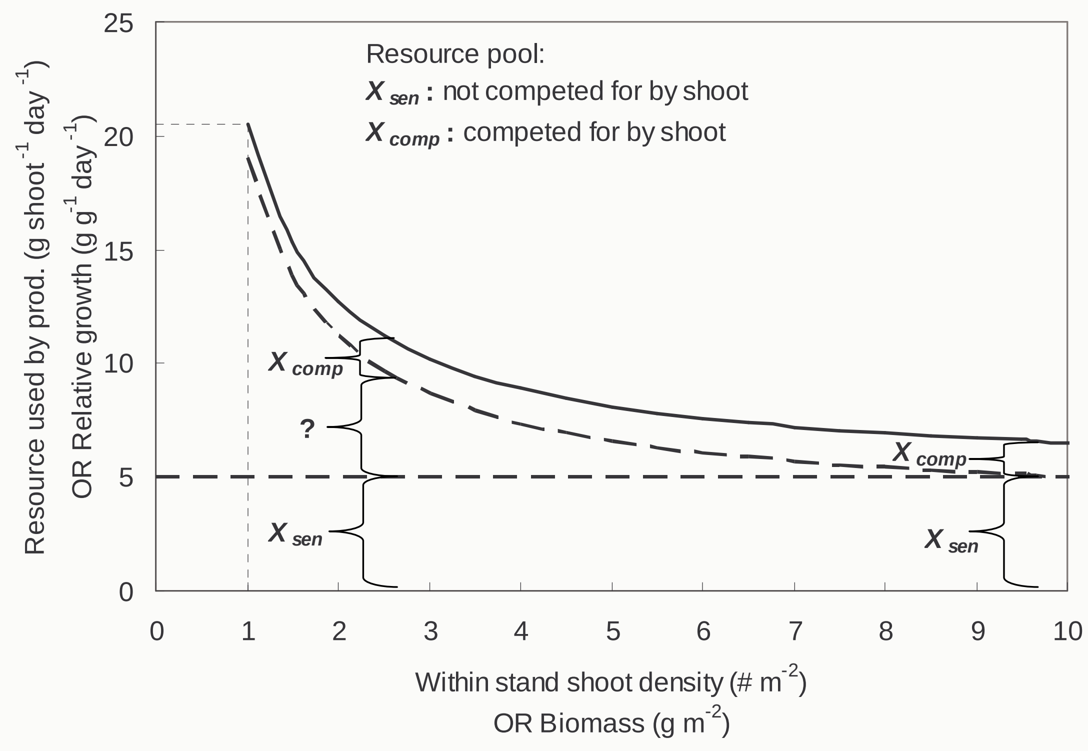


Figure 9.3: Calculation of the relative amount of a shoots' resources competed for (X_{comp}) or not affected by between shoot competition (X_{sen}). The X_{sen} fraction of resources used by the shoot is unaffected by shoot density at high densities, and represents the resources that are not competed for by the shoot. The X_{comp} fraction represents the resources used by the shoot, but which is affected by the shoot density, and thus is competed for by the shoot. At lower densities the X_{sen} fraction may not remain constant as the higher production rates may be associated with higher turnover, which would increase the rate of remobilisation of the resources, and the X_{sen} fraction. It is not possible to determine this fraction, unless there is data for rates of remobilisation and biomass turnover.

Model assumptions

The competition model of low biomass assumes that:

1) all (inter- and intraspecific) individuals are competing for a common resource,
2) the resource is equally available to all the individuals (homogeneity of resource),

3) all plants have similar resource productivities (i.e. that all plants produce the same amount of biomass given the same quantity of resource),

Experiments involving homogenous soils, in small pots, and comparing different planting densities of the same species should avoid the problems listed above. *In situ* evidence for this hypothesis is however subject to these problems.

Model predictions

Specific predictions of this model are, that:

1) Higher biomass of competing plants will reduce the availability of nutrients and thus growth rate of focal plants. Similarly, where a species has multiple connected shoots that each root adventitiously, and show low physiological integration, a higher density of shoots will result in higher within-individual competition for each shoot,
2) Growth of plants should conform to a general hyperbolic function of the form: growth = nutrients available / total biomass competing with the plant, and may include a asymptote term, and
3) An increase in standing biomass, or reduction in access to nutrients, will result in less ability to respond to burial.

The final model prediction tests whether a low biomass of competing plants is beneficial to a partially buried plant, establishing the ecological relevancy of the model.

Testing the competition model of low biomass

Three sets of experiments were undertaken to measure:

1) the ability of *Scaevola plumieri* to grow with and without burial when growing *in situ*, with different densities of shoots of *S. plumieri*, and distances from competing thicket species,
2) the rates of production of a single species grown at different densities. This experiment was conducted on potted *Arctotheca populifolia* seedlings grown at densities of one, two and three plants per small pot, and
3) the *in situ* production rates of five species of dune plants growing naturally, with each species growing at different characteristic biomasses.

In the following pages each set of experiments are reported separately, due to their considerable differences in aims, and methods. A final review of the evidence for the low biomass model of competition is given at the end of the chapter.

EXPERIMENTAL TESTS OF THE COMPETITION MODEL OF LOW BIOMASS

A survey of the factors affecting the production of *S. plumieri* shoots

The competition model of low biomass predicts that given the same resource inputs the production of an individual shoot would depend on the biomass of competing shoots or species present at a site. This model was tested in two comparable experiments: relating a survey of the leaf and stem production of *S. plumieri* shoots on 50 dunes at the Cintsa West dune system to measures of within and between species density and biomass, and a survey of shoots at Old Woman's River conducted using a similar protocol, but experimentally burying half the shoots. More specific hypotheses were tested to determine the influence of the distance to competing vegetation, the size of dunes and burial state on the measured stem and leaf production. It was predicted that: 1) within stands of *S. plumieri*, shoot density will determine the available soil resources and thus the shoot productivity, 2) the distance to non-*S. plumieri* competitors would contribute to competition for soil resources and thus shoot productivity, 3) buried biomass may be remobilised for new growth, thus the initial size of the shoot may serve as a proxy for within shoot resource availability, prior to burial, 4) burial would necessitate increased production, while at the same time may introduce more nutrients into the dune system, and 5) stem and leaf production may be limited and responsive to different factors, particularly stem elongation in response to burial and leaf production in stable conditions of greater resource availability.

METHODS: *S. plumieri* production

Survey of production near Cintsa West

The distribution of isolated *S. plumieri* dunes near Cintsa West, allows a comparison of this species growth at varying densities of *S. plumieri* shoots, and varying distances from a high biomass of stable-dune (thicket) species. A few low biomass herbaceous species grow in *S. plumieri* stands, but account for little of the total biomass (pers. obs.), and were excluded from the analysis.

Fifty dunes, formed by *S. plumieri*, were selected along 2 km of coastline west of Cintsa West. The leaf area production characteristics of the dunes were estimated from production measurements of three shoots measured in the centre of each dune for the spring period (15 July to 5 November 2006). On each shoot the number of leaves produced and the length and width of the 8th leaf (representative of other mature leaves) were measured. The following environmental factors were measured for each shoot: distance from lowest leaf to the sand (used to estimate the minimum time since the leaves of a shoot were last buried), density of *S. plumieri* shoots per dune area, and distance to the nearest thicket vegetation. A more detailed description of the calculation and analysis of the production characteristics of the shoots for this experiment can be found in Chapter 7 (pp. 193).

Experimental manipulation of the burial state of *S. plumieri* shoots at Old Woman's River

Pairs of similar *S. plumieri* shoots greater than one metre distance from each other were selected within stands formed by single *S. plumieri* individuals at Old Woman's River, on 16 November 2006. A random shoot of the pair was placed in a 12 cm diameter PVC tube and buried to above the leaf apice, with freshly blown sand. A range of environments were chosen for the experiments, including areas close and far from non-*S. plumieri* vegetation, and on the seaward-side and landward-side of the foredune. A similar, but more detailed set of measurements to the previous survey were made on 16 November 2006 and 20 February 2007, after which the shoots were harvested. Leaf areas were measured in a similar manner to the previous survey, but included the 8th, 12th and the last leaf on each shoot. Environmental measurements were similar to those measured previously, but also included a rank index of the amount of competitive thicket vegetation for each shoot: 0 = no competitors nearer than 10 m and less than 50% of this area covered by *S. plumieri*, 1 = no competitors nearer than 10 m and more than 50% of this area covered by *S. plumieri*, 2 = competitors > 5 m distant and of small extent (<4 m^2), 3 = competitors > 5m distant and of great extent (>4 m^2), 4 = competitors > 2m distant and of small extent (<4 m^2), 5 = competitors > 2m distant and of great extent (>4 m^2), 6 = competitors < 2 m distant and of small extent (<4 m^2), 7 = competitors < 2 m distant and of great extent (>4 m^2).

Analysis: partitioning of the variance in production explained by environmental factors

Burial, dune position and competition indicators may explain some of the variance in stem and leaf area production of *S. plumieri* shoots at OWR. In addition to these factors, the total leaf area of shoots prior to the burial treatment may be an indication of the resources available to the shoot, and is likely to account for much of the variance in the leaf and stem production after the treatment. Connolly *et al*. (2001) suggest that the initial biomass of plants measured in competition analyses is a vital factor in determining the effect of competition on the plant. For this reason the initial biomass, as estimated by initial shoot leaf area, was included in the analysis as described below. The variance in the initial biomass may also be related to the other competition factors. Therefore, it is of interest to ask: Are leaf and stem production correlated to the initial leaf area of the shoots? If so, does the burial treatment change this relationship? The other measured parameters: position, competitor index, distance to thicket, and within stand shoot density may also affect this relationship, in two ways. Firstly, they could change the elevation of the relationship, statistically affecting the residuals. This would be interpreted as a change in production given the same starting total leaf area, and indicates a change in the resources during the course of the experiment. Secondly, these parameters could result in shift along the relationship, statistically a shift in the fitted values of the relationship. This type of effect would indicate that these factors result in a fundamental shift in the resources available to the shoot in general, not just production after burial. Finally, both shifts could be present indicating an experimental and pre-experimental effect.

An initial Standardised Major Axis (SMA; see Appendix 1) bivariate analysis was done to test for the effect of experimental burial on the relationship of stem or leaf production with initial shoot leaf area. If burial had a significant effect on this relationship, then the variance accounted for by the other factors was partitioned using a multiple regression analysis for the residuals and fitted values within burial treatments. If burial had no effect, a multiple regression analysis was done for the residuals and fitted values with burial treatments combined.

Position relative to the foredune (seaward or landward areas) may relate to the competition predictor variables, or may represent other factors affecting production (e.g. overall resource availability). Thus, a further question is: Does position account for the variation additional to that accounted for by the competition predictor variables? This was answered by conducting a partial-F-test which compared a regression model with position as the only predictor, and a second model with all important factors as predictors. A partial-F-test allows an assessment of whether regression models with and without certain predictors explain statistically different amounts of variation. Multiple regression accounts for variation in the dependent variable in a cumulative manner, thus variation accounted for by the first variable, will not be taken into account when assessing the second variable (Sokal and Rohlf, 1981). This is particularly evident for correlated predictor variables included later in the order of variables for the multiple regression analysis (intentionally the last variable was position). Therefore, large differences in the R^2 value for position between the multiple regression analysis and a single variable regression analysis indicate that the other predictors account for much of the variation for which position accounts.

It was assumed *a priori* that the predictor variables had no interactive effects in the multiple regression analysis. Therefore, the simplest main effects model was fitted, with the predictor order as shown in Table 9.1. The percentage of the total variance in fitted or residual values explained by a predictor was calculated as the SS of the predictor divided by the total SS, and scaled to sum to the adjusted R^2 value. The results table for this section is structured so as to maximise clarity on this procedure of apportioning variation in the relationship between production and initial shoot size (Table 9.1).

RESULTS: *S. plumieri* production

Factors accounting for variation in stem and leaf production at OWR

The initial leaf area of shoots explained between 60 and 80% of the variation in stem and leaf production (Table 9.1). As these variables were highly correlated and both production and initial leaf area may be affected by the same environmental factors, the variation in the residuals or fitted values of these relationships were investigated. Since the initial leaf area was measured before the burial treatment, and the production was measured in response to the burial, therefore: 1) a significant change in residuals indicates an elevational shift in the relationship (an experiment related shift), while 2) a change in the fitted values indicates a shift along the common relationship (a shift that is explained by competitive effects on the initial biomass before the treatments).

The factors affecting the relationships between leaf or stem production with initial leaf area were different (Table 9.1). Burial resulted in a significant increase in stem production, but not leaf area production for similar initial leaf areas (Figure 9.4a and b; a significant slope effect in Table 9.1 for the stem production of buried shoots). Position had similar effects on both the relationships of leaf and stem production to initial leaf area, across burial treatments (Figure 9.4c and d), but an additional decrease in stem production in landward shoots was observed (Table 9.1; significant shifts along common axis and elevation shift for stem production of buried shoots). Little variation in the residuals of the production to initial leaf area relationship was explained, apart from 19% of the variation in the stem production to initial leaf area relationship of buried shoots (Table 9.1). Position accounted for most of the variation in the fitted values of each relationship, in all cases with a smaller production rate in landward shoots (Table 9.1). The distance of the shoot from the thicket, and the competition index accounted for some of the variation in the production to initial leaf area relationships (Table 9.1). These factors combined accounted for 19 to 44% of the variation in the fitted values (Table 9.1). Most of the variation between positions is described by the competition variables (comparison of R^2 values for position in the multiple and single regression analyses). This indicates that low shoot densities and a large distance from competitors may explain much of the increased growth of seaward shoots (Table 9.1). However, for leaf area production and stem production in unburied shoots, the competition variables and position accounted for significantly more variation than just position by itself (Table 9.1; the partial-F tests). Thus, the major effect of competition and position on the growth of the buried and unburied shoots was present prior to the experiment. The initial leaf area was higher for low competition and foredune shoots, resulting in higher growth rates during the experiment.

Table 9.1: The effects of burial, position relative to the foredune, distance to thicket, within stand shoot density, and competition index on the relationship between leaf area or stem length production with initial shoot leaf area. The results represent the effects tested for by a bivariate SMA analysis (burial and position), and multiple regression results for residual and fitted values of these relationships with competition factors (distance from thicket, shoot density and competition index). Significant effects are indicated in bold.

	Relationship:	Leaf area production vs. initial shoot leaf area			Stem length production vs. initial shoot leaf area					
Burial effect on:	Slope	P-value=	0.628		P-value=	**0.011**				
	Elevation		0.462			na				
	Shift[a]		0.847			na				
		Both treatments			**Unburied**			**Buried**		
		R^2	P-value	n	R^2	P-value	n	R^2	P-value	n
	Overall relationship:	0.795	**0.001**	61	0.684	**0.001**	30	0.593	**0.001**	30
Position effect on:	Slope		0.443			0.761			0.879	
	Elevation		0.137			0.629			**0.014**	
	Shift		**0.001**			**0.001**			**0.005**	
Partitioning of variation in:		R^2	P-value	Coef.[b]	R^2	P-value	Coef.	R^2	P-value	Coef.
Residuals by:	Distance to thicket	0.1%	0.683			n.i.			n.i.	
	Within stand shoot density[c]	0.2%	0.560		7.7%	0.076			n.i.	
	Competition index	1.7%	0.100			n.i.			n.i.	
	Position[d]	1.8%	0.088			n.i.		18.7%	**0.011**	-RD
	Total variance explained:	3.8%	0.191		7.7%	0.076		18.7%	**0.011**	
	Position only[e]	0.0%	0.226		n.a.			n.a.		
		Partial Ftest P-value =		0.200						
Fitted values by:	Distance to thicket	10.9%	**0.007**	-	2.9%	0.202		3.3%	0.210	
	Within stand shoot density		n.i.			n.i.			n.i.	
	Competition index	20.4%	**0.001**	-	24.6%	**0.001**	-	12.9%	**0.017**	-
	Position[d]	9.1%	**0.003**	-RD	16.1%	**0.005**	-RD	5.6%	0.104	
	Total variance explained:	40.4%	**0.001**		43.6%	**0.001**		21.8%	**0.025**	
	Position only[e]	29.2%	**0.001**	-RD	32.1%	**0.001**	-RD	18.4%	**0.011**	-RD
		Partial-Ftest P = **0.003**			Partial-Ftest P = **0.034**			Partial-Ftest P = 0.219		

[a] shift along a common relationship; [b] sign of slope coefficient of parameter to fitted or residual values if significant; [c] the reciprocal of shoot density was used for the analysis; [d] the variance accounted for by position after the variance of the other predictors has been removed; [e] the variation accounted for by position with bivariate regression, and compared with the partial-F-test to the variation explained by all predictors combined.

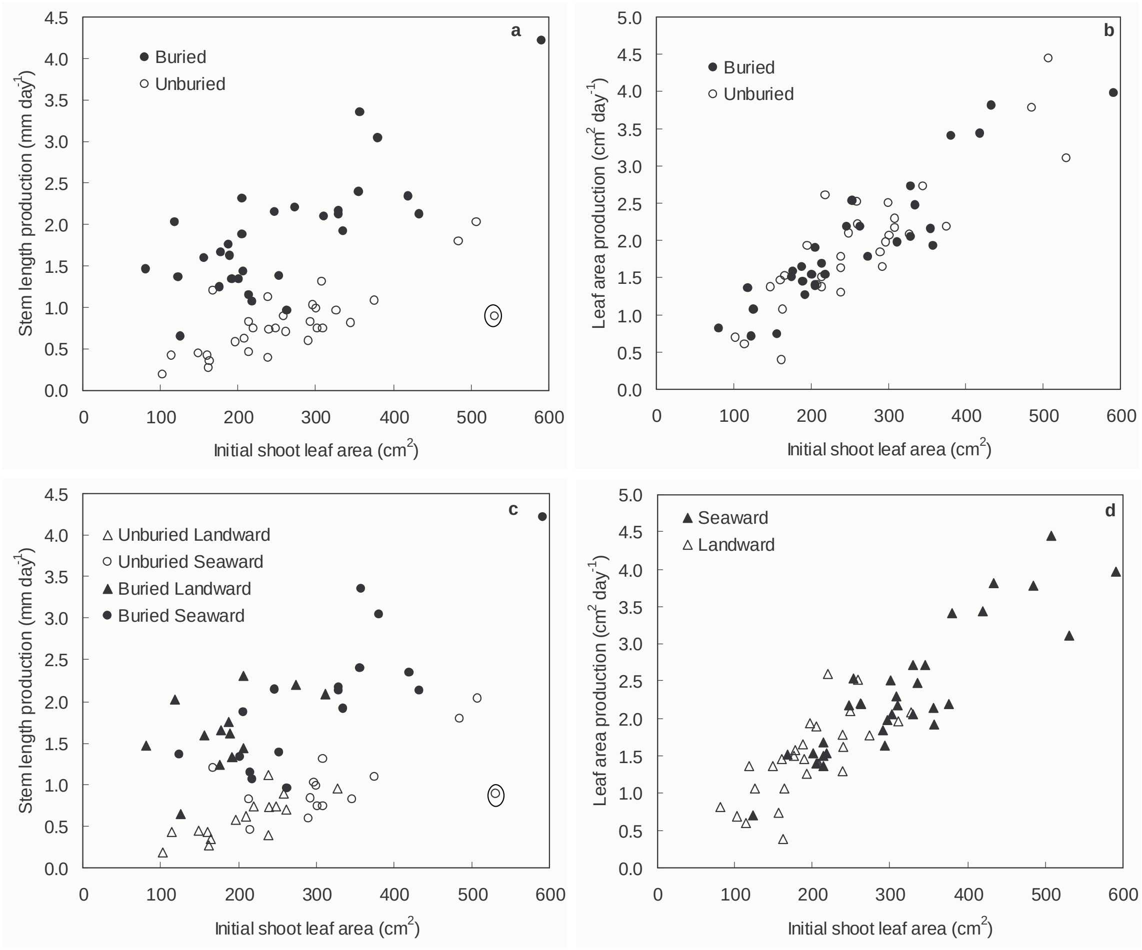


Figure 9.4: Shifts in the stem (a and c) or leaf production (b and d) to initial shoot leaf area relationship, accounted for by burial (a and b) and dune position (c and d). The circled point had undue leverage on regressions of stem production, and was excluded from all analyses.

Factors accounting for variation in leaf production at Cintsa West and OWR

The production data gathered for *S. plumieri* at Cintsa West serves as a confirmatory test of the relationships found at OWR. The competition variables: within stand shoot density and distance to the high biomass thicket, were significantly related to leaf area production at both sites (Figure 9.5a, b and c). There was the same large and significant reduction of leaf area production of shoots within 2.5 metres of thicket vegetation for both sites (Figure 9.5a and b). Similarly, for shoots growing in areas of shoot densities above 15 shoots per metre square, *S. plumieri* showed reduced leaf area production (Figure 9.5c). The relationship of shoot density and distance to thicket species with leaf area production is shown in Figure 9.6.

The length of exposed stem was poorly, but significantly, related to leaf area production in unburied plants at both sites (Figure 9.5d). The production rate of exposed stems was calibrated for 33 shoots at Cintsa West by measuring stem length between the yearly flower scars of this species. These shoots had an average stem production rate of 14.6 cm per year (R^2 = 0.88). Since there is an indication that leaf production decreases with the length of stem below the lowest leaf (Figure 9.5d), this can be related to a rough estimate of the amount of time the stem has grown since the leaves were last buried. The 38 to 52 cm x-intercepts for Cintsa and OWR stems relate to approximately 2.6 and 3.5 years growth, respectively. The effect of burial on the stems of these plants was reported in Chapter 7, pp. 197.

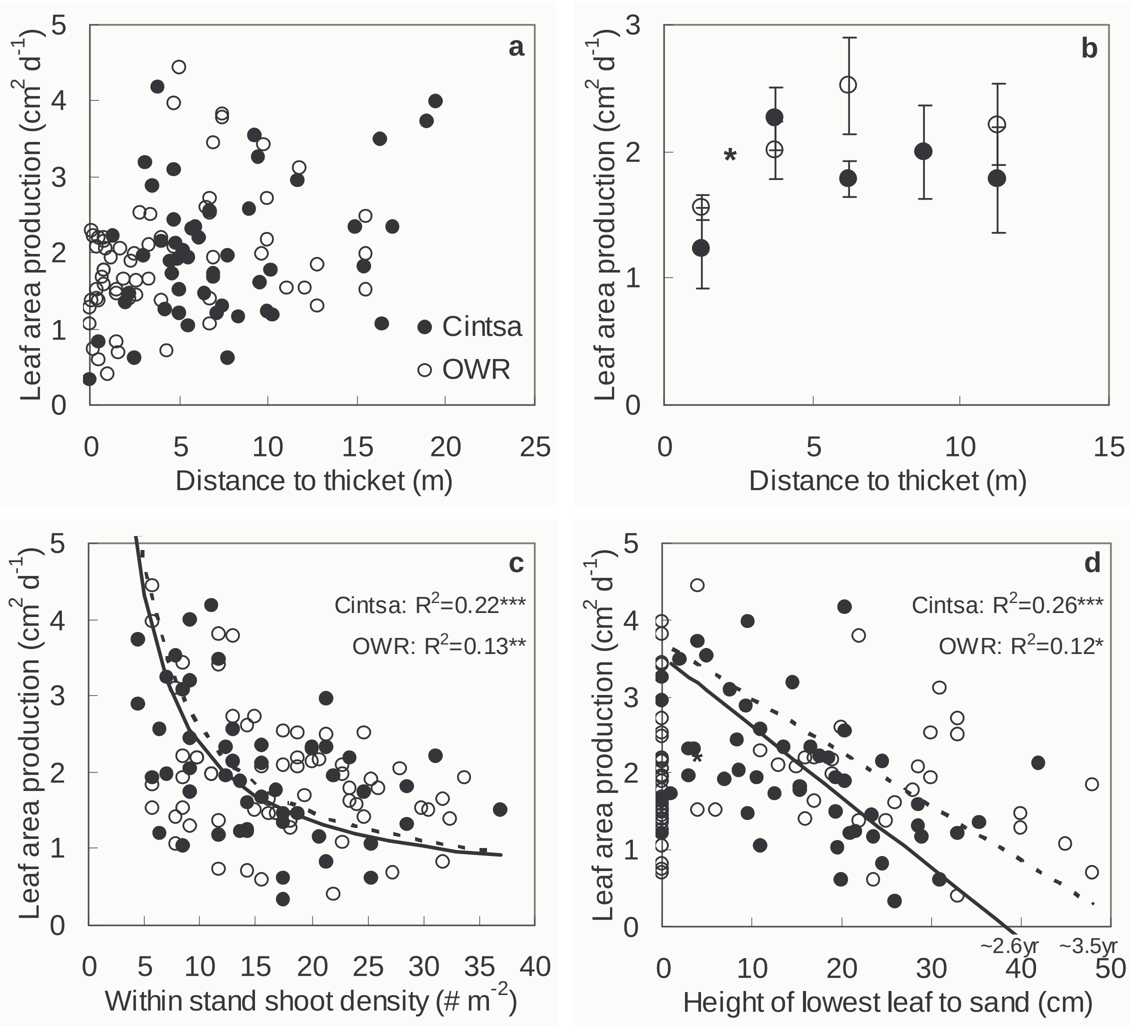


Figure 9.5: Leaf area production related to distance to competitor (a and b), within stand shoot density (c) and height of lowest leaf above the sand (d) for shoots of *S. plumieri* surveyed at Cintsa and OWR. The points in b represent means with standard errors of the mean for 2.5 m intervals where there were $n \geq 3$. * = $0.01 < P < 0.05$; ** = $0.001 < P < 0.01$; *** = $P < 0.001$ and represent the results of a t-test of combined data at the 0.05 level of confidence (b), or significance of the correlation coefficients for SMA regression lines (c and d). The regression lines in d were fit excluding buried shoots (height of zero), all other graphs include buried shoots. For d the intercept values are estimates of the time needed to produce that length of stem.

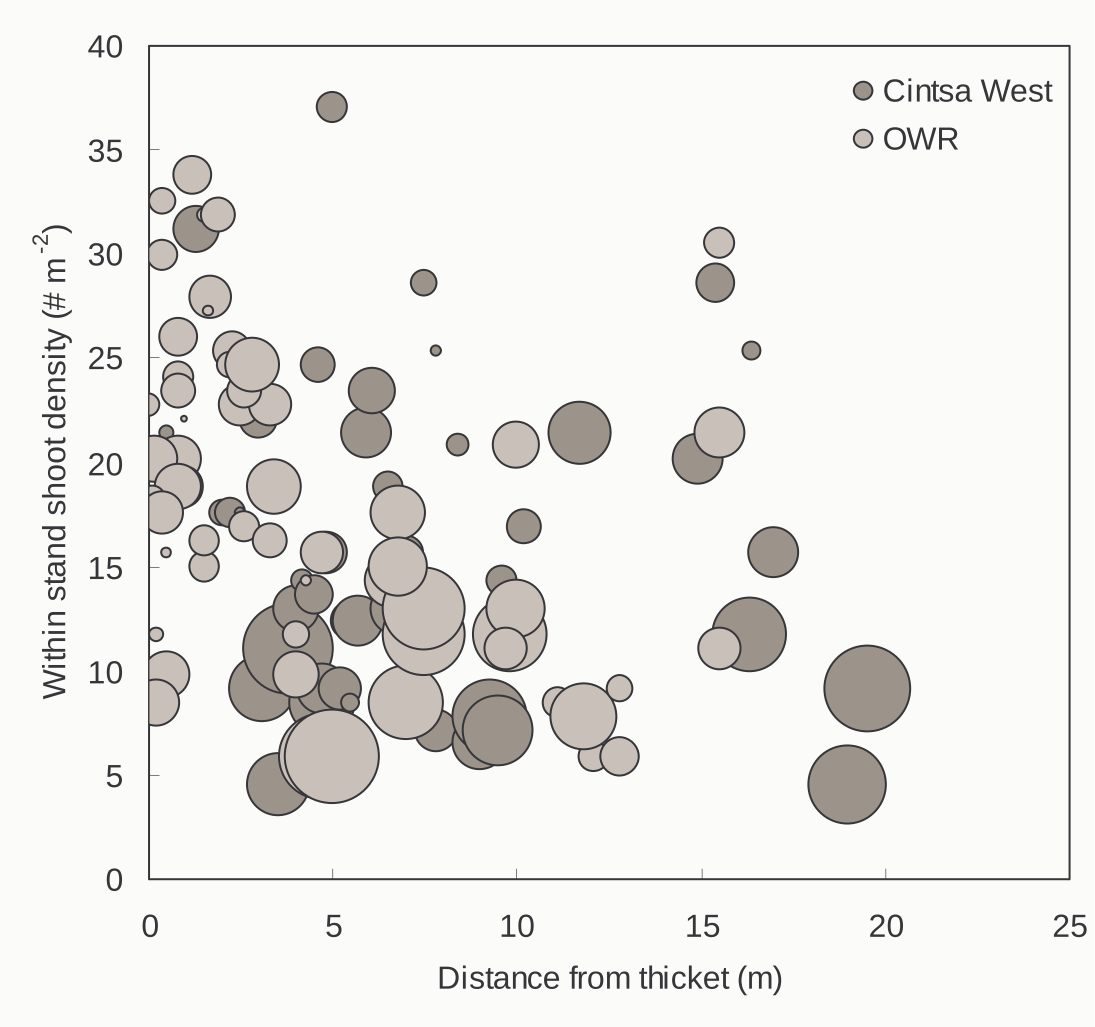
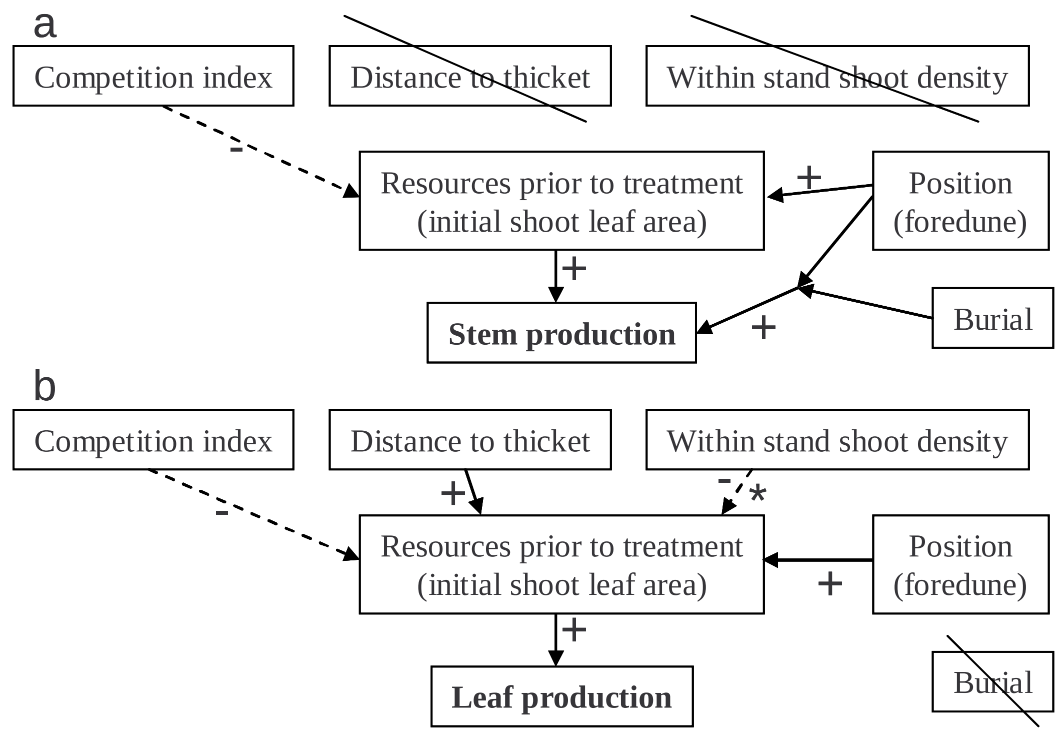


Figure 9.6: Leaf area production (circle size) related to two competition variables: within stand shoot density and distance from thicket. The leaf area production is proportional to the width of the circles.

DISCUSSION: *S. plumieri* production

Within stand and neighbour effects on *S. plumieri* growth

The effects of within stand shoot density and the distance to the nearest thicket area were significant, although they only explained between 10 and 25% of the variation in stem and leaf production (Table 9.1). Stoll *et al*. (2000) put these results in context; the relationship of production to neighbour density is usually triangular, as at high density competition limits production, but at low density high production or low production can occur due to limitations by other factors. Thus, local competition is a limiting factor and not a direct determinant of growth in all circumstances (Stoll *et al.*, 2000). These considerations explain why only about 40% of variation in production is typically accounted for in neighbour analysis (Stoll *et al.*, 1994; Stoll *et al.*, 2000). In this context, the competition model of low biomass may determine the envelope of possible production rates, and not necessarily the actual production within these boundaries. The data does indicate that at low stand densities, at distances greater than 2.5 metres from thicket vegetation, and on the seaward side of the foredunes shoots of *S. plumieri* had significantly higher leaf area production rates. However, when buried, stem production rates were increased and the competition factors account for less of the variation in buried stem production (Table 9.1). This does not necessarily mean that the size of the effect is smaller, rather that less variation is explained by the competition factors, and fewer factors correlate with production. Considering the small amount of variation explained by competition factors it was not considered possible to reliably estimate the size of the effects for the burial treatment. In general, hyperbolic models explained more variation than linear models, consistent with the competition model of low biomass and other studies (e.g. Weiner, 1984).



*significant in individual analyses, not Table 9.1

Figure 9.7: Path diagrams of significant effects of competition factors, position and burial on the initial shoot leaf area, stem (a) and leaf production (b) of shoots of *S. plumieri* at OWR. Negative (dashed lines) and positive (solid lines) influences are shown. Only the interaction between position and burial has a direct effect on production (significant residual shift in Table 9.1), all other effects are via changes in initial shoot leaf area (significant fitted value shift in Table 9.1). Factors with lines through them were not included in the regression models.

Competition factors accounted for variation in the fitted values of the leaf and stem production to initial leaf area relationships (Table 9.1). Only the stem production of buried plants was enhanced in foredune plants due to a shift in the residuals of this relationship. Thus, in general, competition affects the resources available to the shoot prior to burial (measured by initial shoot leaf area), and this in turn affects the rate of shoot growth after burial. Only for the stem production of buried shoots was there a more direct effect of position on production, independent of the initial shoot leaf area. A path diagram is shown in Figure 9.7 summarising these results.

The effect of density on the growth of *A. populifolia*

The competition model of low biomass suggests that when grown at higher densities plants of the same species should have lower growth rates due to reduced available resources. The competition model can be tested by growing plants in small pots, at different densities. The total production of each pot should be similar, but individuals at higher densities will have lowered production. Allometric growth, or plasticity, may result in deviations from this model, as at high densities plants may produce more stem, or less reproductive tissue (Vega *et al.*, 2000).

METHODS: *A. populifolia* density

Plant growth conditions and treatments

Seeds from natural populations of *A. populifolia* were collected (December 2005 to March 2006), air-dried and placed in seedling trays in a greenhouse on 20 March 2006. The germinated seeds were transplanted into 2L pots at densities of 1, 2 or 3 on 3 April 2006. The pots were filled with washed oligotrophic beach sand that was homogenised prior to filling the pots. Pots were placed in random order, and rotated periodically. All of the pots were watered periodically with 200 or 400 ml of tap water. This was adequate to maintain the soil water content in each pot above drought stress levels. Soil water contents were checked periodically with a theta probe (ML2x, Delta-T Devices, Cambridge, UK). The cumulative volume of water added to each pot, and thus, the amount of soluble nutrients was the same across all treatments. Greenhouse conditions were PAR of greater than 1000 μmol m^{-2} s^{-1} for greater than 7 hours per day, a maximum day temperature of 34°C and minimum night time temperature of 15°C.

Census measurements

Nine days after transplanting, the seedlings were considered to be acclimated to the pots, and the first census was done. Subsequently, censi were repeated at two week intervals, for 165 days. After the first census, the pots with the two largest and two smallest plants were removed from each treatment to reduce initial variance (Poorter, 2007). Censi were conducted on each plant within all pots, measuring the lengths and widths of one leaf of each leaf pair, and the total length of stem. Leaf area's were estimated from a regression of leaf length times width versus leaf area for harvested plants ($R^2 = 0.98$, $n = 68$, Leaf area = 0.505*length*width).

Harvest

After the first and fourth census a random sample of five pots was harvested, and after the final census the remaining pots were harvested. Individual plants were separated by placing the pots on a sieve and rinsing the sand from the roots. The root, leaf and stem portions of each plant were weighed after drying in an oven at 60°C for more than 48 hours. The sample sizes for the final harvest and all census dates were 14, 13 and 12 pots for the 1, 2 and 3 densities, respectively.

JIP analysis

An indication of the photosynthetic capacity and resource allocation to photosynthesis was needed to determine if the leaves produced by the plants in the different treatments are of similar quality. JIP-tests involve the induction kinetics of chlorophyll fluorescence after dark adaptation, and are a quick and sensitive qualitative indicator of photosynthetic function and capacity (Strasser *et al.*, 2000). JIP analyses were conducted on the first fully expanded leaves of plants from all pots, the day prior to the final harvest. F_O, F_M, F_V/F_M and *PI(abs)* were calculated for each leaf from measurements made using a PEA analyser (Hansatech Instruments, King's Lynn, Norfolk, UK) after placing the leaf in a dark-adaptation leaf clip for 30 minutes. A transient showing the change in fluorescence was measured during a one second illumination of a leaf section with high light of 650 nm. For more details see Strasser *et al*. (2000) and references therein. F_O gives a measure of the amount of inactive PSII reaction centres in a leaf cross section. F_M is a measure of the maximum excitation of PSII. F_O and F_M are used to calculate $F_V/F_M = (F_M-F_O)/F_M$ which is the maximum potential proportion of PSII's that are used to provide reductant for later photosynthetic processes. F_V/F_M values near 0.8 represent optimal non-photoinhibited photosynthetic tissue. *PI(abs)* was designed as a indicator of the performance of the light absorption, electron transport and heat dissipation components of the photosynthetic machinery (Strasser *et al.*, 2000). Empirically, *PI(abs)* is a good indicator of production and photosynthetic differences between plants (Hermans *et al.*, 2003). Caveat: as there is no published empirical validation of the JIP models used in this analysis to calculate *PI(abs)*, we regard the results as a quick and useful *qualitative* insight into the leaf photosynthetic machinery.

Analysis

Two measures of response were investigated: the pot total leaf area or mass production in a pot, and the average leaf area or mass produced per plant. To avoid pseudoreplication in the 2 and 3 planting density treatments, the average leaf area per plant of each pot was used rather than the counting each plant as a replicate. Where this approach was inappropriate (analysis of biomass allocation) a single plant from each pot was randomly chosen for inclusion in the analysis.

RESULTS: *A. populifolia* density

Planting density resulted in large total and relative growth differences between treatments (Density effect on *RGR*: $F_{2,30} = 9.845$, $P < 0.001$; Figure 9.8a and b), such that plants growing at a low density were twice to three times the size of plants growing at higher densities. The total biomass in each pot was the same for all treatments (Density effect on total biomass: $F_{2,30} = 0.014$, $P > 0.817$). The total leaf area of plants or per pot had similar trends (Figure 9.9a and b). The total leaf area of the plants at the end of the experiment were higher in the more dense treatments, consistent with the differences in initial plant leaf area (Figure 9.9b). This effect was attributed to the unequal starting biomass between treatments, probably due to the differing contribution of seed nutrient reserves between treatments.

Are there allocational consequences for the increased growth rate in the low density treatment?

All treatments fell on the same nonlinear relationship between final leaf and root mass (Figure 9.10a). Thus, larger plants, in less the dense treatments, had greater root mass relative to leaf mass. Stem mass was related to total plant mass by a single linear relationship for all treatments, indicating that there was no shift towards more stem mass at higher densities (Figure 9.10b).

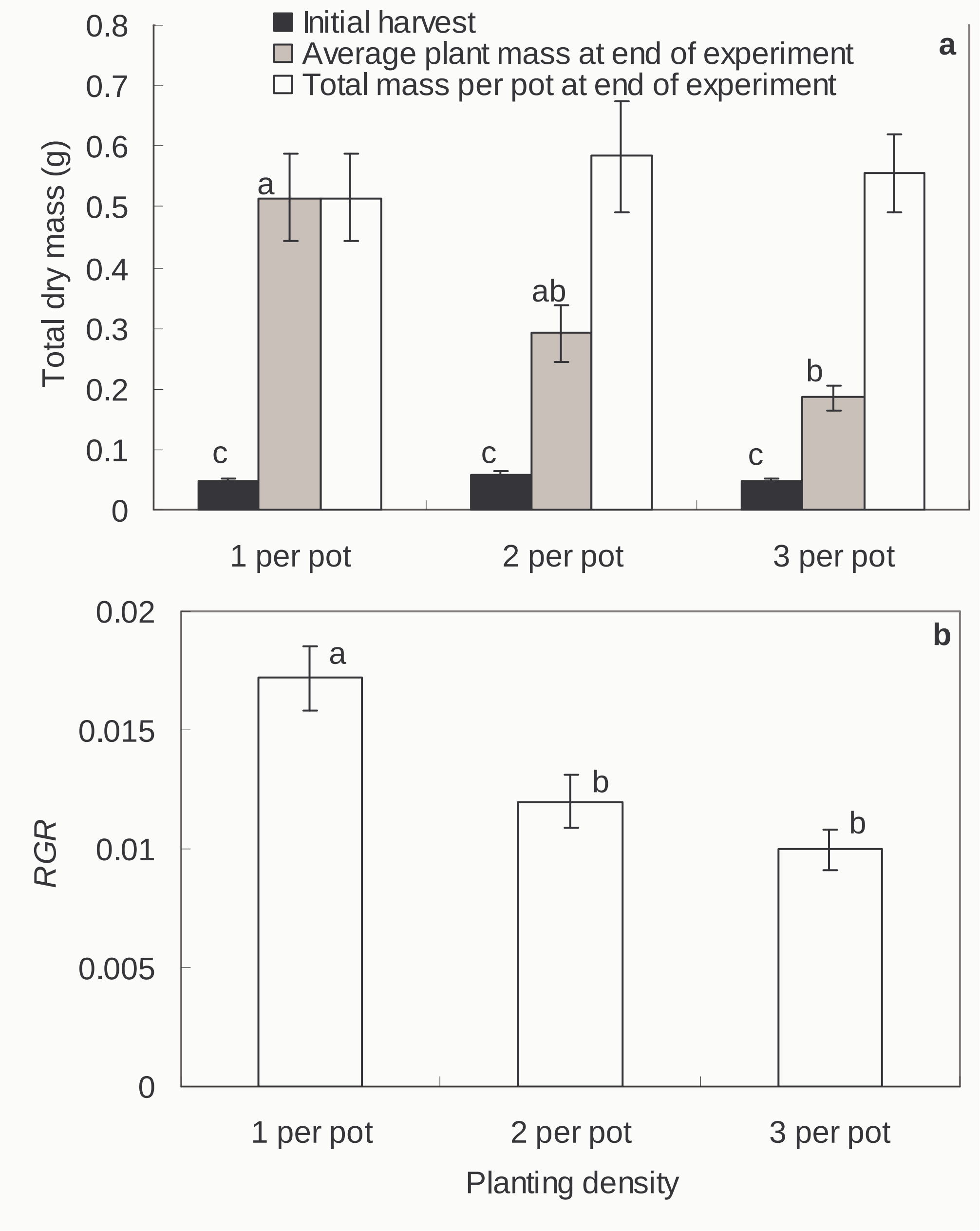


Figure 9.8: Dry mass of harvested *A. populifolia* seedlings at the start and end of growth in pots at three densities (a), and relative growth rates (g g^{-1} day^{-1}) of average plants from each planting density (b). Error bars are standard errors of the mean, n = 9-13. Letters represent significant differences between densities found with Tukey HSD *post hoc* comparisons.

The slopes of both of these relationships, between densities, were not significantly different (*P* > 0.90 for both relationships). Similarly, there were no differences in elevation between densities for either relationship (*P* > 0.40 for both relationships). However, the three density treatments were significantly shifted along the shared curves for both sets of relationships (*P* < 0.001). The small size of the seedlings and the lack of an increase in stem allocation suggest that little light competition occurred.

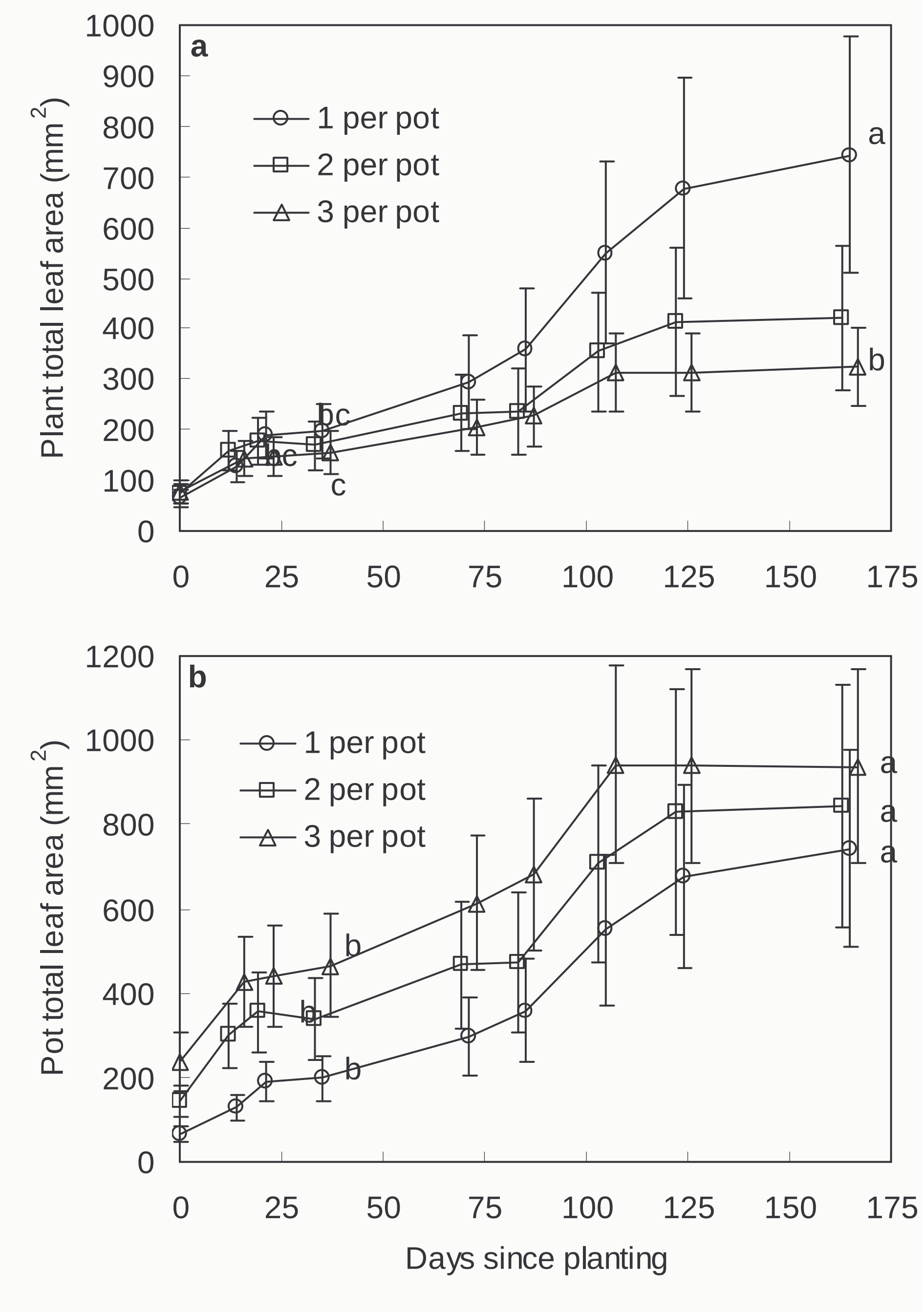


Figure 9.9: Leaf area production on a per plant basis (a) or a per pot basis (b), for three different planting densities. Data points have been shifted ± 2 days for sake of clarity. Error bars are standard errors of the mean (n = 9 to 13). Repeated measures ANOVA was done for the days of harvesting (35 and 165 days), these results are indicated by letters representing significant differences.

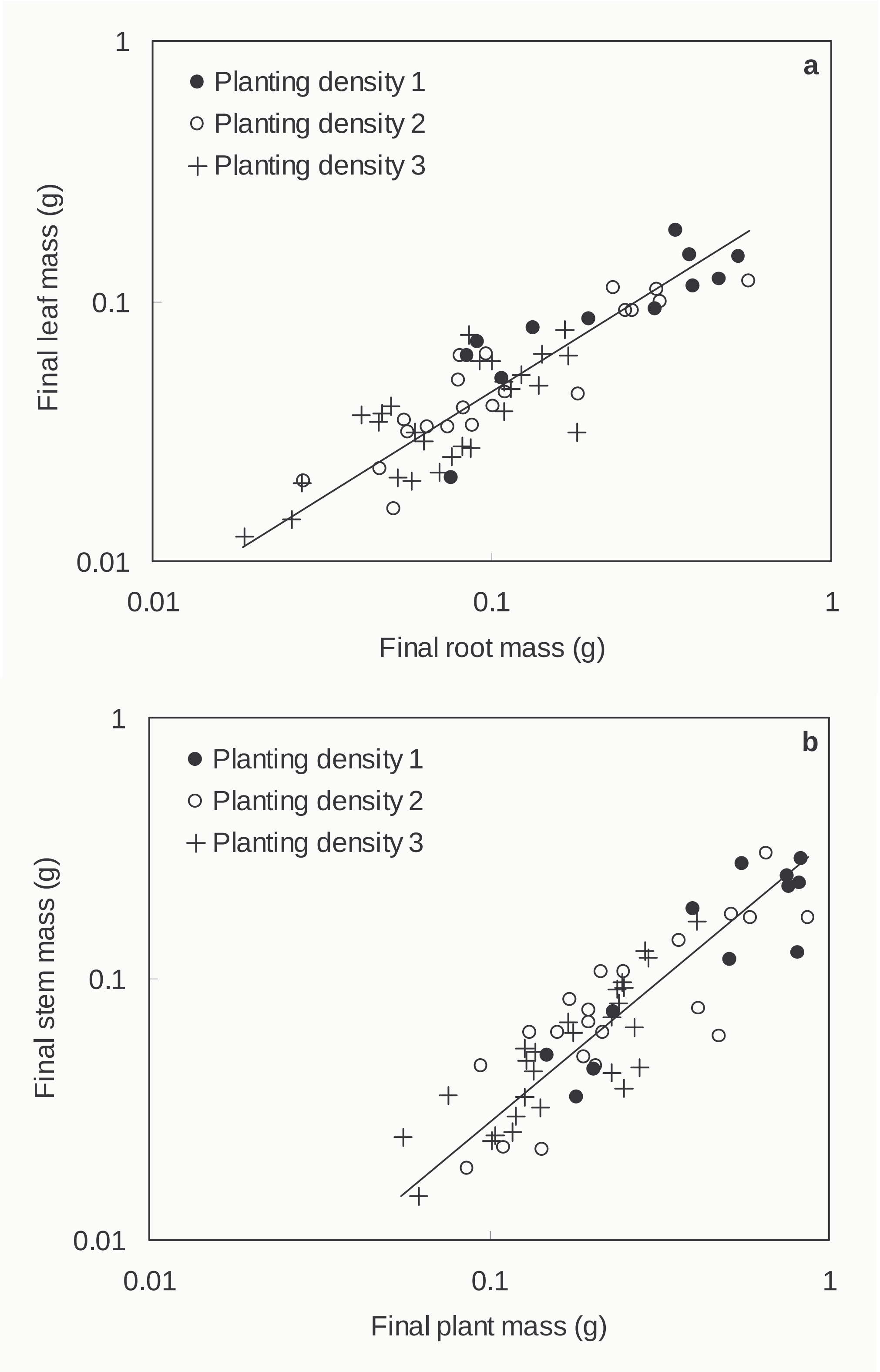


Figure 9.10: Allometric relationships between leaf and root mass (a), and stem and total plant mass (b) measured at the final harvest for three planting densities. An SMA line fit to all the data is presented. The equations for these lines are: leaf mass = 0.294*root mass$^{0.815}$, R^2 = 0.78, n = 60, stem mass = 0.342*plant mass$^{1.09}$, R^2 = 0.78, n = 60.

Is the biomass produced in each treatment of similar construction cost?

The total biomass produced in pots of each planting density, must be of the same construction cost for the observed similar biomasses to support the competition model. Specific leaf area (1/*LMA*) is related to other important leaf parameters that also give some indication of leaf construction cost (Wright *et al.*, 2004). Similarly, the JIP-test is sensitive to changes in leaf photosynthetic properties (Strasser *et al.*, 2000). Neither, the JIP-test parameters or *SLA* showed large significant differences between treatments (Table 9.2). F_M was 11% higher in leaves of plants planted at the highest density relative to plants at the lowest density. The small F_M difference and lack of a difference in the performance index suggest that the leaves of the treatments are of similar chlorophyll content (F_O indicates PSII concentration) and photosynthetic capacity.

Table 9.2: *SLA* and JIP-test measurements made on the first fully expanded leaf one day prior to the final harvesting.

Density	*SLA*	F_O	F_M	F_V/F_M	*PI(abs)*
1 per pot	113±5	398 ±11	2390 ±65 b	0.796 ±0.005	21 ±1.4
2 per pot	116±6	436 ±16	2551 ±53 ab	0.796 ±0.004	23.4 ±1.7
3 per pot	107±6	424 ±11	2644 ±71 a	0.799 ±0.004	19.6 ±1.7
F^a	0.588	2.32	4.13	0.19	1.39
P>	0.564	0.114	**0.025**	0.832	0.263

[a] degrees of freedom were 2,54 and 2,33 for the *SLA* and JIP-test related F statistics, respectively.

DISCUSSION: *A. populifolia* density

Greenhouse grown *A. populifolia* had reduced growth at higher planting densities (Figure 9.8). This effect was additive, such that pots across all planting densities supported the same total biomass. Disproportionate allocation of biomass to the stem, at high planting densities could also affect the testing of the competition model. Namely, if plants growing at higher planting densities allocated more biomass to stem, then this would be a complication, as these plants would have a lost an opportunity cost related to producing roots or leaves. However, this was not the case, as stem mass was isometrically related to total mass and a similar relationship was found for all treatments. The higher planting densities showed reduced growth rates, but produced leaves of similar photosynthetic capacity (Table 9.2) and had similar biomass allocation patterns (Figure 9.10a and b). Thus, the increased growth that could be associated with the competition model was not at the expense of other aspects of plant growth.

Interspecies field test of high growth rate related low biomass

The results presented in the previously in this section support the competition model of low biomass, namely: 1) the *in situ* growth of *S. plumieri* shoots was limited by the density of shoots within the same individual, and the distance to the nearest area of high biomass, however burial appeared to change these effects, and 2) pots of *A. populifolia* seedlings, had uniform total biomass when grown at different densities, and individual seedlings had higher growth rates in the less dense treatments. Thus, the competition model could play a role in determining the high growth rates of low biomass dune species, although other factors may also allow increased burial responses (Chapter 7). Is high growth and low biomass a general trend in the dune ecosystem? And, is it ecologically relevant? In order to test this, the growth and biomass of five species of dune plants were monitored for varying periods. These species were selected as each had a characteristic biomass, and these species represented a large range of biomasses.

METHODS: Field test of the competition model

Production

The leaf area, and stem production of seven plots of *A. populifolia*, *S. plumieri*, *M. cordifolia* and *M. muricata* were measured for a year period, between 7 February 2004 and 14 February 2005. In order to expand the number of low biomass species studied, *Hebenstretia cordata* was also measured during the midsummer of the following year, between 4 December 2005 and 14 January 2006. Plots of each species were demarcated by driving 50 cm long steel droppers into two corners of a metre square quadrat. Within each of these plots three shoots (except for *M. muricata* and *H. cordata* plots) were marked with wire, such that newly produced leaves could be counted as increases in the number of leaves above the wire. Similarly, leaf loss was determined by successive counts of the number of leaves below the wire. The wire was moved up the stem if there were few leaves below the wire. The average leaf area of leaves on each shoot was measured on each occasion, by measuring the length and width of 5 to 8 of the leaves present on the shoot. Leaf area was accurately estimated for each species by a correlation of leaf length times width with leaf area (*A. populifolia*: $R^2 = 0.98$ for 24 leaves by LA = 0.790*L*W, *S. plumieri*: $R^2 = 0.99$ for 655 leaves and LA = 0.625*L*W, *M. cordifolia*: $R^2 = 0.97$ for 533 leaves and LA = 0.664*L*W). These relationships were generated for a large amount of harvested leaves by measuring leaf area, length and width using image analysis software (WinDIAS, Delta-T Devices, Cambridge, U.K.). Leaf production and loss were measured on 6 dates for the four species during the year measurement period. On each measurement day entire shoots were harvested from areas adjacent to the plots. Harvested shoots were dried for 3 days at 60^oC and weighed. Specific leaf area and aboveground leaf to stem mass ratio were use to calculate stem and leaf mass production, from the leaf area production rates. Production of reproductive structures was estimated as the mass ratio of reproductive structures to leaves, measured on the harvested shoots, multiplied by the leaf mass production for each period.

M. muricata and *H. cordata* have small leaves that were not practical to count. Instead, numerous 5 cm long segments of stem were initially marked and subsequently harvested when the other species were censused. The increase in mass, of the leaves, stem and reproductive structures, distal to the marker were used as estimates of production relative to the leaf and stem mass of representative 5 cm long segments harvested on the first day.

Biomass

The biomass of each plot was estimated from previously established relationships between biomass and: cover (*M. cordifolia*: biomass = $10^{(0.0148*\%cover+1.80)}$, $R^2 = 0.91$, n = 10; *A. populifolia*: biomass = 5.65*%cover-21.1, $R^2 = 0.95$, n = 7) and plant volume (*M. muricata*: biomass = (1522*plant volume+411.2)/plant area, $R^2 = 0.95$, n = 3). The biomass of *S. plumieri* was estimated by multiplying the density of shoots, by the average shoot mass of the harvested shoot for each plot, adjusted for the average leaf area of the three measured shoots. This procedure was shown to be a relatively accurate method of determining biomass ($R^2 = 0.95$, n = 13). The biomass of *H. cordata* plots was estimated by matching pairs of similar plots. One of the matched plots was randomly chosen and harvested, the other was used for monitoring leaf and stem production. These plots were not immediately adjacent to each other, mitigating the negative effect of the harvest on neighbouring plants. Individual shoots of *A. populifolia* and *H. cordata* were clumped and locally abundant. For these species the definition of biomass is important (Ripley and Pammenter, 2004b). Biomass was defined conventionally where these species occupied the whole of a metre square quadrat, or alternatively as the mass of these plants divided by the area occupied by the canopies of these species.

RESULTS: Field test of competition model

In order to validate the production and biomass data measured in this study, and add *I. pes-caprae* as a further low biomass species, this data was compared to the similar data of Ripley (2002), for the OWR site. As that two year study had comparable production and biomasses to those measured in this study this data was included in the analysis (Figure 9.11). A single hyperbolic relationship was found between production and biomass for all species, apart from the short-term data for *H. cordata* (Figure 9.11). Linearization of this relationship, by plotting relative growth against the reciprocal of biomass, allowed this relationship to be parameterised (inset of Figure 9.11). The intercept of the linearised relationship was not significantly different to zero, although here is a strong indication that high biomass plants have relative growth significantly above zero. The hyperbolic nature of this relationship is such that a 50 to 70% reduction in biomass was associated with a 90 to 60% increase in relative growth. The intercept and hyperbolic nature of this relationship result in the growth rewards for a reduction in biomass increasing disproportionately with decreases in biomass (Figure 9.11).

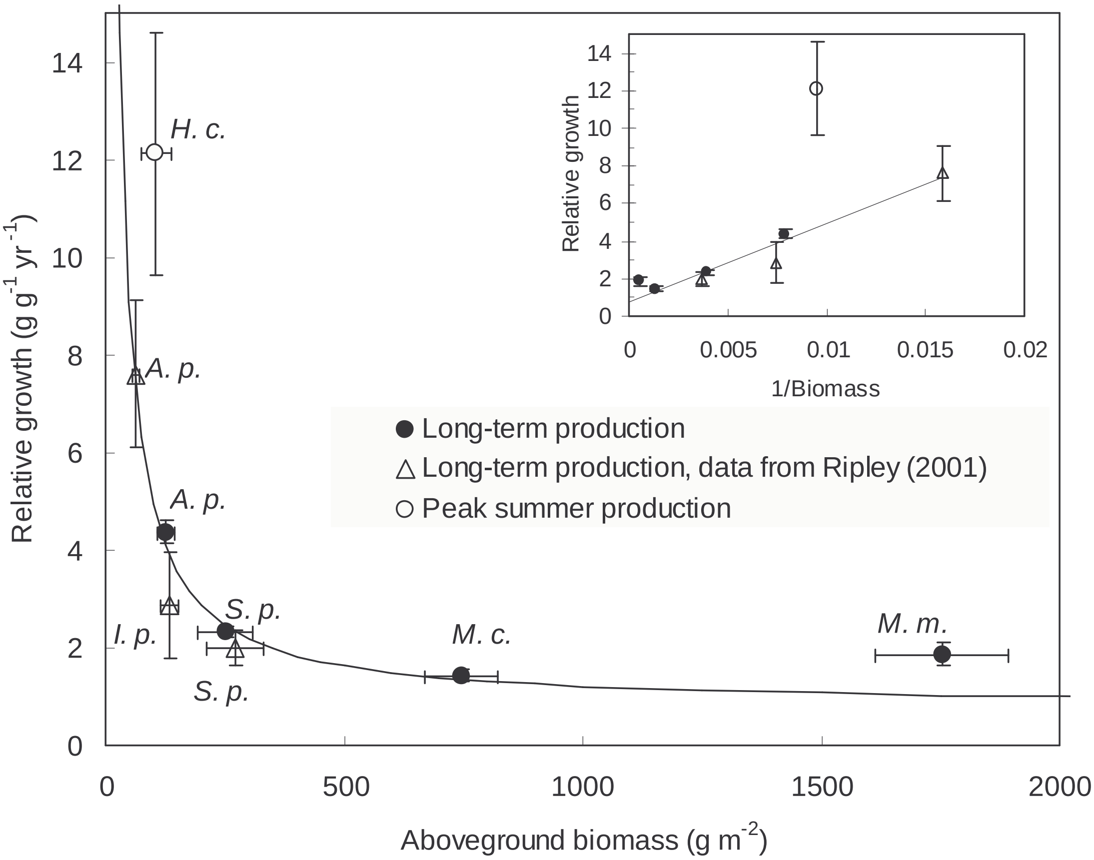


Figure 9.11: The relationship between relative growth and aboveground biomass of six species of dune plants at OWR, including long-term data from Ripley (2001). Parameterisation of the simple competition model of low biomass was done by fitting a SMA line to the relative growth and reciprocal of biomass (inset). Once the model parameters were estimated, the model could be drawn on the main graph. The summer data for *H. cordata* fell off the relationship, and was not included in the SMA regression model.

DISCUSSION: Field test of competition model

A possible explanation of the strong change in production with biomass may be that the species have different soil nutrients. However, the soils measured in Chapter 2 (Table 2.2.2) correspond to the quadrats in which production measurements were made. The soils from under the four species were similar in nitrogen content, and this parameter was independent of biomass ($R^2 = 0.07$, P = n.s., n = 12 samples).

The intercept of the relative growth to the reciprocal of biomass relationship is likely to be different to zero (Figure 9.11 inset). The Standardised Major Axis (SMA) regression did not have a significant intercept, but in the case of both high biomass species, the average relative growth was significantly greater than zero (Figure 9.11).

Therefore, there is the strong indication that there is a second component to the competition model of low biomass. *Vis-à-vis* in addition to the increased availability of resources at low biomass, there is a significant amount of resources that are not competed for by biomass, and contribute a relative growth rate of about one (at high biomass). This result is consistent with remobilisation of resources from senescing biomass to new growth (see Theory, pp. 274). This effect may account for the observed intercept. Apportioning of the observed response between the competition effect, and the remobilisation of resources effect, is not possible, given just this data set. However, the amount of remobilised resources from senescing leaves was measured for these species (Chapter 8). Thus, back-of-the-envelope calculations may give an idea of the importance of the remobilisation effect. By multiplying the proportion of nitrogen remobilised from leaves of the four species by the relative growth and comparing this value to the total relative growth an estimate of the contribution of remobilisation to the relative growth can be attained (Table 9.3). This calculation assumes that growth is primarily determined by nitrogen dynamics. Additionally, it is necessary to assume that there was no net increase in biomass over the course of the experiment, and therefore turnover equals the relative growth. These assumptions may be reasonable for the two mobile-dune species, and for *M. cordifolia* these assumptions make no difference to the calculations, as this species has 0% remobilisation (see Theory and Figure 9.3 for more explanation of these calculations).

Table 9.3: Rough budget of nitrogen supply compared to the relative growth of the species. Rather than compare growth in nitrogen units, growth has been expressed in the original units of relative growth, and thus is comparable across species.

Species	Relative growth $g\ g^{-1}\ yr^{-1}$	Nitrogen remobilisation efficiency proportion	~Growth supported by N remobilisation $g\ g^{-1}\ yr^{-1}$	~Growth supported by N other than that supplied by N remobilisation $g\ g^{-1}\ yr^{-1}$
A. populifolia	4.37	73.1%	3.20	1.18
S. plumieri	2.33	52.1%	1.21	1.11
M. cordifolia	1.43	0%	0.00	1.43
M. muricata	1.86	25.9%	0.48	1.38

Although these calculations are rudimentary, they show that remobilisation may entirely account for the observed interspecies differences in relative growth (Table 9.3). Interestingly, this budget indicates that all species have similar net requirements for nitrogen. As discussed in the Theory section, these estimates are maximum estimates of nitrogen remobilisation, and minimum estimates of growth which is determined by competition. Thus, at this extreme, remobilisation can account for 73% to 0% of the nitrogen required for growth, and soil nitrogen pools which are competed for could account for 23% to 100% of the nitrogen required for growth. In absolute terms these estimates suggest that all species share a similar net amount of resources which derive from the soil, and are competed for.

DISCUSSION: REVIEW OF THE EVIDENCE FOR THE COMPETITION MODEL

A number of conditions are required for the competition model to be relevant to coastal dune plants.

The ecological context of the dune environment requires that:
1) *Higher growth rates are needed to outgrow sand burial*
The four main study species were partially buried in the experiments of Chapter 5 and all survived considerable burial. However, only two of the species showed a quick and large growth response to the burial (Figure 5.1). Thus, repeated partial burial would result in the other two species surviving less burial in the long-term, and this corresponded with the typical positions of these species along the burial gradient. This result is consistent with other typical dune coloniser species that have increased growth after burial (Maun, 1998). That competition affects the response to burial was specifically tested (see discussion below).
2) *Belowground resources limit plant growth and the response to burial*
The two mobile-dune species, that had high growth rates in response to burial, were nutrient limited and this affected their response to burial (Figure 5.1). The other two species showed little response to nutrient addition. Other belowground (or aboveground) resources may limit their growth. These may also be incorporated into the competition model of low biomass. For instance, low cover would result in low total soil water deficits, and a lack of drought (e.g. Ripley and Pammenter, 2004a). The competitive effect of a high biomass of plants within 2.5 m of a shoot of *S. plumieri* was assumed to be a belowground interaction. While this seems likely a shading effect may also account for some of the competition effect. However, most of the monitored shoots that were growing near competitors, were not within the

canopies of the competitors (pers. obs.). Thus a shading effect may not be an important effect for the plants monitored in the *S. plumieri* experiment.

For studies involving a number of species, the results are only comparable if:
3) *Between the typical dune positions of the species there are no major spatial nutrient gradients*
There were no gradients in soil nitrogen content, between the stands of the four species studied here (Chapter 2.2; Table 2.2.2). Thus, this factor was unlikely to result in the observed changes in production, between species (Figure 9.11).
4) *Between species and within species (at different biomasses) gross nutrient use efficiencies (of limiting nutrients) or root mass specific absorption rates remain constant.*
The four species studied here must have different nutrient use efficiencies and root specific absorption rates. Indeed, the observed change in relative growth with species biomass, is equally possibly explained by leaf nitrogen remobilisation as the competition model of low biomass (Table 9.3). In the greenhouse experiment with *A. populifolia* there was strong support for the competition model, where there were no differences in soil nutrients, and seedlings were all of one species. Similarly, the intraspecific *S. plumieri* surveys avoided comparing species of different nutrient use efficiencies. In these experiments the competition model was also supported.

The models are applicable to very simple ideal plants, deviations from this should be considered such as:
5) *That clonal integration does not result in major translocation of carbohydrates or nutrients from unburied to buried shoots.*
Clonal integration, or the interconnectivity of shoots, may result in translocation of resources to buried shoots of *S. plumieri* (Chapter 7, Figure 7.13). The homogeneity of burial at the shoot scale (Chapter 2.1; Figure 2.1.5), the observed competitive effect of high densities of shoots of the same individual (Figure 9.5c) and localised response to fertiliser (pers. obs.), may indicate a relative lack of integration of nutrients physiology between shoots of *S. plumieri*.

Summary of evidence for the competition model of low biomass

In general, a low aboveground biomass had a positive effect on the stem and leaf production of shoots of *S. plumieri* (Figure 9.5). However, these results indicated that the stem and leaf production of *S. plumieri* respond to different competition factors, limitations, and interactions between factors (Figure 9.7). Stem production is positively affected by burial (Figure 9.4) and nutrient addition when buried (Figure 5.1), but negatively affected by the time since burial last occurred (Figure 9.5d). Leaf production is positively affected by nutrient addition under both burial and unburied conditions (Figure 5.2), low shoot density of *S. plumieri* (Figure 9.5), the proximity of other large competitors (Figure 9.5), but not the presence of a nitrogen-fixer (Figure 6.3, although this did affect *M. muricata*). Both stem and leaf production were increased in seaward shoots (Figure 9.4), and since this is not necessarily entirely related to competition (Table 9.1), may indicate other sources of nutrients for mobile-dune plants. Thus, aspects of the competition model of low biomass may be applicable to buried and unburied individuals of *S. plumieri*.

A. populifolia seedlings grown at different densities had growth consistent with the competition model. Thus, all pots had similar total production, but this was divided up amongst the number of seedlings in the pots (Figure 9.8a). The treatment densities had little effect on the allocation of leaf, stem or root mass, thus the competition model appears to have no other negative consequences for *A. populifolia* seedlings.

Species of different typical biomass had production rates consistent with the competition model: hyperbolically increasing production at low biomass (Figure 9.11). This establishes the high production-low biomass pattern for a range of species, measured *in situ*. However, an alternative hypothesis was shown to be plausible, based upon measurements and calculations of the nitrogen remobilised from senescing tissue. Thus, it is possible that both or either of the competition model or remobilisation of nutrients could account for the observed relationship. Never-the-less the competition model was shown to influence the growth of plants whether looking at between plant or within plant competition, for the mobile-dune species *A. populifolia* and *S. plumieri*.

What were the sizes of the effects of the competition model on growth?

The competition variables explained up to 40% of the variation in the leaf production rate of *S. plumieri* (Table 9.1). Individually, each competition variable explained a small (10% to 25%), but significant, part of the variation in the leaf production rate (Figure 9.5). The analysis of the residual and fitted values of the leaf area production to initial leaf area relationship, indicated that in all cases these competition effects on leaf area production occurred through their influence on the initial shoot leaf area (Figure 9.7). While only a small amount of the variation is accounted for by these variables, the size of the effects of competition on leaf area production were large. Across the range of measured within stand shoot densities the average *S. plumieri* leaf area production increased from 1 to 4.5 cm^2 $shoot^{-1}$ day^{-1} (Figure 9.5c). Similarly, the average leaf area production increased significantly from ~1.5 to ~2.2 cm^2 $shoot^{-1}$ day^{-1} when a measured shoot was more than 2.5 metres from a large competitor. Stem production was significantly, but less clearly, influenced by competition than leaf area production (Figure 9.7).

Does competition limit the production of plants under burial conditions?

Interspecies competition and within individual between shoot competition were shown to have a strong negative effect on the stem and leaf growth of unburied plants (e.g. Table 9.1). However, under burial conditions stem production appeared to be less affected by competition than for unburied shoots. The position and competition variables still explained significant variation in the fitted values of the stem production to initial leaf area relationship (Table 9.1). This may indicate that the effect of competition on stem production is via competitive effects on initial shoot leaf area, which translate to different resources available to the shoot to respond to burial. Thus, competition affects burial responses indirectly. There appears to be a shift in growth towards greater production under burial conditions i.e. no change in leaf area production, but a significant increase in stem length production (Figure 9.4a and b). This response is largely unaccounted for by position and competition factors. Thus, competition does affect the burial response, but other factors, like the ones suggested in Chapter 7 are necessary to explain a large part of *S. plumieri*'s burial response.

The role of competition and burial in the dune succession

A large biomass of a species, or neighbouring species, places a clear strong competitive limitation on the growth of a focal shoot of *S. plumieri*. This then has an effect on amount of biomass the species supports per shoot, indirectly affecting the burial response. In the greenhouse, density treatments of *A. populifolia* seedlings demonstrated that a high growth rate could be achieved at a low density, without affecting other aspects of the seedlings growth. Across five dune species of different typical aboveground biomass growth increased hyperbolically with decreasing biomass, also supporting the competition model. However, in the latter experiment a second possible explanation was that measured nutrient remobilisation capacities of the species could account for the observed relationship. Thus, the competition model does account for the *in situ* growth of *S. plumieri* under buried and unburied conditions, and the results of greenhouse manipulations of *A. populifolia*, but may or may not account for the *in situ* growth differences of a range of species.

The following is offered as an example of the competition model in an ecological context: If a certain amount of production is required to survive a series of burial events, then three plants growing adjacent to each other would have lower production rates than a single plant, and may not survive the burial. Should one of these plants survive the burial, then this plant would grow faster, at the low density, and survive greater future burial. Thus, between individuals, the competition effect would result in a burial filtering of plants in areas of high biomass. Within a plant, greater biomass would also result in reduced growth (e.g. the responses *S. plumieri* shoots to within stand shoot density) and thus similar filtering of shoots of a single plant (e.g. Maun and Lapierre, 1984). A plant with high turnover or other mechanism that maintained a low biomass would also have higher production rates under burial conditions.

It seems clear that without burial acting in such a manner so as to remove plants that cannot grow fast, plants with low biomass would have a disadvantage competing with larger neighbours. Thus, it is vital that burial is included in models of dune succession as the presence of this disturbance allows the survival of plants with adaptations that would be clearly disadvantageous in other circumstances.

Chapter 10

Do dune plants have optimal growth geometries during partial burial?

Optimal geometry of growth of dune plants: Theory — Simulation of effect of burial on measured plant geometries — Dune shape and volume formed by different species — Do dune plants form steep dunes, and benefit from this by reducing growth needed to respond to burial?

INTRODUCTION

Burial is an important process in the extremely mobile substrate of foredune and transgressive dune ecosystems (Moreno-Casasola, 1986; Hesp, 1991). Dune plants may be tall enough (~10 to 40 cm) that burial events (~0 to 20 cm) result in partial burial of the plant. Thus, after partial burial stem elongation must occur before the next burial event, so that the plant can maintain photosynthetic leaf area above ground (Maun, 1998). In addition to repeated partial burial, the low nutrient and extreme conditions found on coastal dunes limit plant growth in response to burial (Maun, 1998). Under these limiting conditions, adaptations that minimise the cost (or length) of stem elongation required to maintain leaves above ground allow plants to better respond to partial burial. The ability of coastal dune plants to respond to partial burial determines the position of dune species on dune zonations (Maun and Perumal, 1999), and their presence in areas where little vegetation survives (Hesp, 1991).

Studies in which burial is measured in terms of height, inherently consider burial as a vertical phenomenon. Dunes, by definition, are not flat (Hesp, 2002), thus assuming that burial acts in a vertical manner is a simplification. Theoretically, this assumption ignores the fact that optimal growth of plants on steep dunes could save up to 18% of the resources required by vertically growing shoots (see **Theory**). In addition, it is not known whether plants grow in such optimal geometries on non-horizontal surfaces.

Sand accumulation on vegetated dunes is a complex process, determined by plant size, plant cover and wind velocity (Buckley, 1987), dune profile (Bagnold, 1941), and other factors, and may described by non-linear dynamics (Baas, 2002). If plants are able to grow more efficiently on steeper dunes, then plants which have characteristics causing steeper dunes to form will be advantaged by non-vertical growth.

Strong SW and NE winds blow parallel to the coastline of the study area (Eastern Cape Province, South Africa) during winter and summer, respectively. These winds result in the formation of tall (2 to 20 m) reversible transverse dunes, characterised by high rates of sand burial, particularly on the steep dune slip faces (Wasson and Hyde, 1983). As the lack of vegetation in these dune fields is largely due to extreme sand burial, the vegetation that is found in the dune fields must be adapted to survive such conditions. The ability to outgrow rapidly building dunes and dune slip faces, whilst minimising the cost of growth, is an important adaptation of plants in these dune fields (Plate 10.1 for an example).

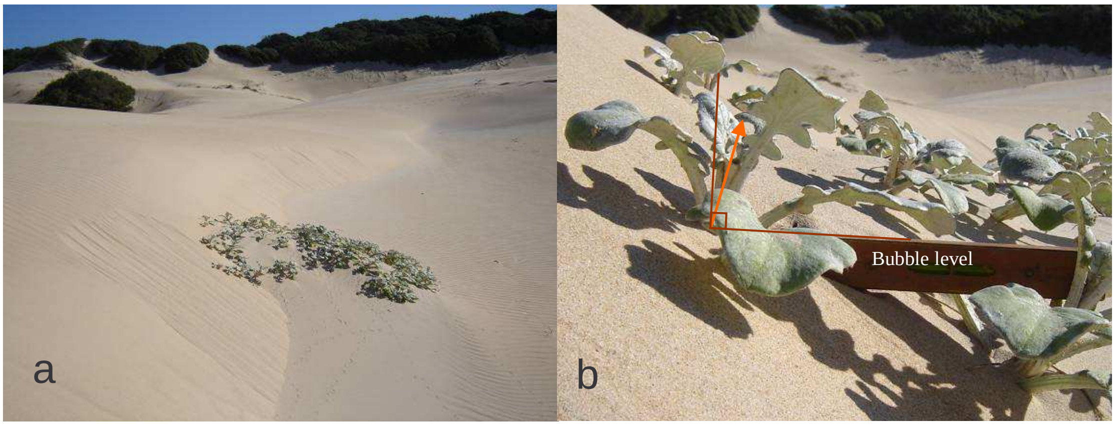


Plate 10.1: Photographs of an *Arctotheca populifolia* plant encountering an advancing slip face (a), and a close up of the same plant on the slip face (b) the dune is 33^{o} from horizontal. A horizontal line is extended from the bubble level to a stem, the growth trajectory of the stem and a vertical line are shown.

The aim of this section is to address the issue of plant growth under non-horizontal partial burial conditions theoretically, and test the hypotheses that: 1) upon partial burial, shoots modulate growth towards optimal growth trajectories, relative to the dune surface on which the shoots are growing, 2) plants that form steep dunes increase the potential advantage gained from optimal growth.

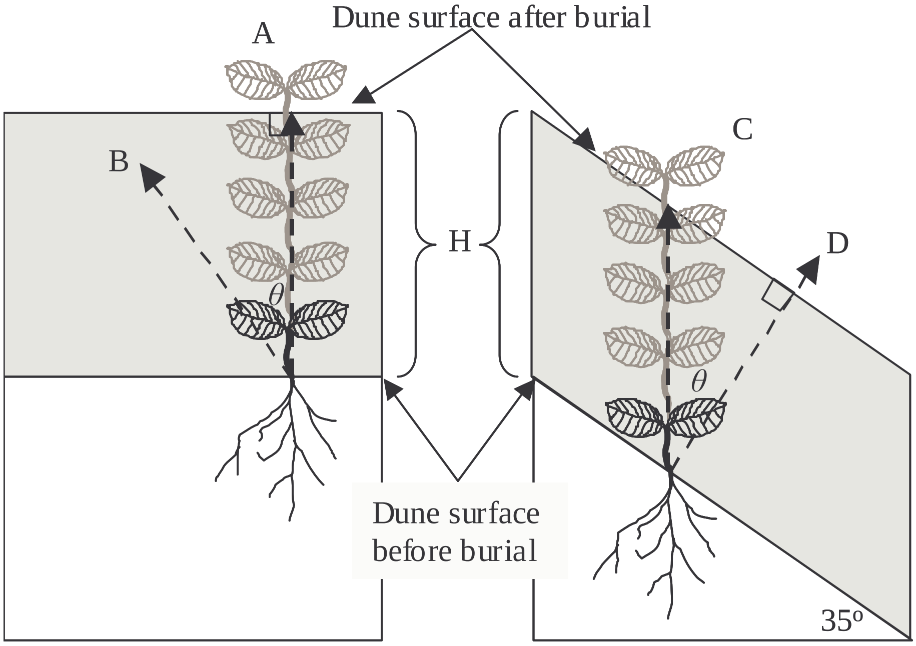


Figure 10.1: Demonstration of stem elongation needed to maintain exposed leaf area during continuous sand accumulation for plants growing vertically (A), and at 35° from vertical (B) on a flat dune, and for plants growing vertically (C) and perpendicular to the dune surface (D) on the slip face of a dune. H represents the vertical depth of burial, and θ the angle of growth needed to grow normal to the dune surface (in the case of D, θ is the same as the angle of repose of the slip face of the dune, 35°). All growth vectors (dashed lines) are of the same length.

THEORY

Coastal dune pioneers are often short and herbaceous, consisting of a number of shoots that may be interconnected below the surface of the sand (Doing, 1985). Due to their small size, the local dune surface on which a shoot grows may be approximated by a plane, in the case of both a slip face, and other dune surfaces (measurement of the profiles of vegetated dunes greater than 40 cm in height, suggested that dune slopes are relatively constant over distances of approximately 1 metre; data not shown). Gradual or episodic burial occurs on this plane, resulting in the partial burial of the shoot. Plants encountering burial have limited resources with which to grow (Maun, 1998). Gradual or episodic burial events occur on this plane, resulting in partial burial of the shoot. Plants encountering partial burial have limited resources with which to grow (Maun, 1998). Thus, an optimal growth response must minimise the amount of stem elongation necessary to maintain photosynthetic leaf area above the sand surface. The angle of shortest growth (θ) for a partially buried plant, is perpendicular to the dune plane, where θ is equal to the slope of the dune from horizontal (Figure 10.1).

Thus, the distance of shortest growth, S_{opt}, is:

$$S_{opt} = H * cos(\theta) \qquad \text{(eqn 10.1)}$$

where, H is the vertical depth of burial and θ is the angle of the dune surface from horizontal (and the angle of the optimal plant growth from vertical).

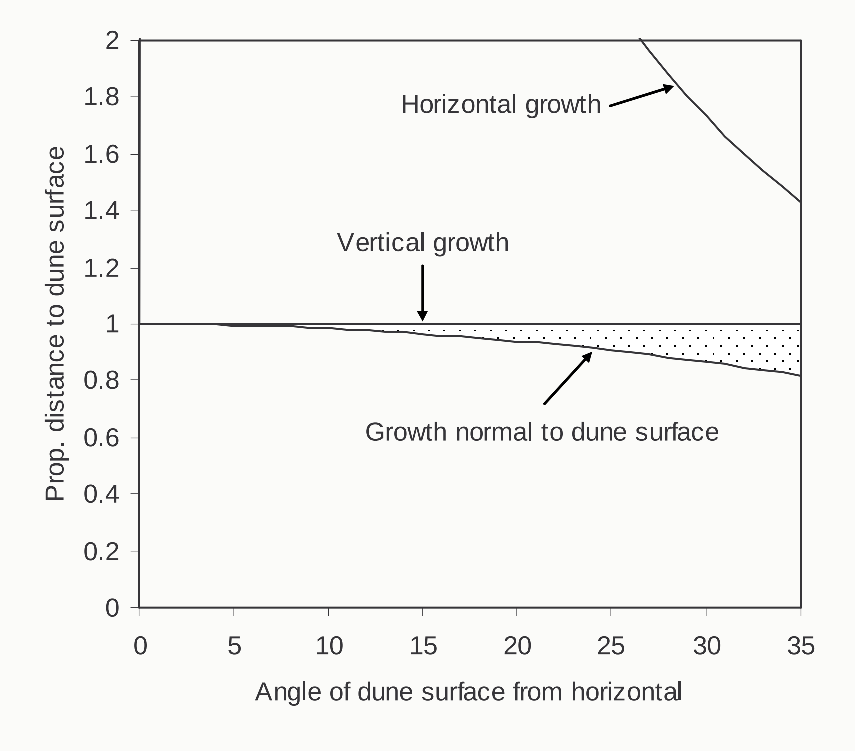


Figure 10.2: Growth distance required for a repeatedly partially buried plant to maintain leaf area on a dune surface with three growth trajectories, optimal, vertical, and horizontal, related to the slope of the dune surface. The shaded region represents the “saving” in growth for plants growing normal to the dune surface relative to vertical growth. Distances are proportional to vertical growth, given a vertical burial of unit 1.

As the angle of the dune surface increases from horizontal the optimal distance of growth becomes considerably shorter than that of vertical growth (Figure 10.2). However, the angles that sand dunes can form are limited by the angle of repose of the sand medium. The critical angle of repose, the angle at which sand starts avalanching and forms slip faces, is relatively constant with similar values of 30 to 35^{o} being found in lacustrine, coastal and desert dunes (Carrigy, 1970). For buried dune plants the greatest angle that can yield the shortest growth trajectory is thus 35^{o} from vertical. At this extreme the length of optimal growth is 0.82 of that for vertical growth (equation 10.1).

Clearly, plants undergoing burial cannot maintain leaf area by growing horizontally, which is a typical growth form of many dune creepers growing on stable substrates (e.g. *Ipomoea pes-caprae*). On quickly accumulating dune surfaces, at the angle of repose, horizontal growth represents a stem elongation of 1.45 that of vertical growth (74% greater growth than optimal), while on less steep dunes the necessary horizontal growth length increases to infinity. Under stable conditions these considerations may not apply as light competition may result in vertical growth, or resource heterogeneity and low soil nutrients may result in horizontal growth.

More generally, local dune surfaces are better represented by a series of contiguous planes, than the simple model presented in Figure 10.1. Thus, the angle and direction of growth must be compared with the angle and aspect of the local dune surface to determine whether growth subsequent to partial burial has been optimal. However, the reasoning that applies to the simple model is also valid for this more complex three-dimensional model. For instance, the two dimensional model for a plant being buried on a slip face (Figure 10.2), can be extended to three dimensions. Clearly, growth does not have to be perfectly normal to the dune surface, for the shoot to grow more than 10% shorter than a vertical shoot.

Sand movement and plants interact to form dunes

Regardless of slope, dunes of equal horizontal area require the same volume of sand to achieve similar vertical depths of burial (the areas of the shaded rectangle and parallelogram in Figure 10.1 are the same). Hence for a given volume of sand, plants that form steeper dunes are buried by the same vertical height as occurs in less steep dunes. This suggests the intriguing possibility that plants with the ability to form dunes of steeper angles and have the ability to grow optimally, although buried by the same vertical height for a given volume of sand, have the potential advantage of requiring less growth to reach the dune surface. The windward side of un-vegetated dunes often has a shallow profile (Tsoar, 1985, Kroy *et al.*, 2002), in the range of angles at which plants gain little from growing non-vertically (0 to 10^{o} in Figure 2). Therefore, non-vertical growth is advantageous only on dune slip faces, or, if plants form dunes that are steeper than 10^{o} (i.e. steeper than those that are not vegetated.

This model of growth under non-flat burial conditions may be extended to other ecosystems where sand movement is a common disturbance: desert dune plants and burrowing animals (especially in a submarine environment) are potential examples.

MATERIALS AND METHODS

Survey measurements

Three species of common coastal dune plants were surveyed to determine whether these species respond to partial burial optimally, relative to burial conditions. The species selected constitute a major component of the flora of mobile foredunes and the large transgressive dune fields found along the Eastern Cape coast of South Africa (Lubke, 1983): *Arctotheca populifolia* (Berg.) T. Norl., a fast growing pauciennial daisy of short (ca. 0-20 cm) stature; *Scaevola plumieri* (L.) Vahl., a perennial species that forms large foredunes, and has vertical growing shoots, interconnected belowground; and *Ipomoea pes-caprae* (L.) R. Br., a fast growing creeper.

Plants were surveyed in March and April 2007 when both of the dominant SW and NE winds occur. Sites included the large transgressive dune fields north of Kleinemonde, Kasouga and Old Woman’s River, and in the mobile foredunes at Old Woman’s River, in the Eastern Cape province, South Africa (33^{o}25’to 33^{o}45’S and 26^{o}35’ to 27^{o}15’E). These dune fields have a bimodal rainfall pattern with spring and autumn peaks, and range from 600-700 mm of rain per year (Stone *et al.*, 1998).

Surveyed shoots were divided into three categories: 1) partially buried shoots on any dune surface less steep than 30^{o}, defined by having the lowest leaves under the sand surface; 2) partially buried shoots, growing on dune slip faces (surfaces at the angle of repose, 30-35^{o} from horizontal); 3) unburied shoots, which had the lowest leaves more than 5 to 10 cm above the sand surface. These categories will be referred to as partially buried, slip face and unburied shoots, respectively. The internode lengths of all species elongate in response to burial, thus this was used as an indication of how recently burial had occurred. Recently buried shoots that showed no stem elongation were not included in the analysis. As *S. plumieri* can form large dunes consisting of only a few individuals (Barker *et al.*, 2002), no more than four random shoots in each category were measured per dune, often these were on different aspects. These samples could be considered independent, as there was no detectable pattern associated with sampling more than one shoot per dune. This was determined using a using a runs test, which calculates the observed number runs (sequences of similar data), in comparison with the number of runs expected from a random pattern (Sokal and Rohlf, 1981). Stands of dense vegetation, and shading, might affect the growth trajectories of these species (pers. obs.), therefore only stands with a low density of shoots were surveyed. Shoots were surveyed by measuring the direction of growth (D_p) and angle from horizontal (A_p) of the leaf-bearing portion of the stem. In the case of partially buried shoots, 10 to 15 cm of the stem was exposed by careful excavation and this trajectory was measured. The aspect (D_d) and angle from horizontal (A_d) of the dune surface were measured directly around the selected shoot. Angles and directions were measured with a plumb-line clinometer and magnetic compass, respectively. Dune surfaces were measured by placing a small flat board on the sand to approximate the dune surface.

Burial simulation

To determine whether stems were growing optimally, the length of stem required to survive repeated partial burial events was compared to the length that would have been produced by an optimal stem. In order to do this, the length of stem elongation required to maintain a shoot above the sand surface was simulated for a vertical burial event of one unit height. Measured stem growth trajectories were used to model this growth distance (S_{act}). S_{act} was compared to that of a theoretical optimal shoot growing normal to the measured angle and aspect of the dune surface (S_{opt}).

The following simulation of the burial allowed such a comparison: The measured dune surface was defined as a three-dimensional plane on which the shoot's actual growth trajectory and the theoretical optimal trajectory were compared relative to a vertical burial event of one unit height. When buried by a one unit vertical burial, the optimal length of shoot (S_{opt}) necessary to maintain the shoot above the dune surface is given by the cosine of the angle of the dune surface from horizontal (equation 1). The actual length of shoot (S_{act}) that the measured stem must grow was calculated by using R^3 vector geometry (D. Orpen, pers. comm., 2007), which results in the following equation:

$$S_{act} = \frac{H * \cos(A_d)}{\begin{array}{l}\sin(A_d) * \sin(D_d) * \cos(A_p) * \sin(D_p) \ldots \\ \ldots + \sin(A_d) * \cos(D_d) * \cos(A_p) * \cos(D_p) + \cos(A_d) * \sin(A_p)\end{array}}$$

This simulation could be applied to all three categories of measured shoots forming a test of whether there is a shift in growth trajectory under partial burial conditions, relative to unburied conditions. This simulation allowed actual lengths (S_{act}) to be compared to a value of 1 (1 indicates vertical growth, <1 optimisation away from vertical growth, >1 little optimisation). The value of S_{opt} for a dune surface represents the shortest length of growth that S_{act} could attain for that dune surface.

Survey of dune shape

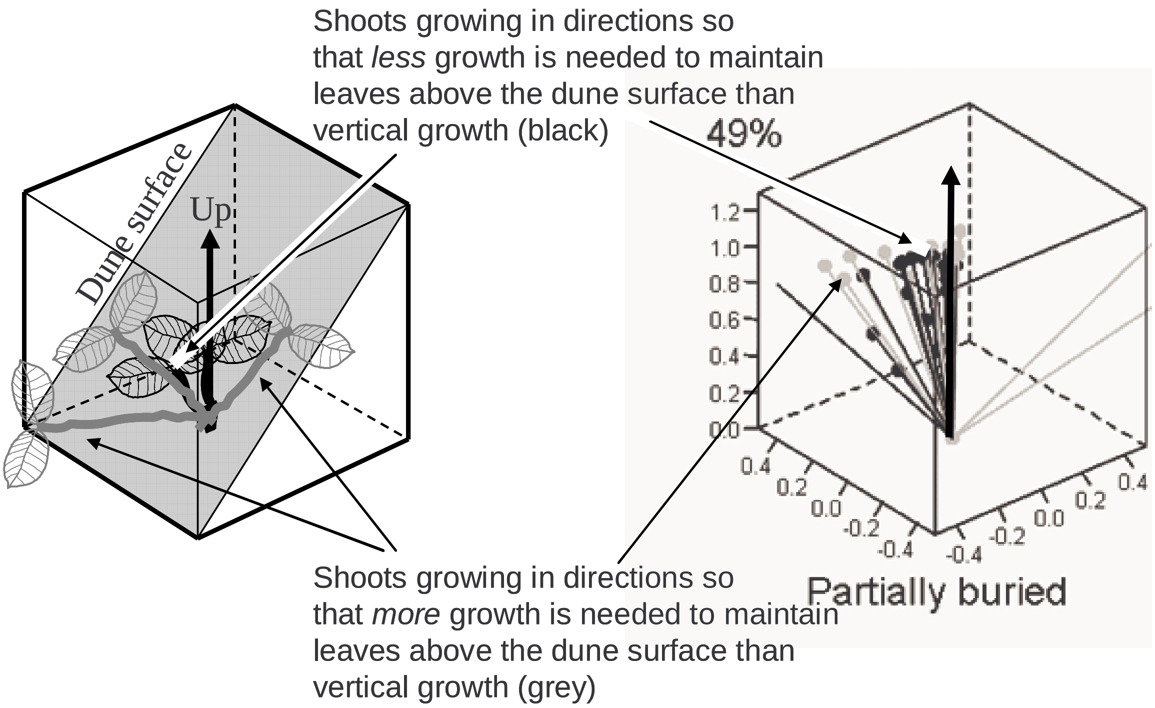
A survey was made of the profiles of dunes formed by the presence of *A. populifolia*, *S. plumieri*, or no vegetation. Few distinct *I. pes-caprae* dunes were present in the survey area. The survey was conducted on the 6 and 13 May 2007, on the well-developed transverse dune field north east of Old Woman's River (27^{o}09'E, 33^{o}29'S) and Kleinemonde (27^{o}05'E, 33^{o}33'S). Transverse profiles of dune steepness were measured including the tallest point on a dune, in the direction of the predominant wind, for 13 to 15 isolated dunes of each type across a range of sizes. A clinometer attached to a 50cm flat board was used in the survey.

Statistical analysis

Analysis was done using the R statistical program version 2.4.1 (R Development Core Team, 2006). The simulated growth vectors required for a shoot to remain above the dune surface were plotted in 3-D coordinate space, taking the origin as the initial shoot starting point, and rotating the dune surfaces to a common direction. Transformations were not able to reduce the high degree of skewness and the heterogeneity of variances in the data for the unburied, partially buried, and slip face stems. For this reason rank-based statistics, and the median, and inter-quartile ranges were used in box and whisker plots as measures of central tendency and statistical dispersion. The inter-quartile range (the range between the first and third quartiles) defines the box length, and the whiskers are the range of the data excluding outliers (Verzani, 2005). The non-parametric rank-based Wilcoxon two sample test (R Development Core Team, 2006) was used to determine differences in the length of stem elongation required to maintain leaf area above the sand surface, between partially buried and unburied shoots, for each species. To test whether the growth trajectories of buried shoots on dune slip face's resulted in more optimal growth than vertical growth (=1), a one sample t-test was performed testing whether the mean was significantly less than one. If samples failed the Shapiro-Wilk normality test, a distribution free sign test was performed (Verzani, 2005), to test for differences of actual growth from one. The previous analysis was repeated to determine whether optimal growth was dependent on dune aspect. The data for each species was divided into north- and south-facing dunes and the mean or median S_{act} compared to a value of one (dune aspect was corrected for the current magnetic declination). In this analysis all partially buried (partially buried and slip face) shoots were used if the dune angle was greater than 10^{o} (the dune angle at which growing normal to the dune surface starts to become shorter than vertical growth, Figure 10.2). Standardised major axis regression, as implemented in SMATR (Falster *et al.*, 2006), was used to determine the lines of best fit and differences in slope and elevation between dune shape parameters for dunes formed in the presence of *A. populifolia*, *S. plumieri*, or no vegetation.

RESULTS

The majority of measured stems (97.5%) had growth trajectories away from the dune surface on which they were growing. Thus, only 2.5% of stems were growing with trajectories that would result in complete burial if repeated burial events occurred. These 11 stems (of 434) were all unburied stems of *S. plumieri*, and *I. pes-caprae*, and were excluded from further analyses.



Shoots growing in directions so that *less* growth is needed to maintain leaves above the dune surface than vertical growth (black)

Shoots growing in directions so that *more* growth is needed to maintain leaves above the dune surface than vertical growth (grey)

Figure 10.3: An illustration of the features of Figure 10.4. The growth trajectories of a large number of measured shoots are shown on the right, and correspond to shoots growing on a dune surfaces as depicited on the left. All of the shoots shown have been rotated so that the dune surface on which they were measured has the same aspect as the diagram on the left.

Graphically, the partial burial of shoots of all species resulted in a large shift towards vertical growth (Figure 10.4; the interpretation of this figure is illustrated in Figure 10.3). Unburied shoots, if repeatedly partially buried, would require large growth distances at their current trajectories (>2 times the vertical distance) in order to maintain leaf area above the sand surface. The three species displayed different growth under unburied conditions, with: *S. plumieri* having many shoots close to vertical, *I. pes-caprae* had a large variation in growth trajectories from vertical to horizontal, and *A. populifolia* had relatively vertical growth with many shoots orientated towards the dune aspect. Shoots that were partially buried on slip faces had more growth trajectories nearer to optimal than vertical growth (Figure 10.4).

Do plants optimise growth under partial burial conditions?

In a general manner, Figure 10.4 indicates that stems optimise growth to shorter growth trajectories after partial burial. Statistically , partially buried shoots of all three species showed a significant shift in growth towards more optimal trajectories, relative to unburied shoots (Figure 10.5), represented by the decrease in the stem elongation values depicted in this figure. A large number of unburied shoots showed clear un-optimal growth (>>1) under the simulated burial conditions (percentages in Figure 10.4 and values >> 1 in Figure 10.5).

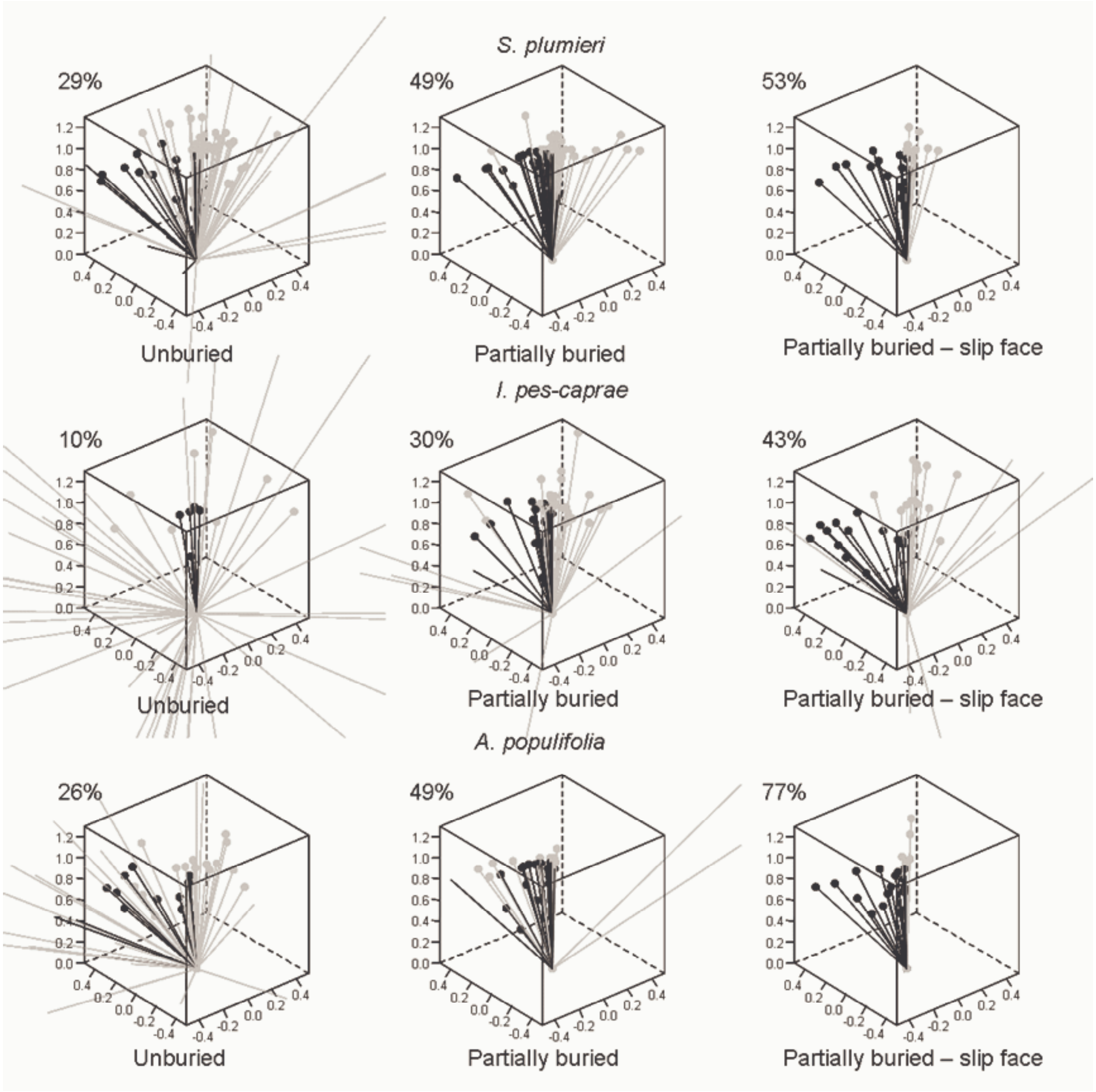


Figure 10.4: Growth vectors for three species of partially buried (on dune slopes of 0-30^{o}), partially buried on slip faces (30-35^{o}) and unburied stems (0-35^{o}), required to maintain leaf area above the dune surface after a simulated burial event of one unit vertical height. Growth trajectories were measured relative to the dune surface on naturally occurring stems. The direction of growth was normalised relative to the dune aspect for all stems; the dune aspect of all stems is the same as illustrated in Figure 10.3. Black vectors represent shoots growth closer to optimal than vertical growth (<1), grey vectors are shoots as, or less, optimal then vertical growth (≥1), and vectors without plotted points, exceed the plotted axes. The percentage of vectors closer to optimal than vertical growth is given in the top left corner of each panel. See previous Figure (10.3) for an illustration of the features of these graphs.

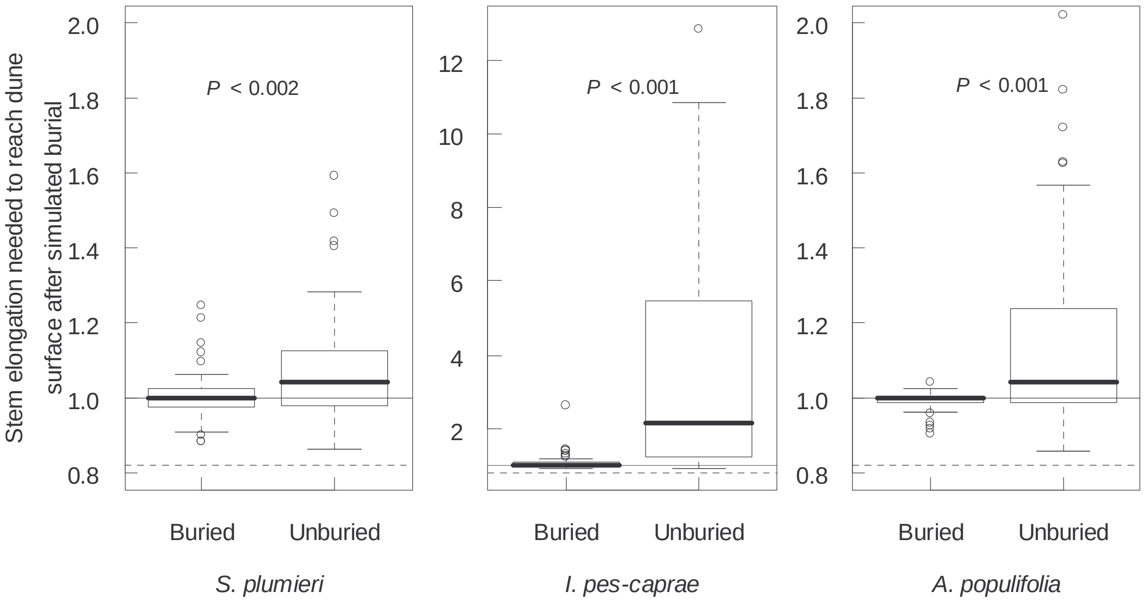


Figure 10.5: Box and whisker plots of the median stem elongation distance needed to maintain leaf area above the dune surface, for partially buried and unburied stems of three species, after a simulated burial event of one unit height. P-values represent the results of Wilcox rank sum tests for differences in centre between partially buried and unburied shoots. Values of 1 and 0.82 represent vertical growth (solid line), and optimal growth on a dune slip face (dashed line), respectively.

Do plants grow more optimally than simple vertical growth?

A. populifolia and *S. plumieri* stems grew significantly closer to optimal than that expected from the null hypothesis of vertical growth (Figure 10.6). *I. pes-caprae* showed three distinct types of growth on slip faces: 27% of shoots were more optimal than 0.9, a number of shoots had vertical growth, and a number of shoots were growing at oblique angles to the dune surface. All species had a number of shoots that showed considerable non-vertical optimisation close to that expected for a slip face (Figure 10.6).

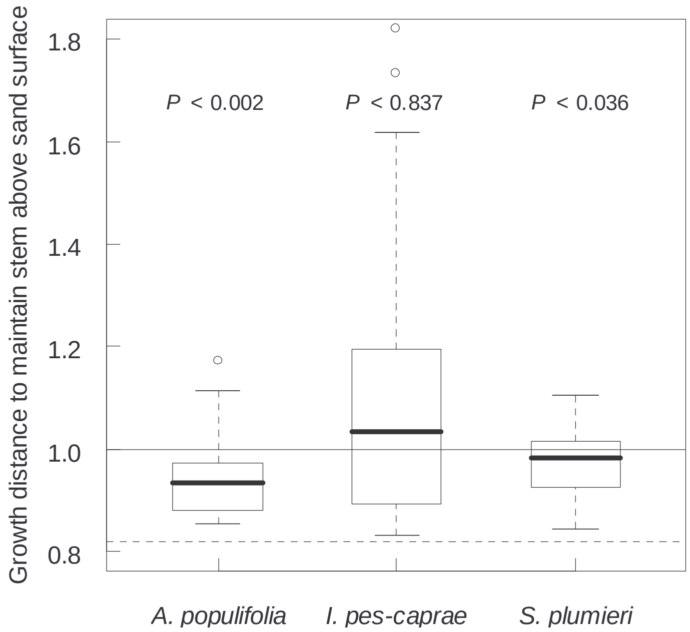


Figure 10.6: Box and whisker plots of the median stem elongation distance needed to maintain leaf area above the dune surface, for partially buried stems of three species growing on dune slip faces. Values of 1 and 0.82 represent vertical growth (solid line), and optimal growth on a dune slip face (dashed line), respectively. P-values represent one-sample sign tests (*A. populifolia*, and *I. pes-caprae*) or a t-test (*S. plumieri*) for the median or mean being less than one.

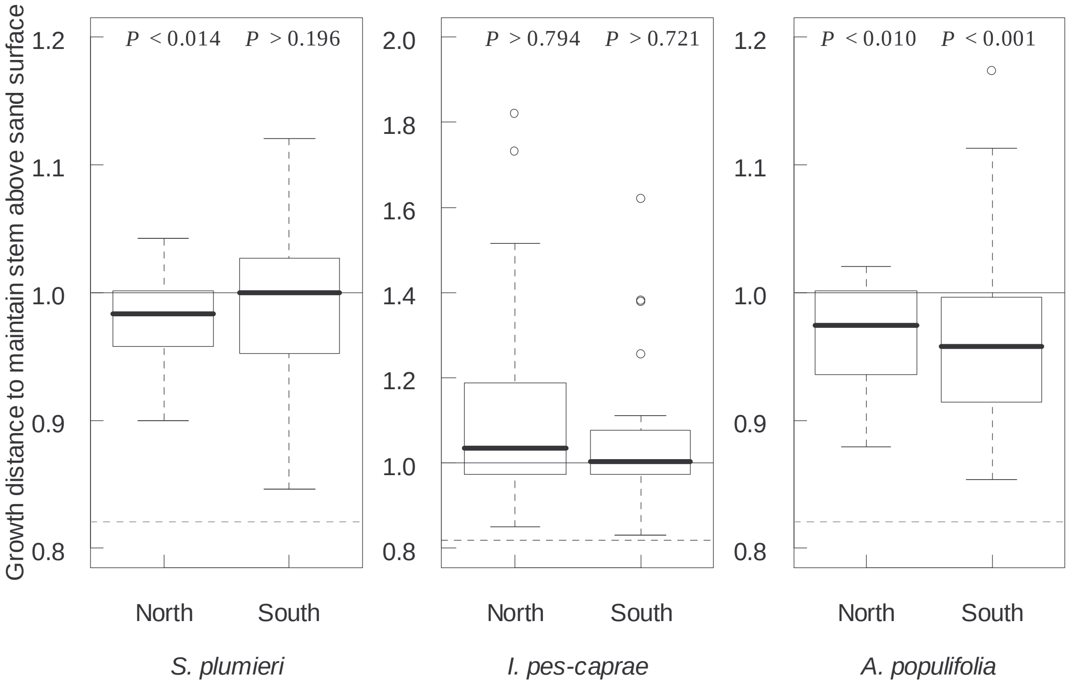


Figure 10.7: Box and whisker plots of the median stem elongation distance needed to maintain leaf area above the dune surface, for partially buried shoots on north or south facing dunes, growing on all dune slopes greater than 10°. Vertical growth (solid line) and the most optimal growth possible (dashed line) are shown on the figure. P-values represent a one sample sign test (*I. pes-caprae*, north) or t-tests (all other comparisons) for the median or mean being less than one.

As species may have leaves and shoots arranged to maximise solar interception, the southerly latitude of the dunes (ca. 33°S) may result in dune aspect playing a role in determining the direction of growth of shoots (*S. plumieri* in particular, pers. obs.). For this reason the optimality of actual growth compared to vertical growth (1) for partially buried and slip face shoots was determined for north- and south- facing dunes separately (Figure 10.7). *S. plumieri* displayed optimal growth on north-facing dunes, but not on south-facing dunes. *A. populifolia* displayed true optimisation of growth away from vertical growth, on both aspects.

Dune size and shape

The size of un-vegetated dunes was considerably larger than even the largest vegetated dunes (Figure 10.8). However, the range of dune angles and heights of vegetated and un-vegetated dunes overlapped considerably. An effort was made to find *isolated* un-vegetated dunes of comparable area to the vegetated dunes.

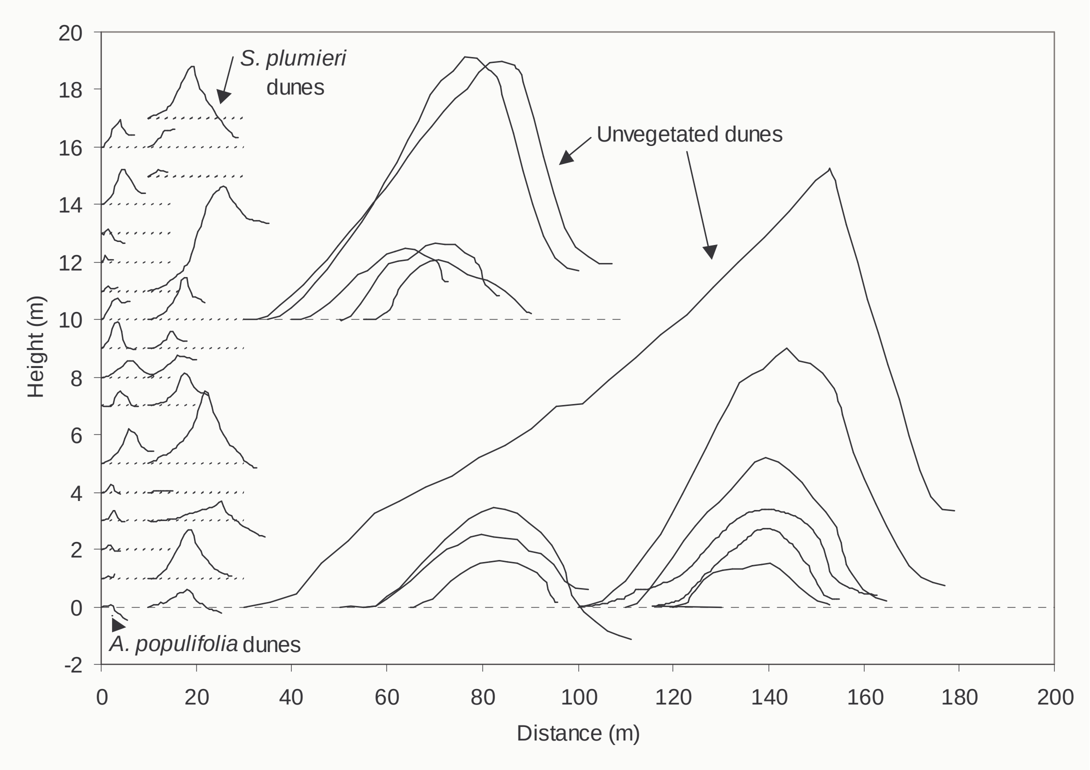


Figure 10.8: Height profiles of isolated dunes formed in un-vegetated areas, or by individuals of *A. populifolia* and *S. plumieri*.

As there is a critical dune size needed for a slip face to form on un-vegetated dunes (Bagnold, 1941; Kroy *et al.*, 2002), it follows that for a given region the size of a dune determines the maximum angles that form on a dune and the overall shape. For this reason, dune size needed to be taken into account when comparing the average steepness and maximum height of three dune types (Figure 10.9).

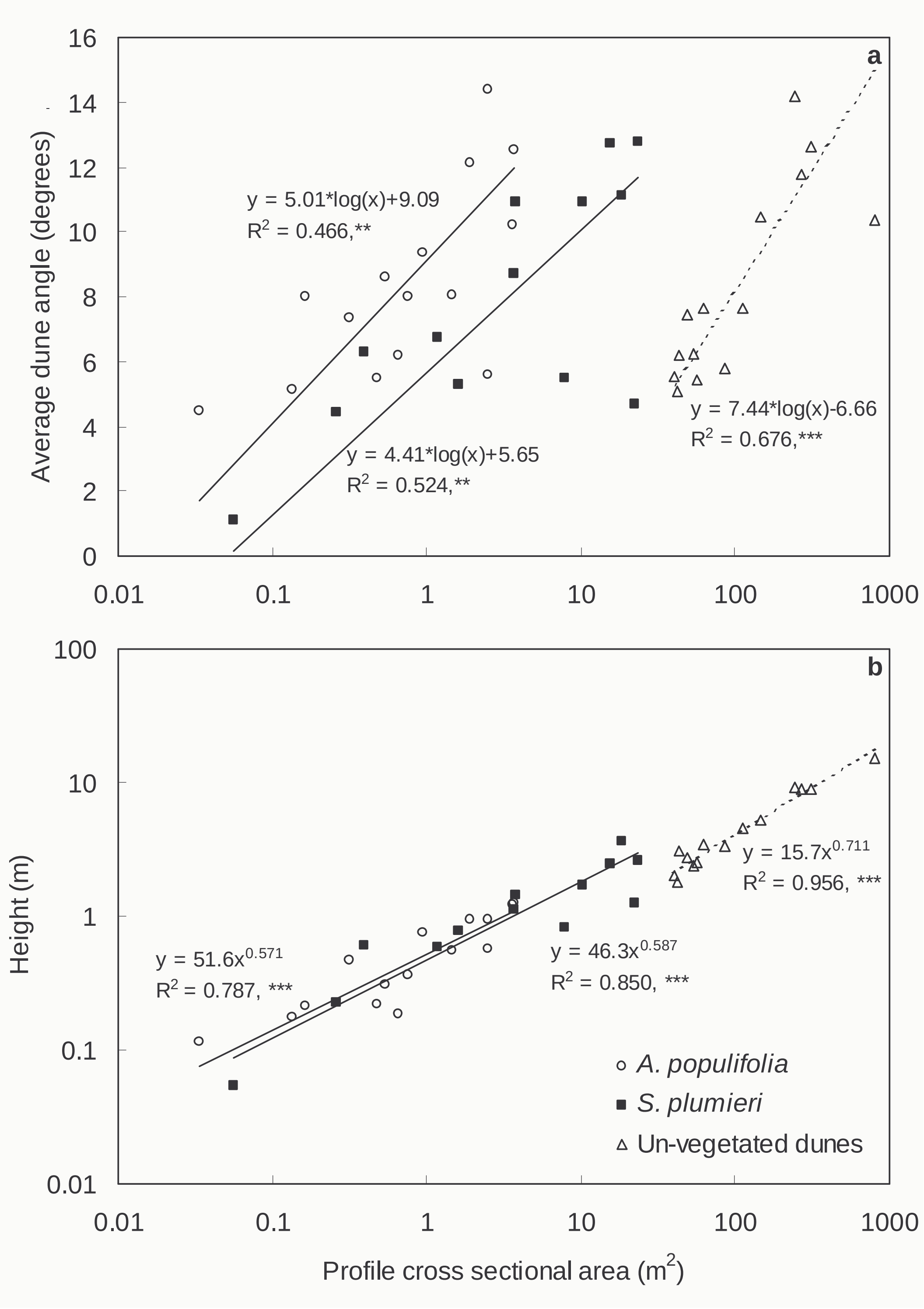


Figure 10.9: Average dune steepness (a) and maximum height (b) related to dune size as estimated from the dune profile cross-sectional area, for isolated *A. populifolia*, *S. plumieri* and un-vegetated dunes. Logarithmic and power functions (see figure) were fit and compared using linear standardised major axis regression on log transformed data. ** = $0.001 < P < 0.01$; *** = $P < 0.001$.

S. plumieri and *A. populifolia* dunes had significantly different logarithmic relationships between the dune size and average angle (Figure 10.9a). Standardised major axis regression analysis found that dune types differed in the size of dunes at which a particular angle was achieved ($P < 0.001$; significant elevation shift). All dunes, apart from the four smallest, formed angles greater than ten degrees, the angle above which optimal growth is shorter than vertical. Vegetated dunes developed slip faces at less than half the dune size for which slip faces were observed on un-vegetated dunes (data not shown). *A. populifolia* dunes were significantly steeper than *S. plumieri* dunes for a given plant size (data not shown). A comparison of dune size with maximum height found that un-vegetated dunes were significantly shorter than equivalent sized vegetated dunes (Figure 10.9b; a significant difference in the coefficient, but not the exponent between dune types; $P = 0.168$ and $P<0.001$, respectively). The predicted height of the largest *S. plumieri* dune (23.6 m^2 cross-sectional area) was 2.96 m and 1.48 m for an equivalent sized un-vegetated dune.

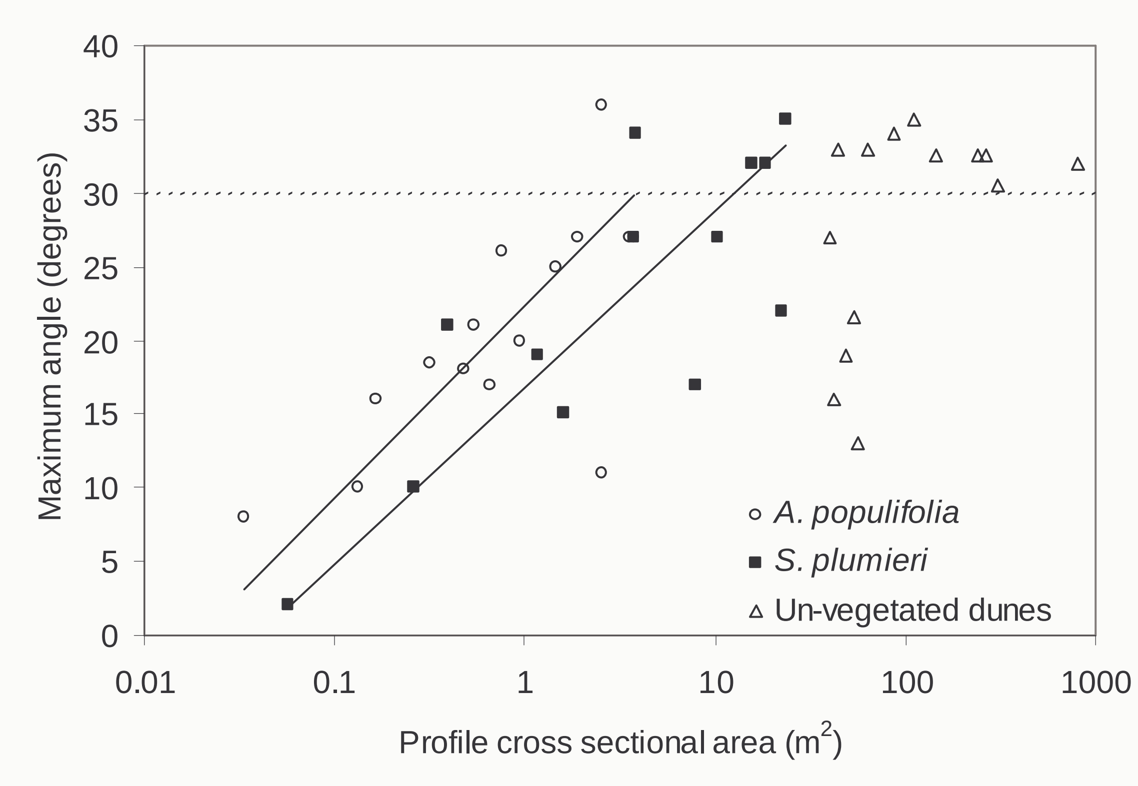


Figure 10.10: Maximum dune steepness related to dune size as estimated from the dune profile cross-sectional area, for isolated *A. populifolia*, *S. plumieri* and un-vegetated dunes. The minimum angle of slip face formation is shown (dotted line).

The maximum angle that the dunes formed was significantly related to the profile area of the vegetated dunes (Figure 10.10; *A. populifolia*, $R^2 = 0.65$, n = 13, $P < 0.003$; *S. plumieri*, $R^2 = 0.65$, n = 13, $P < 0.003$). The size at which a vegetated dune gained a slip face was related to which species formed the dune (*A. populifolia* dunes at a profile cross sectional area of 3.8 and *S. plumieri* dunes at 12.8 m^2). Due to the lack of small un-vegetated dunes it was not possible to estimate the size at which these dunes developed slip faces (the smallest dune with a slip face had a profile area of 44 m^2).

DISCUSSION

Do plants optimise growth under partial burial conditions?

Unburied shoots of *S. plumieri*, *I. pes-caprae*, and *A. populifolia* show a range of growth trajectories, with 46, 16 and 58% of shoots close to optimal if buried in a simulation (Figure 10.5). Partially buried shoots of all species had greater than 68% of shoots within 10% of optimal growth under the simulated circumstances (Figure 10.5). These results indicate that all species are able to modulate growth to near optimal trajectories under partial burial conditions. This optimisation allows, *A. populifolia* and *S. plumieri*, to use approximately 10% less stem to maintain photosynthetic leaf area above the sand surface, while *I. pes-caprae* used ca. 50% less stem.

An inducible strategy of growing close to optimal, and away from an accumulating dune surface is clearly utilised by the three species examined here. However, the above analysis can not distinguish between simple vertical growth upon burial (more optimal than horizontal), and the true optimal growth normal to the dune surface.

Do plants grow more optimally than simple vertical growth?

Vertical growth is optimal on horizontal dunes, but as the angle of the dune increases towards the extreme of a dune slip face, vertical growth reaches 18% greater than optimal (Figure 10.1, 10.2). For this reason, the simulated growth distances of partially buried slip face shoots were compared to a value of one, the distance the stem must grow if vertical. The median simulated growth distances for *S. plumieri* and *A. populifolia* were significantly less than one, suggesting that these species grew normal to the dune surface, rather than vertically (Figure 10.6).

Observations of the growth of *S. plumieri* indicated that this species grows facing a northerly direction under unburied conditions. On north-facing dunes, growth normal to the dune surface coincides with the direction that plants grow to maximise sunlight interception. Thus, optimisation of growth for partial burial could be an artefact of aspect and plant heliotropism. To account for this partially buried shoots (partially buried and slip face shoots, where the dune slope was greater than 10^o) growing on north- and south-facing dunes were compared separately, to vertical growth. *S. plumieri* showed no optimisation beyond vertical growth on south-facing dunes (Figure 10.7). *A. populifolia*, the species most restricted to mobile-dunes, shows true optimisation, growing normal to the dune surface on both north- and south-facing dunes (Figure 10.7).

The mechanism allowing growth normal to the dune surface remains unclear. Heliotropism may account for the optimal growth of *S. plumieri* on the north-facing dunes, but does not account for *A. populifolia*'s growth on south-facing dunes. Schumm (1964; as cited in Marsh and Koerner, 1972) suggests that sand-creep causes annuals to have down-slope bent stems on slip face surfaces. Similarly, the mechanically weak stems of these dune species may be shifted down-slope by the frequent small sand avalanches that occur on dune slip faces. This may passively result in more optimal growth perpendicular to the sand surface. As optimisation appears to occur in partially buried plants on surfaces less steep than that which causes avalanches this mechanism seems unlikely (Figure 10.7, and Figure 10.4 the buried shoots of *A. populifolia*). An alternative is that this phenomenon may be an artefact of the competitive arching growth suggested by Givnish (1986). A down-slope arching growth form was found to minimise height growth, and maximise light interception for understorey herbs growing at angles on steep slopes (Givnish, 1986). In the case of coastal dune plants such non-vertical growth would have a double advantage of maximising light gain and burial survival, while minimising the cost of producing stem. Other passive or active mechanisms may be needed to explain this phenomenon.

Do dune species form steeper dunes?

The ability to form steep dunes in conjunction with non-vertical growth optimisation may be a characteristic that allows coastal dune plants to survive in areas of high sand mobility. This hypothesis is supported as the dunes formed by *A. populifolia* and *S. plumieri* were as steep as un-vegetated dunes of greater than ten times the size (Figure 10.9a). The shape of the dunes formed by each species corresponds well to the non-vertical growth optimisation that the plants achieve: *I. pes-caprae* forms shallow dunes (Hesp, 2002), and was unable to optimise growth on steep dunes (Figure 10.6); *S. plumieri* formed steep dunes and was able to optimise growth on north-facing aspects (Figure 10.7 and 10.9); *A. populifolia* formed the steepest dunes, at smaller plant sizes and dune volumes to either *S. plumieri* and un-vegetated dunes (Figure 10.9a), and showed true ability to optimise growth on steep dunes.

The shape of vegetated dunes varies with the particular characteristics of a species (Hesp, 1989). A clear relationship exists between the cover/density of a plant, and the height and length of the dune that forms (Hesp, 1989, his Figure 6). This explains why *A. populifolia* formed a steeper dune than the less dense *S. plumieri* (Figure 10.10). Sparse, porous plants often result in sand deposition leeward of the plant forming shadow dunes (type 1a; Hesp, 1989). By causing deposition distant to the vegetation, shadow dunes may be advantageous for plants, effectively avoiding burial. More dense plants cause deposition within the plant cover, resulting in the formation of a convex dome (type 1b; Hesp, 1989). This is the dune type of the isolated vegetated dunes studied here, but these dunes may coalesce into undulating ridges (Hesp, 1989). The third dune type (2a) of Hesp (1989) is formed by the deposition of sand parallel to the shore. This dune type forms a long continuous ridge vegetated by seedlings at a particular point on the shore, such as the storm ware mark. The fourth dune type (2b) occurs in areas of deposition due to colonisation by seedlings or rhizomes from adjacent vegetated dunes. In this case a ramp is formed up to the existing dune. Thus, the isolated type 1b dunes studied here are a single type of the many possible vegetated dune formations. However, the growth model suggested in this section, is not limited to convex dome dunes, rather it is applicable to any situation where a plant is buried. For instance, the average dune angle for a survey of five large *S. plumieri* dunes within the vegetated dune field at OWR was 19^o (measured at 36 points). Five belt transects at this site, ranging from the first vegetation, to forest vegetation found that the average dune angle was 17^o (measured at 75 points). Thus, in more complex vegetated dune field at OWR the majority of plants are growing on steep surfaces, where optimal post-burial growth is not vertical.

Steeper vegetated dunes may result in taller dunes relative to the volume of sand trapped. This would result in negative consequences for plants growing on these dunes, as increases in dune height necessitates greater stem production over the lifetime of the plant. The relationship between the stem produced over a plant’s lifetime and the volume of sand trapped by the plant was approximated by comparing maximum dune height and the cross-sectional area of dune profiles, for vegetated and un-vegetated dunes. These parameters were strongly related (Figure 10.9b, $R^2 = 0.79$ to 0.96) in the form of a power function (height = a*cross-sectional area^b). Dunes formed by *A. populifolia* and *S. plumieri* had similar relationships, but differed significantly to un-vegetated dunes, which were approximately half the height relative to cross-sectional area of the vegetated dunes (Figure 10.9b). Therefore, a negative consequence of steep dunes is that coastal dune species must grow to greater heights in the long term than would be expected if these species formed less steep dunes. Studies of the rates of dune formation of vegetated and un-vegetated dunes would be of great interest. The formation of steeper dunes by vegetation results in both negative and positive consequences for the growth of coastal dune plants.

Other instances of growth optimisation

A number of studies report that dune plants switch from laterally spreading rhizomes to vertical growth under partial burial conditions, a clear optimisation for plants growing on shallow dunes (*Ammophila breviligulata*, Disraelis, 1984, and Maun, 1985, and *Calamovilfa longifolia*, Maun, 1985). The latter author presents a tantalising diagram (Figure 7 of Maun, 1985), in which *C. longifolia* is depicted growing closer to perpendicular to a steep dune surface, than vertical. In addition, that author suggests that this densely growing species commonly forms “embryo dunes shaped like hummocks with steep lake ward slopes of 20-30^o” (Maun, 1985). Could *C. longifolia* also display this optimal non-vertical growth?

Do dune plants build ‘sand castles’, allowing them to survive extreme burial?

Coastal dune plants form steep dunes at smaller sizes than un-vegetated dunes. The shape of these dunes overlap with the range of slopes for which non-vertical growth results in minimal growth during partial burial. At least one species achieves this optimal growth and forms steep dunes. Thus, these ‘sand castles’ may be a means of achieving minimal growth in the short term under the extreme burial conditions of coastal dunes.

SECTION 5: SYNTHESIS

Chapter 11
Synthesis

This chapter attempts to synthesise the diverse results of the preceding chapters by considering a number of broad questions applied to the coastal dunes of the Eastern Cape:

- What are the dynamics of nitrogen on coastal dunes?
- What are the dynamics of nitrogen within dune plants?
- What is the general cause of zonation?
- What adaptations are useful in the high burial and nitrogen-limited dune environment?
- What environmental and physiological factors affect the growth of *S. plumieri*?

What are the dynamics of nitrogen on coastal dunes?

The mobile-dune species were nitrogen limited, therefore: Does this have large implications for the response of these species to burial? Undoubtedly. These species showed an increased growth in response to burial with full nutrient and nitrogen addition (Chapter 5 and 7). The leaf and photosynthetic physiology of *Arctotheca populifolia* and *Scaevola plumieri* was highly efficient relative to the nitrogen used (Chapter 8). *S. plumieri* was shown to have a variety of traits allowing a response to burial: remobilisation of buried leaf resources and ability to access nutrients in the new sand volume, all characteristics that allow a greater burial response under nutrient limited conditions (Chapter 7). Finally, between and within-individual competition for soil resources limited the growth of *S. plumieri* under unburied conditions (Chapter 9). Under burial conditions the influence of competition was present, but reduced, probably due to influence of alternative sources of nutrients that are unavailable during unburied conditions (e.g. remobilisation of buried leaf resources and ability to access nutrients in the new sand volume).

The mobile- and stable-dune species studied here differed in the degree to which nitrogen limited growth (Chapter 5). This shift was tentatively associated with the ability of *Myrica cordifolia* to fix nitrogen, and the presence of the roots of *Metalasia muricata* under stands of the nitrogen-fixer (Chapter 5). Furthermore, *M. muricata* was shown to be able to benefit from the presence of a nitrogen-fixer, while *S. plumieri*, was unable to access this source of nitrogen (Chapter 6). Thus, nitrogen limitation is spatially heterogeneous across the dunes, dependent on the presence of a high biomass of nitrogen-fixing species in the stable-dunes (this is illustrated in Figure 11.1). May this trend be of greater importance on other dune systems?

From these results it seems that the following partial explanation of zonation is reasonable: The salt sensitivity of the nitrogen-fixing symbiosis may exclude nitrogen-fixers from the mobile-dunes (e.g. Cordovilla *et al.*, 1994; Sande and Young, 1992). Thus, the poor soils of the mobile-dunes limit the growth of mobile-dune species that must grow to survive burial. Consequently the mobile-dune species must have nutrient use efficient characteristics to survive. Thus, species that do not have these characteristics (*M. muricata*, e.g. Table 8.2) are limited to areas of the dunes that have higher nutrient levels, and less burial - the areas where nitrogen-fixers are present. This model certainly seems to function with the four dune species studied here, but it is uncertain how general the model is to other species at this site, or globally. *S. plumieri* is the most dominant dune forming species, *M. cordifolia* a natural, dominant and abundant nitrogen-fixer, and *M. muricata* contribute high biomass to the stable-dunes. Thus, this zonation model is important as it defines the growth of much of the biomass, and ecosystem forming (dune-stabilising) species on Eastern Cape coastal dunes, even if the model is limited to just these three species.

What are the dynamics of nitrogen within dune plants?

The nitrogen-limited mobile-dune plants had physiologies that enabled them to use nitrogen efficiently, and remobilise nitrogen from senescing leaves. These plants were also highly productive for the amount of nitrogen invested in the leaves. This strategy is consistent with these plants being nutrient limited whilst requiring high production rates. A number of results indicate that these same plants did not respond to nutrient addition or any other treatment with an increased allocation to leaf nitrogen concentration (Figure 5.3, Table 7.4, and Figure 7.16). This lack of response may indicate an interesting strategy, namely allocating any excess source of nitrogen to the production of new tissue. The survival advantage for allocating excess resources to new growth is clear for a nutrient limited and burial disturbed environment. Given that these species have extremely high photosynthetic rates, and are close to light saturated at the observed photosynthetic rates (Figure 8.12), there is likely to be little advantage to further increasing leaf nitrogen content. This is the dichotomy that Vos *et al*. (2005) established for maize and potatoes (potatoes have leaves of uniform photosynthetic capacity and allocate nitrogen to new leaves as do the mobile-dune plants). This may underlie the pattern where the branching of *S. plumieri* is limited to shoots that have increased nutrient resources (Chapter 6 and 7, data not shown).

What is the general cause of zonation?

Zonation can be defined by the spatial change in species presence, or in terms of vegetation characteristics, such as production. Numerous spatial change or production related hypotheses were tested in this thesis, and included: 1) the effects of seawater inundation, 2) the stem tissue density hypothesis, relating species competitive and burial response abilities, 3) the spatial heterogeneity of facilitation by a nitrogen-fixer species, and 4) the spatial nature of inter- and intra-plant competition as a part of the competition model of low biomass. The importance of each of these factors in influencing species growth and survival is consistent with the modern holistic view of what factors cause zonation on coastal dunes (e.g. Maun and Perumal, 1999; Barbour *et al.,*1985). For instance, the inverse correlation between stem elongation in response to burial and height competitive ability has the potential to explain the presence of two zones, one of short stature vegetation in an area of mobile sand, and the second in an area of stable-dunes and increasingly tall vegetation. However, the clearly non-random distribution of species within each of these zones remains generally unexplained by the stem tissue density hypothesis. The spatial heterogeneity of species presence and biomass production within the mobile and stable-dune zones, can then be further explained by some of the other hypotheses, such as competition, the presence of nitrogen-fixers, and within the mobile-dune zone episodic seawater inundation (see the schematic representation of these processes in Figure 11.1).

These four processes that cause spatial heterogeneity could potentially be further linked. For example, a high biomass of competing shoots or plants was shown to have a small negative effect on the burial response of *S. plumieri*. Thus, burial and competition may interact with burial reducing the amount of competing biomass and result in increased growth. Alternatively, plants that evenly space shoots and avoid high within-individual shoot densities would be able to grow faster in response to burial than other more densely growing plants. The low nutrients of dune soils would be a third interacting factor, limiting plant growth in response to burial, and enhancing the importance of competition. Other interactions between these processes may also occur.

Salinity, competition, facilitation, burial, water relations, salt spray, soil-pathogens and herbivory play roles in causing dune zonation in various parts of the world. This study confirms the importance of a number of these processes, whilst providing new insight into the mechanisms of how these factors influence plant zonation. Thus, the zonation and succession of dune plants is clearly multi-factorial. However, the stem tissue density model may potentially explain a larger proportion of the variation in species distribution than the other models for Southern African coastal dunes. The stem tissue density model explains a large amount of the dune zonation pattern caused by two environmental gradients, but is based upon the economical and physical consequences of a single plant trait. Thus, the parsimony and explanatory power of this model make it highly alluring.

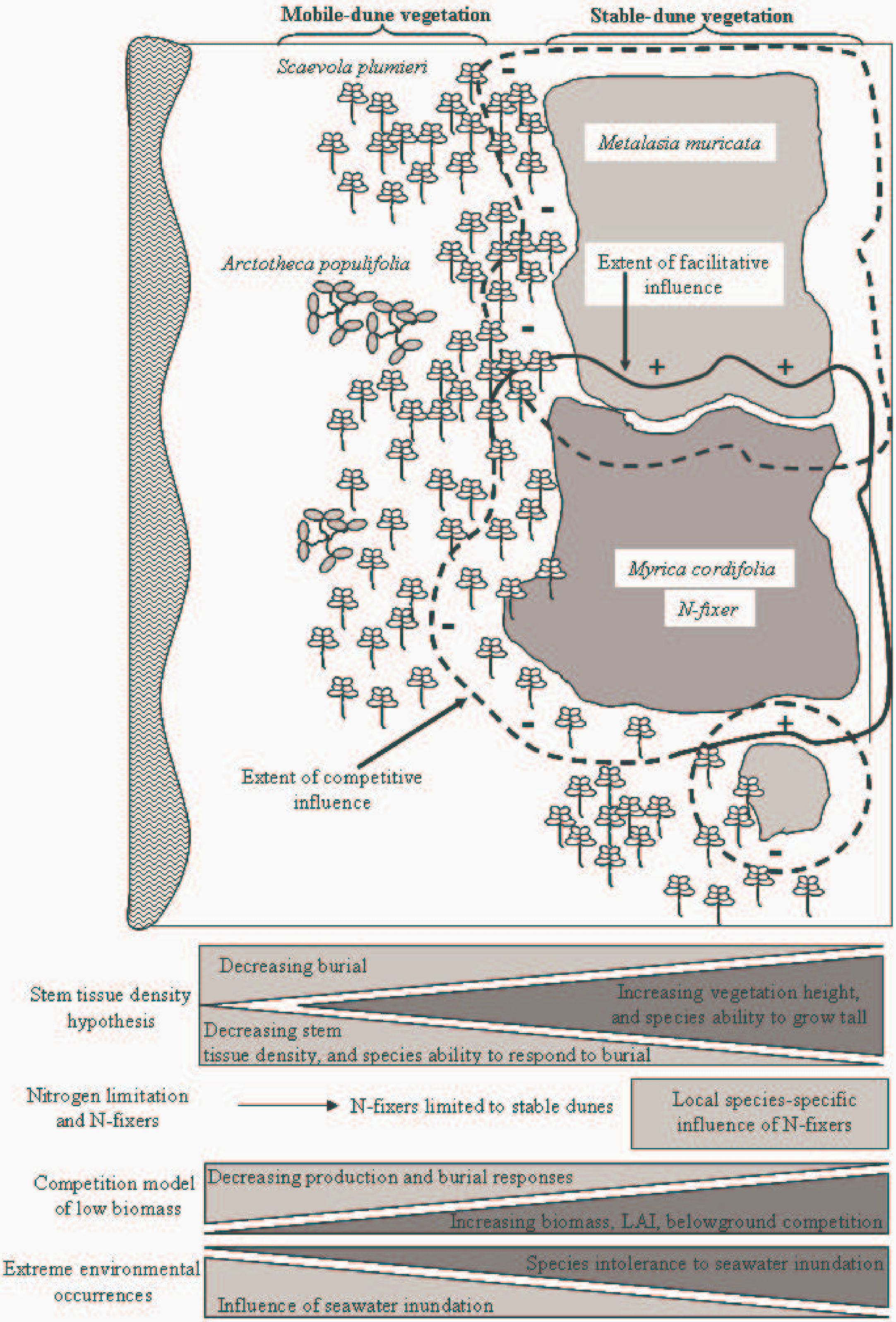


Figure 11.1: Schematic diagram of causes of species zonation and spatial heterogeneity of growth. See text for explanation.

What adaptations are useful in the high burial and nitrogen-limited dune environment?

Adaptations that maximise stem length production, or leaf area production whilst minimising the amount of carbon, nitrogen or dry mass incorporated in this new tissue would be greatly beneficial to plants under high burial and nitrogen-limited conditions. Thus, by changing the way in which resources are distributed an enhanced response to burial can be achieved with the same resource use. Decreases in specific leaf area and leaf nitrogen content are classic examples of these reduced construction costs (Table 7.4). Dune plants, particularly *S. plumieri*, display these shifts, at the expense of a slight reduction in photosynthetic rate after burial (Figure 7.16). Thus, there is a small negative consequence to having enhanced leaf area production. The mobile-dune species have a high requirement for growth in a nutrient limiting environment, consequently the leaves of these species have a fundamentally low nitrogen cost relative to the carbon fixed as indicated by a high photosynthetic nitrogen use efficiency (Figure 8.6). In addition to a low nitrogen cost, the leaves of these species are further efficient through high rates of remobilisation of nutrients from senescing leaves (Table 8.2).

Dune plants display a large allocation shift resulting in increased stem production with burial, thus changes in stem costs would also be beneficial. Stem mass specific length (*MSL*; cm g^{-1}) is a measure of stem cost, analogous to *SLA* for leaves. This parameter has not been widely studied in the burial response literature, even though it may be a vital trait in dune plant ecology (e.g. Chapter 4). The components of *MSL*, stem tissue density (measured as dry mass to fresh volume) and stem width, contribute to the mechanical strength of the stem (e.g. Niklas, 1992). Under burial conditions the plant has a reduced need for mechanical support, and thus a large impediment to reducing *MSL* is removed. Clear reductions in stem width (*S. plumieri*; Figure 7.15) and stem tissue density (many species; Figure 4.13) were shown to result after burial treatments. Thus, mass specific length potentially plays an important and previously unrecognised role in dune species' stem elongation response to burial.

Considering the high growth rates that the mobile-dune species achieve in these nutrient limiting conditions these species must possess characteristics conferring high nutrient use efficiencies or low tissue production costs. The suite of characteristics presented in Chapter 8, are generally consistent with the different burial responses of the species studied in Chapter 5. Under stable conditions the two mobile-dune species had shorter leaf life spans (Table 8.2), lower stem tissue densities (Figure 4.13) and higher photosynthetic nitrogen-use-efficiencies (Table 8.6) than the stable-dune species. These traits are consistent with low stem and leaf production costs allowing the mobile-dune species to achieve high stem and per leaf area production rates. In particular, *A. populifolia* had a high photosynthetic nitrogen-use-efficiency (compared to the species in Field and Mooney, 1986) and extremely low stem tissue density. In combination, these traits may explain the high rate of stem and leaf production that *A. populifolia* achieves under stable and burial conditions, and which are necessary to survive in the mobile-dune area. *S. plumieri* had a contrasting response to that of the fast growing *A. populifolia,* as this species maintained bare stem above the sand surface (Table 5.1) allowing it to have an inducible growth response to burial, but only when its leaves are buried (Figure 7.17 and 7.20).

The mobile-dune species had the most efficient resorption of nitrogen from senescing leaves, and in addition the levels to which the nitrogen was reduced, the nitrogen resorption proficiency was lowest in the stable-dune species (Table 8.2). All species, apart from the nitrogen-fixing *M. cordifolia,* had the ability to reduce nitrogen in senescing leaves to contents that fall within or at the low extreme (*S. plumieri*) of the "nitrogen resorption proficient" range of Killingbeck (1996). A consequence of the fast growth rates (and high rates of burial) of the mobile-dune species was that leaves had a short longevity, resulting in a potentially large loss of nitrogen to litterfall. Thus, the high efficiencies of nitrogen resorption in the mobile-dune species are important in allowing long shoot nitrogen retention times and higher nutrient-use-efficiencies (Berendse & Aerts, 1987) in these nutrient limited plants. The ability of mobile-dune species to reduce costs of stem and leaf production appear to play an important role in the ability of these plants to survive in these extremely disturbed and nutrient limited environments.

What environmental and physiological factors affect the growth of *S. plumieri*?

S. plumieri is the most common dominant plant on the mobile sand dunes along the coastlines of much of Africa, and other tropical regions. This species is typically one of the first species encountered landward of the storm-water mark, and can extend far back into the vegetated landward areas of a dune zonation. The burial responsive growth of *S. plumieri* is generally responsible for the formation of large dunes in the mobile-dune zone, and for this reason is potentially the most important species for the study of dune stabilisation in tropical areas. With this in mind the study of the burial response and environmental responses of this species were included in many parts of this thesis. What affects this species growth (Chapter 5, 6, 7 and 9)? What characteristics allow this species to respond to burial (Chapter 5, 7 and 10)? What is the nature of the burial that this species typically encounters (Chapter 2.1)? What follows is a quick summary of the major findings (Figure 11.2).

The growth of *S. plumieri* was consistently shown to be nutrient limited (nitrogen), particularly when partially buried. Surprisingly, the presence of a nitrogen-fixing species did not enhance the growth of nearby shoots, although this did facilitate the growth of another species (Chapter 6). Rather, between- and within-plant competition were shown to be important determinants of the growth of individual shoots of this species (Chapter 9). While this competition was an important determinant for the leaf area production of all shoots, the stem production of buried shoots was less (but significantly) related to these factors (Chapter 9). The implication may be that buried shoots have access to new resources, reducing the effects of competition. Thus, the competition model of low biomass may well account for some of the growth limitation of this species, but other factors must be involved when determining this species growth in response to burial (Figure 11.2). The large number of factors determining this species growth may result in the low coefficients of determination (R^2) for each factor. However, the effects of single factors on shoot growth of this species where remarkably similar between independent survey’s of *S. plumieri* growth (at OWR and Cintsa; Chapter 9), an important confirmation of the strength of the findings.

The physiological mechanisms of burial response where shown to be important determinants of growth, in addition to various environmental factors (Figure 11.2). These mechanisms were compared in a number of quantitative experiments, allowing a rigorous test of past and new hypotheses. A new hypothesis, that of remobilisation of resources in buried leaves, was shown to account for much of the burial response of *S. plumieri*. Past hypotheses, such as the importance of new nutrients in freshly blown sand, were shown to be less important. These results indicate that a large number of physiological mechanisms may account “reactive” growth, a blanket term used to describe and enhanced growth in response to burial (See Figure 7.19).

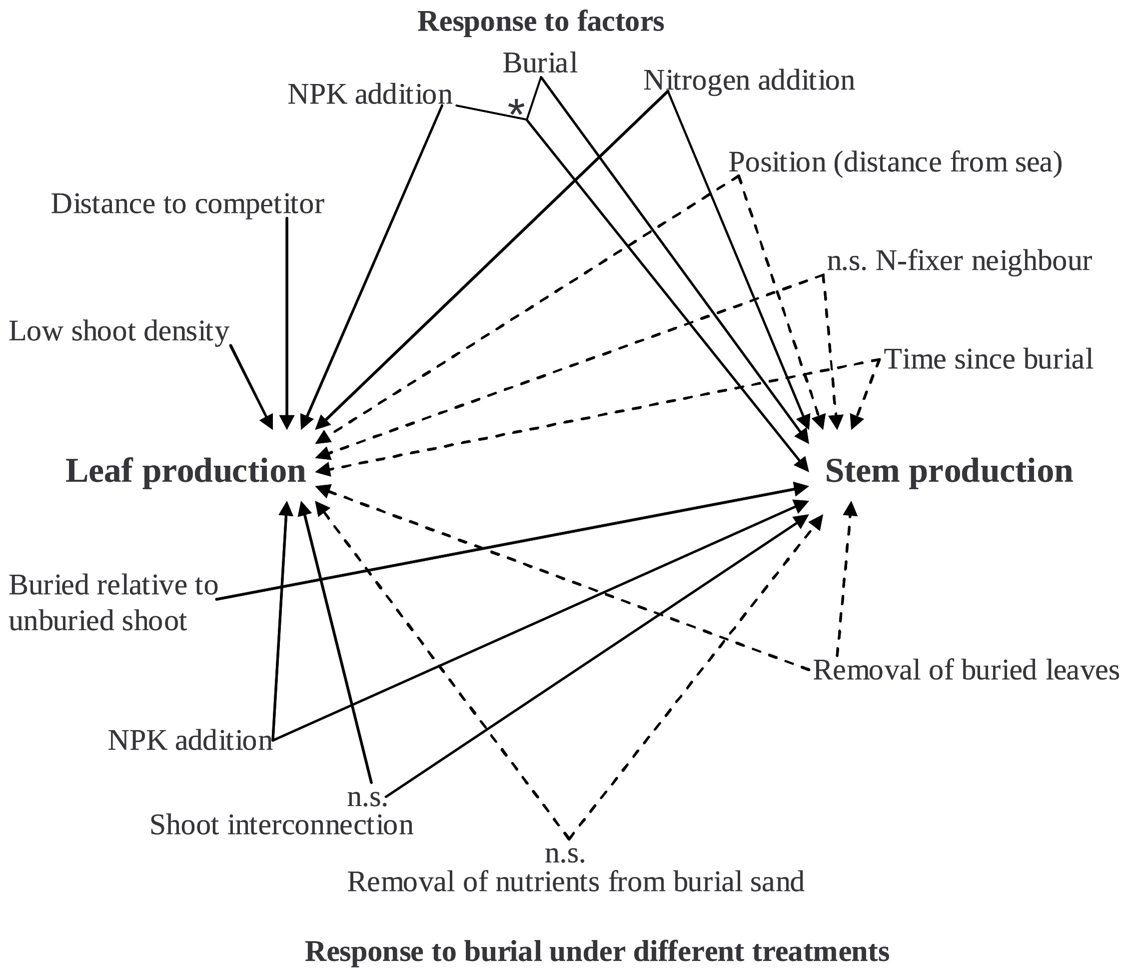


Figure 11.2: Factors and treatments that affect the leaf and stem production of *S. plumieri***.** Positive (solid lines) and negative effects (dashed lines) are indicated, as well as not significant, but consistent effects (n.s.) and interactive effects (asterisk).

Final words

Interested readers should consider this as a starting point for new discourse in dune ecology, not an end. The idea that vertical growth is not necessarily optimal could be integrated into the increasingly popular models of sand dune formation (e.g. Baas, 2002, and Kroy *et al.*, 2002). The proposed inverse trait correlation may not be universal to causing zonation in dune ecosystems, but the need for an inverse trait correlation to explain species responses to a gradient is an important unused zonation mechanism in the dune ecology literature.

This thesis has shown that dune plant zonation and survival on environmentally harsh coastal dunes is mediated through characteristics that relate specifically to minimising the costs of producing growth. Reduced nitrogen and carbon costs of growth, and characteristics that influence nutrient supply were shown to be of great importance to survival and particularly relevant in this nutrient limited environment. A critical approach was taken to ideas presented in the literature, and has resulted in the formation of a large number of new ideas about coastal dune plant ecology. This, more holistic, view of the dune environment emphasises the importance of the economies of carbon and nitrogen, and their interaction in determining which plants are present on the dunes.

CONCLUSIONS

- **Chapter 2.1:** Burial, by sand, occurred frequently in small magnitude events, relative to the height of the plants. The occurrence of larger important burial events was related to periods of high-speed winds, was seasonal (summer maximum), and was found to occur within 10 days of the previous burial event, for this site. The magnitude of burial was only heterogeneous over larger scales (>>1m), and with respect to the aspect of the dune. Summer easterly winds were the most important source of potential sand transport, contrary to the westerly winds that predominate in nearby dune fields (Alexandria).
- **Chapter 2.2:** The tolerance of numerous species to an extreme inundation by seawater, corresponded to the position of these species along the dune zonation gradient. The four main species studied were exposed to different amounts of salt spray, but the species that typically had the highest salt spray exposure also had the shortest leaf life spans. Thus, the effects of salt spray may be related to spatial gradients as well as differences in the time with which leaves can accumulate salt.
- **Chapter 2.3:** Nutrient levels in dune soils from around the world are extremely low, consistent with nitrogen limitation for most soils, and phosphorus limitation in conditions of greater leaching. Similarly, coastal dunes in the Eastern Cape have low nutrient contents, while dune plants show indications of varying nutrient limitation.
- **Chapter 2.4:** Dune soils have low soil water contents, but high water availability due to the nature of the soil suction curve. In general, areas with low leaf area index at this study site have been shown to have sufficient water to supply the plant throughout the year (Ripley *et al*. 2002). For this study, higher biomass dune species had no consistent differences in midday water potential, or pressure volume curve parameters to the low biomass dune species. The xerophytic characteristics of the leaves of many dune plants (succulence, thickness, thick cuticles) are better ascribed to halophytic and heliophytic characteristics. Indeed, succulence partially explains the sequence of species' zonation on these dunes, and tolerance of species to seawater inundation.

- **Chapter 3:** The current facilitation models of the zonation of species on coastal dunes, often lack: 1) explicit mechanistic reasons for differential species tolerance of conditions along the environmental gradient, and 2) a reason for the lack of foredune species in "later" dune communities.
- **Chapter 4:** Stem tissue density results in the observed differential ability of a species to respond to burial, as species with a low stem tissue density are able to produce more stem for a given investment in dry mass. Concurrently, stem tissue density determines the mechanical elasticity of stems of these species, resulting in a mechanistic inverse correlation (tradeoff) between the ability of a species to respond to burial and the maximum height to which the species can grow. This tradeoff was shown experimentally for 8 dunes species, and is implied in the measurements made of all ~30 species present on dunes in the Eastern Cape. Stem tissue density relates strongly to these species presence in mobile-dune areas, or areas of tall vegetation. The inverse correlation fulfils both of the criteria that current dune zonation models lack.
- **Chapter 5:** Nutrient availability was shown to limit the growth of the two mobile-dune species, *A. populifolia* and *S. plumieri*, and the responses of these species to burial. Thus, mechanisms that increase the supply of nutrients to these two species (nitrogen-fixation, reduced competition, shifts in biomass allocation) or characteristics that shift nutrient allocation in response to burial (buried leaf nutrient remobilisation, release of stored nutrients, reduced nutrient costs of producing tissue) are likely to be important in determining how these species survive burial. The two stable-dune species, *M. cordifolia* and *M. muricata*, were not responsive to nutrient addition, and less responsive to burial than the mobile-dune species. These results confirm that dune species have differential abilities to respond to burial, based upon where on the burial gradient they are typically present. In addition, nutrient dynamics have the potential to play and important role in determining the ability of a species to respond to burial, and the mechanisms of burial response.
- **Chapter 6:** The presence of nitrogen-fixing species (*M. cordifolia* and *Acacia cyclops*) in the stable-dune area may explain the lack of fertiliser response of the two stable-dune species measured in the previous chapter. Nearby

nitrogen-fixers enhanced the growth of *M. muricata*, but the burial responsive species, *S. plumieri*, was not positively influenced by the presence of a nitrogen-fixer. Thus, nitrogen-fixers may be competitive or facilitative to neighbouring plants, depending upon the species studied. As nitrogen-fixers are found in the stable-dune, and burial responsive species are unable to benefit from growing near a nitrogen-fixer, nitrogen-fixation does not appear to affect the responses of mobile-dune species to burial.

- **Chapter 7:** A literature survey tested whether dune species show enhanced biomass accumulation in response to burial. While this widely reported response was found for a large number of species, the enhancement of biomass accumulation was limited to shallow burial depths relative to the height of the plant. A consistent shift from root to shoot allocation was found for species that were able to enhance growth in response to burial, but not for burial-unresponsive species. Experiments on the burial responsive species, *S. plumieri*, showed that this species had the following responses to burial: 1) increased internode length, increased stem production, but little change in leaf production. Mechanisms whereby the plant gained the extra resources needed to respond to burial were: 1) reduced stem width and density, 2) remobilisation of resources in buried leaves, 3) reduced reproductive allocation, 4) stimulation of adventitious rooting in the new soil medium, gaining a small amount of nutrients from this sand, and 5) reduced costs of leaf production and photosynthesis. Season influenced the magnitude of the burial response, such that during the summer period of greatest burial, the burial response was considerably larger than winter. Burial responses were partially similar to complete shade etiolation responses, but the latter response lacked a net increase in biomass production.
- **Chapter 8:** The two nutrient limited mobile-dune species were shown to have highly efficient carbon metabolism relative to the nitrogen used. These plants had high whole plant nitrogen use efficiencies as indicated by long retention times of nitrogen in the plant, relative to short leaf life spans. The mobile-dune species maintained high photosynthetic rates through extremely high stomatal conductances, thus using readily available water over limited nitrogen resources. The partitioning of the photosynthetic enzymes and apparatus was

relatively well partitioned, with little excess nitrogen invested in the enzymes than that needed to achieve the observed photosynthetic rates. The whole plant- and photosynthetic-nitrogen use efficiency of these species were considerably closer to optimal than the nitrogen-fixer, *M. cordifolia*.

- **Chapter 9:** A theory of dune plant growth was proposed, which may allow plants could respond to burial by occurring in an environment of low biomass of competing plants/shoots. The growth of shoots of *S. plumieri* was shown to be related to many factors including: 1) the position on the seaward (+) or landward (-) aspect of the foredune, 2) burial state (+stem production for buried shoots), 3) distance to nearest large thicket species (-within 2.5 metres distance), and 4) the density of *S. plumieri* shoots within a stand, taken to be a single individual. Therefore, competition, both between species and within an individual, plays a role in reducing shoot production. However, it remains less clear how competition affects the burial responses of plants.
- **Chapter 10:** The geometry of the growth of dune plants in response to partial burial was shown to be optimal, in a manner not previously anticipated. Partially buried shoots of *A. populifolia* grew perpendicular to the dune surface, and as a result required reduced stem length production to maintain leaf area above the sand surface. The two dune species able to grow non-vertically, or optimally, on non-horizontal dune surfaces also formed considerably steeper dunes.

APPENDICES

CONTENTS

APPENDIX 1

Notes about statistics

In any ecological study the nature of field experiments, variation, costs, demonic and non-demonic intrusions (Hurlbert, 1984) necessitate some statistical compromises. In some cases, sample sizes are relatively small while variation is high. Parametric tests are sensitive to these conditions, while alternative non-parametric tests are unable to test even simple factorial experimental designs. Within these limitations, the author has attempted at every step to remove pseudoreplication (Hurlbert, 1984), reduce the chance of type II errors, and use parametric statistics only when conditions are appropriate.

The debate concerning ratio data and the complications of using this means of describing data (Berges, 1997; Poorter and Nagel, 2000), has not gone unnoticed by the author. Consequently, in all obvious cases: size related allometry, specific leaf area's, and photosynthetic nitrogen use efficiency, the use of ratios has specifically been considered. In these cases, if an allometric (rather than isometric) relationship is found, an ANCOVA or the relevant bivariate analysis (Falster *et al.*, 2006 and Warton *et al.*, 2006) has been conducted to avoid biased conclusions. Standardised major axis (SMA) regression is particularly useful (see Warton *et al.*, 2006 for a thorough review of the subject). This procedure fits lines to data that allow better parameterisation of models, as the lines are fitted to minimise the sum of squares of residuals to the regression line, while accounting for x variable measurement error. In some cases this is preferable to minimising the residual vertical distances of the y parameter, as for ordinary least squares regression (OLS). Unfortunately, available SMA statistical programs do not fit full statistical models comparable to OLS ANCOVA models. Thus, SMA analysis is limited to linear effects models with no treatment interactions. Importantly, the type of regression adopted, does not affect the calculation, or interpretation, of the correlation coefficient. Fortunately, one characteristic of these two fitting procedures is that OLS and SMA regression lines are more similar as the correlation coefficient increases. Therefore, in some instances OLS ANCOVA models have been used where R^2 values were high (>0.85), when SMA models would have been preferable, but not possible to implement at this stage in statistical program development (e.g. Chapter 5).

Allometry, allocation, plasticity, and ANCOVA

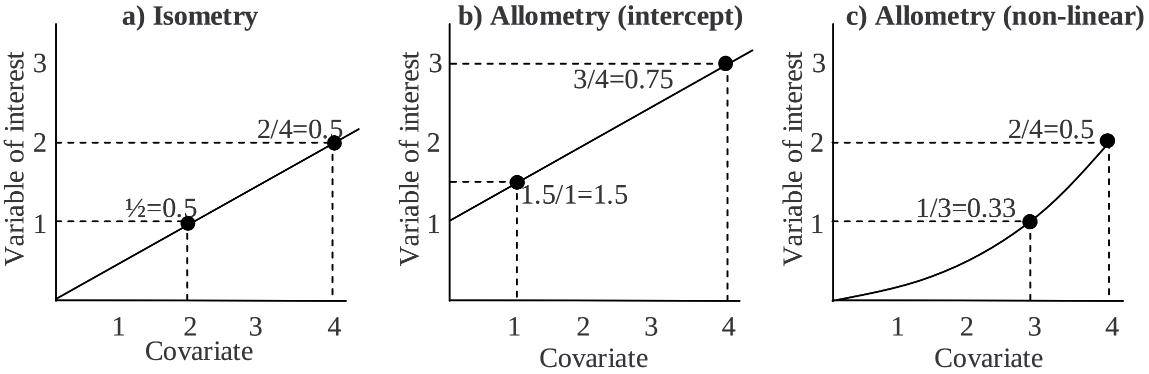


Figure 12.1: Isometric (a) and two types of allometric bivariate relationships: a linear relationship with a non-zero intercept (b), and a non-linear relationship (c). In each diagram two points are drawn falling on the same line or curve. For each point the ratio of the variable of interest to the second variable has been calculated to demonstrate the effect of these three types of scaling relationships on the values of the ratios. Only the isometric relationship (a) maintains the same ratio at all sizes of the covariate, although for the allometric curves the two points fall on the same fundamental relationship.

Allometry is the measurement of scaling, allocation is the partitioning of resources, and plasticity is the ability to change, and these are important and interrelated concepts. As each concept involves the interaction of two or more continuous variables, these three concepts are best analysed using ANCOVA. These concepts are used throughout this thesis; consequently a quick introduction was included here.

The way in which resources are allocated (plasticity) changes with plant age or size (McConnaughay and Coleman, 1999; Poorter and Nagel, 2000). Thus, a second variable (age or size, etc) may confound the interpretation of allocational plasticity, as different treatments may be compared across different values of this second variable. Allometric trajectories describe these size relationships, and when used in conjunction with ANCOVA, may allow an insight into the reasons behind shifts in allocation.

Differences, between two groups, in mass ratios (plasticity) can arise for two different reasons: apparent and true plasticity (McConnaughay and Coleman, 1999). Apparent plasticity occurs when two treatments (or groups) have different sizes, but share a single allometric trajectory between the two parameters. This size separation results in different allocation (ratios) between the two parameters for each group, due to the size differences in combination with the effects of the allometric trajectory (Figure 12.1b or c). The implication of this is that if these groups were of the same average size, they would have the same allocation pattern. Thus, upon discovering apparent plasticity, one could ask: Why do the groups have different sizes (or ages)? True plasticity occurs when treatments result in fundamentally different trajectories between two parameters. However, these relationships may intersect, in which case the allocation difference between the groups is, again, dependent on size. Differences in plasticity and allocation are contingent on the ecological context in which these changes act. Apparent plasticity is ecologically relevant when two groups are functionally separated along a shared allometric trajectory. Conversely, true plasticity could be ecologically irrelevant if two groups share the same allocation due to the position of the groups at an intersection of their two separate allometric trajectories. Poorter and Nagel (2000) make an important point: that both true and apparent plasticity can be ecological significant and it is ecological significance which should guide our interpretation of allocational plasticity. It is noted that there is a lack of consensus on the techniques used to account for allocational plasticity (e.g. Poorter and Nagel, 2000 and McConnaughay and Coleman, 1999). The technique suggested by Poorter and Nagel (2000), that of plotting a mass fraction against total mass, was not adopted here, as it may confound the analysis of the data by plotting aspects of the same variable on both axes. The techniques used by McConnaughay and Coleman, (1999) are more widely used in the literature and where adopted here, although they may have less statistical power (Poorter and Nagel, 2000).

These concepts may be applied to a broad variety of ecophysiogical problems. Competitive allocation between aboveground and belowground production, the biomass allocation between two compartments, reproductive effort may be determined by age, and photosynthetic rates relative to leaf nitrogen contents are all examples for which these concepts are useful.

Allometry

A strictly proportional relationship between two variables is termed an isometric relationship (Figure 12.1a). Mathematically, this means that the ratio of variable A to variable B is the same for any size of B. For allometric relationships this rule does not hold, for one of two reasons. The first is that the relationship between the two parameters could have an intercept other than the origin (Figure 12.1b). An example is the ratio of assimilation to leaf nitrogen content, termed photosynthetic nitrogen use efficiency (Evans, 1989). The second reason for a relationship to be allometric is that the relationship may be non-linear (e.g. the relationship of leaf length to surface area; Figure 12.1c). Most of the literature uses the word allometry to refer to a relationship of the form $y = ax^b$ where b is significantly different to one (linear; e.g. Warton *et al.*, 2006). Allometric scaling is conveniently linearised by applying logarithm transformations to both variables. Isometry, allometry due to different intercepts, and non-linear relationships are demonstrated in Figure 12.1a, b, and c. The allometric nature of the relationship between two variables determines the effect of size on the relationship. Since many of these relationships are logarithmic in form, allometric differences can have disproportionately large effects on ecophysiological processes, and therefore it is vital to detect and account for allometric effects.

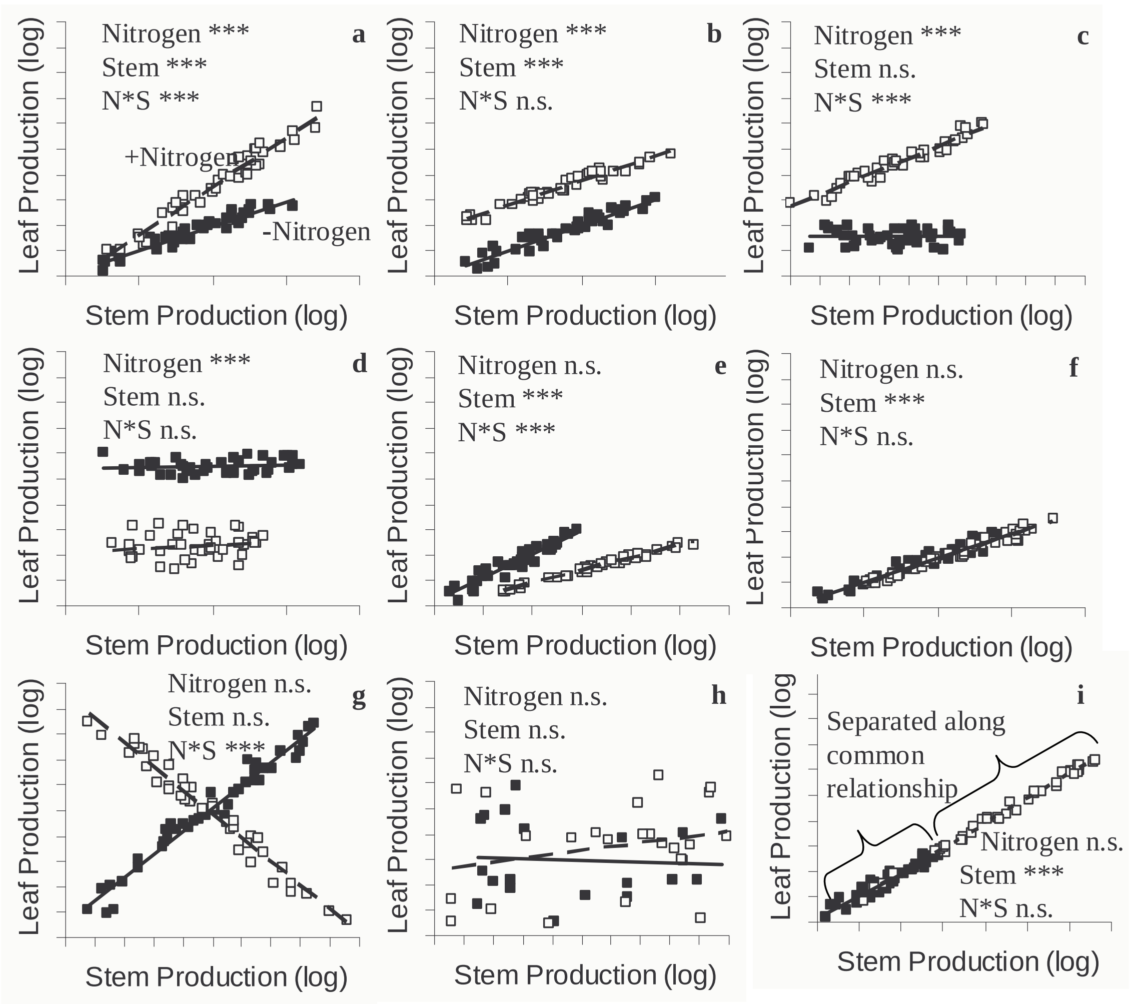


Figure 12.2: Schematic diagram to illustrate the eight possible ANCOVA results (a-h) for a bivariate relationship. The bivariate relationship depicted here is between the logarithms of leaf and stem production with two nitrogen treatments. If the interaction between treatment and size is significant, then this implies the treatments have different slopes. If this is the case then the significance of the main treatment effect cannot be interpreted simply, *vis* the effect of the treatment is dependent on the size of the covariate (for instance compare a, c, e, and g). An additional possibility is to test for a shift along a common relationship (i).

ANCOVA: Testing for differences in allometry, and true or apparent plasticity

In all linear regression and ANOVA analyses, higher order interactions signify that the generality of main effects or lower order interactions are dependent on the values of the factors. In the initial analysis of an ANCOVA, a significant factor-by-covariate interaction indicates that the effects of the factor, while significant, are dependent on the value of the covariate (Engqvist, 2005). A significant factor-covariate interaction is statistically equivalent to saying that lines describing each group have different slopes. For instance, the relationships depicted in Figure 12.2a, b, e, and g, where the effect of nitrogen is less, or non-significant, at a specific value of stem production.

The ANCOVA technique is dependent on the covariate explaining a significant amount of variation in the variable of interest. If there is no relationship between the variable and covariate, it is inappropriate to perform an ANCOVA, or try to detect allocation differences in this manner (Figure 12.2d and h). Such cases are suggestive of a lack of limitation or coordination of the allocation of biomass between the two compartments being analysed.

The procedure used for an ANCOVA is to test whether groups have significantly different slopes (factor-by-covariate interactions; Figure 12.3). If there are significant factor by covariate interactions a classical ANCOVA can not be done, and the main effects can not be interpreted simply. If all groups share a common slope, classical ANCOVA is done, where a model is fitted to the data with the factor-by-covariate interaction terms removed. Significant main effects in the latter type of analysis can be interpreted as different groups having different y-intercepts. Since the slopes are the same, these differences are maintained at any value of the covariate. This procedure is equivalent to conducting an ANOVA on the residuals of the relationship between a variable and a covariate. Engqvist (2005) gives a short and simple review of appropriate ANCOVA procedures.

True and apparent plasticity relates to the significance of main effect and factor-by-covariate interaction terms (Figure 12.3 describes the process of determining true and apparent plasticity from an ANCOVA). True plasticity is indicated when a factor has a significant main effect, in the absence of a factor-by-covariate interaction, or when there is a significant factor-by-covariate interaction (e.g. Figure 12.2a, b, c, e, and g). Apparent plasticity is indicated when there are no factor effects, but the groups are significantly separated along a common allometric relationship (Figure 12.2i). Two methods can indicate such a shift, *post hoc* to an ANCOVA which shows that the groups share a common relationship (e.g. the results in Figure 12.2f or i): 1) by conducting an ANOVA including only the y variable and factors, however, this is very conservative and will not take into account the covariate, and 2) using the values of the covariate and y variable, and the formula for common relationship, a value can be calculated describing the points position along the common relationship, these points can be used in an ANOVA comparing shifts along this shared axis. The SMA software of Falster *et al*. (2006) offers this procedure. For ANCOVA's this procedure is poorly reported, and was not used in this thesis. Fortunately, in the cases where these tests were necessary, the shift along the common relationship was sufficiently large, that the initial conservative analysis was sufficient to demonstrate a significant difference (Appendix D of Warton *et al.*, 2006, has some discussion of this issue).

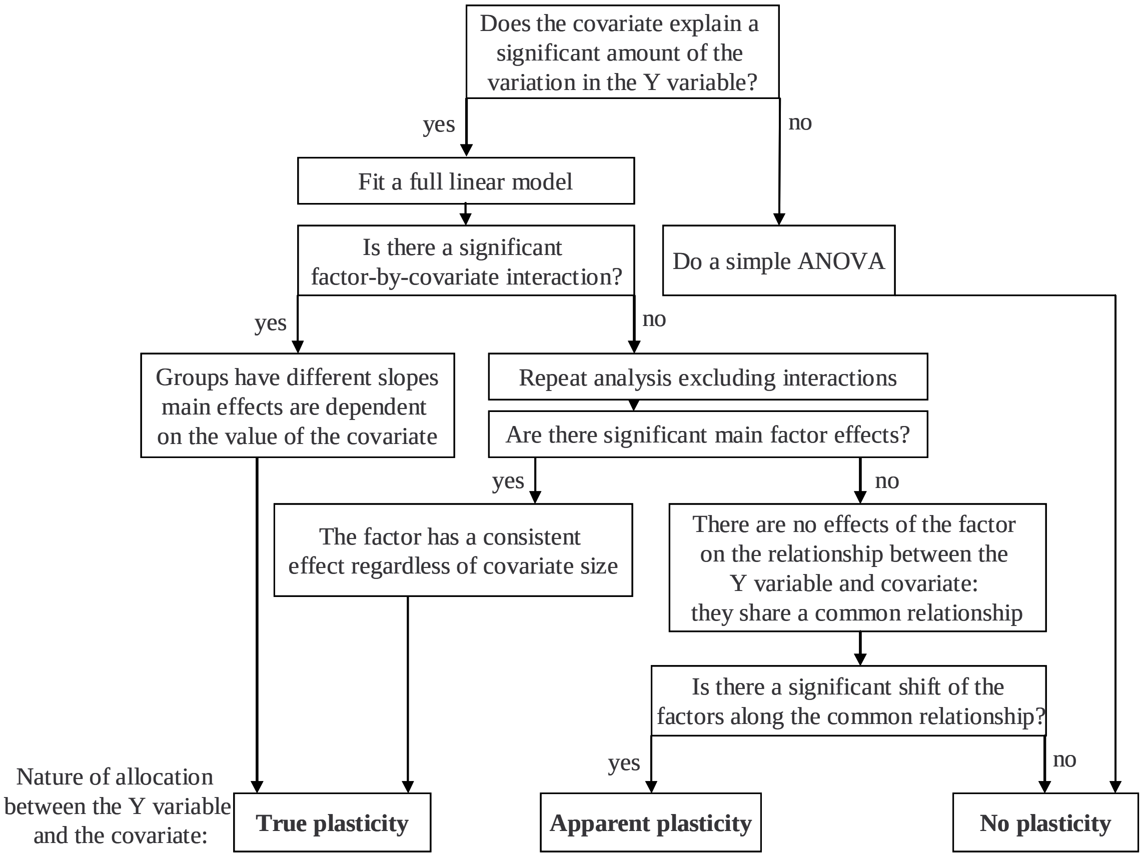


Figure 12.3: Flow chart of the use of ANOVA or ANCOVA to determine whether allocational differences between two variables (a Y variable and covariate) are due to true or apparent plasticity, or whether plasticity is not present. This scheme is applicable to ANCOVA analysis, in general, although the concept of plasticity may not be relevant. In analyses with two or more factors, the interaction between these factors must be retained, and only the factor-by-covariate interactions removed in the second repeat analysis.

APPENDIX 2

Explanation of the requirements for an inverse correlation to exist between plant maximum height and rate of stem elongation after burial

In the introduction of Chapter 4, it was stated that “inorder for increases in ρ_{wet} [and ρ_{dry}] to have an effect on plant height, E must relate allometrically, or linearly (with an intercept), to ρ_{wet}.” These are vital requirements for the stem tissue density hypothesis to hold, and these requirements can be demonstrated in the following manner:

Analytically, a power relationship (E=a$\rho_{dry}{}^{f}$), would allow a linear (E=a$\rho_{dry}{}^{1}$), or an increasing allometric relationship (E=a$\rho_{dry}{}^{f}$, where f>1) to exist between E and ρ_{dry} (See Figure 4.2). Thus, relating l_{crit} to l_{elong}, by combining the equations given in Figure 4.2 gives the following absurdly complex solution:

$$l_{crit} = C\left(\frac{a\left(\dfrac{B}{\pi\left(\dfrac{e}{2}\right)^{2} l_{elong}}\right)^{f}}{\dfrac{mB}{\pi\left(\dfrac{e}{2}\right)^{2} l_{elong}} + c}\right)^{\frac{1}{3}} d^{\frac{2}{3}}$$

By keeping a, b, C, d, and e constant and varying one or both of f or c, it can be shown that l_{crit} and l_{elong} are inversely correlated, only if: 1) there is a positive intercept (c) in the relationship between ρ_{wet} and ρ_{dry}, or 2) the exponent (f) is greater than 1 in the ρ_{dry} to E relationship, or 3) both of these conditions hold, with some variation in the values of these two parameters for which an inverse relationship occurs. As the fitted values for f and c were 2.26 and 0.97, and significantly different to 1 or 0, this implies that an inverse relationship must exist between maximum height and stem elongation rates, assuming that the variables a, b, C, d, and e are relatively constrained for coastal dune plants.

APPENDIX 3

Estimation of NUE_{leaf} by leaf nitrogen and nitrogen remobilisation efficiency

Berendse and Aerts' (1987) give the following equation to calculate whole plant nitrogen use efficiency (NUE):

$$NUE = P_N / MRT \qquad (1)$$

Berendse and Aerts' (1987) formula for plant NUE is difficult to parameterise as the mean retention time of nitrogen (MRT) represents the mean lifespan of nitrogen in the plant and P_N the nitrogen productivity. Values for MRT could be measured by an analysis of nitrogen isotopes. However by two modifications of this general formula, a new concept, NUE_{leaf}, is defined which can be estimated by the easy-to-measure leaf nitrogen content (L_{leaf}) and nitrogen remobilisation efficiency (R).

The concept of whole plant NUE can be extended to include the components, NUE_{leaf}, NUE_{stem}, NUE_{root}. NUE_{leaf} is of particular interest as most of a plant's nitrogen is in the leaves (Chapin, 1989), and this is the nitrogen that is most directly involved in productivity. Thus, similar to NUE:

$$NUE_{leaf} \approx P_{Nleaf}/MRT_{leaf} \qquad (2)$$

This formula also has the same limitations as the formula for whole plant NUE, as measuring MRT_{leaf} would require nitrogen isotope analysis. However, if MRT_{leaf} was replaced by its components leaf longevity (L_{leaf}) and nitrogen remobilisation efficiency (R) this formula would be more simple to parameterise.

Inductively, as $R \rightarrow 0$, then $MRT_{leaf} \rightarrow L_{leaf}$
and $R \rightarrow 1$, then $MRT_{leaf} \rightarrow \infty$
Where → means "as a [variable] tends to".
Thus inductively,
$MRT_{leaf} = L_{leaf}/(\text{any polynomial form of } 1\text{-}R)$ (3)

A simple iterative computer model of this process, whereby a shoot with sequentially produced leaves has an *R* reallocation of nitrogen to the newly produced leaf and L_{leaf} confirms that the most simple form of equation 3 gives the same results and iterative model (Figure 12.4) and is the correct formula for estimating MRT_{leaf}. Thus:

$$MRT_{leaf} = L_{leaf}/(1-R) \qquad (4)$$

This relationship implies that the *MRT* of leaf nitrogen increases hyperbolically as *R* tends towards 1. Thus, the remobilisation efficient leaves of *Arctotheca populifolia* (73.1%) result in 3.7 times longer MRT_{leaf}'s than L_{leaf}. The nitrogen remobilisation efficiencies reported by Harte and Pammenter (1983) of ca. 80% for *Scaevola plumieri* result in MRT_{leaf} of 4.15 years. Thus, this formula for MRT_{leaf} indicates an important ecological phenomenon.

Deductive proof of equation 4 from a similar model of this process was achieved using series mathematics by David Orpen (pers. comm. 2005).

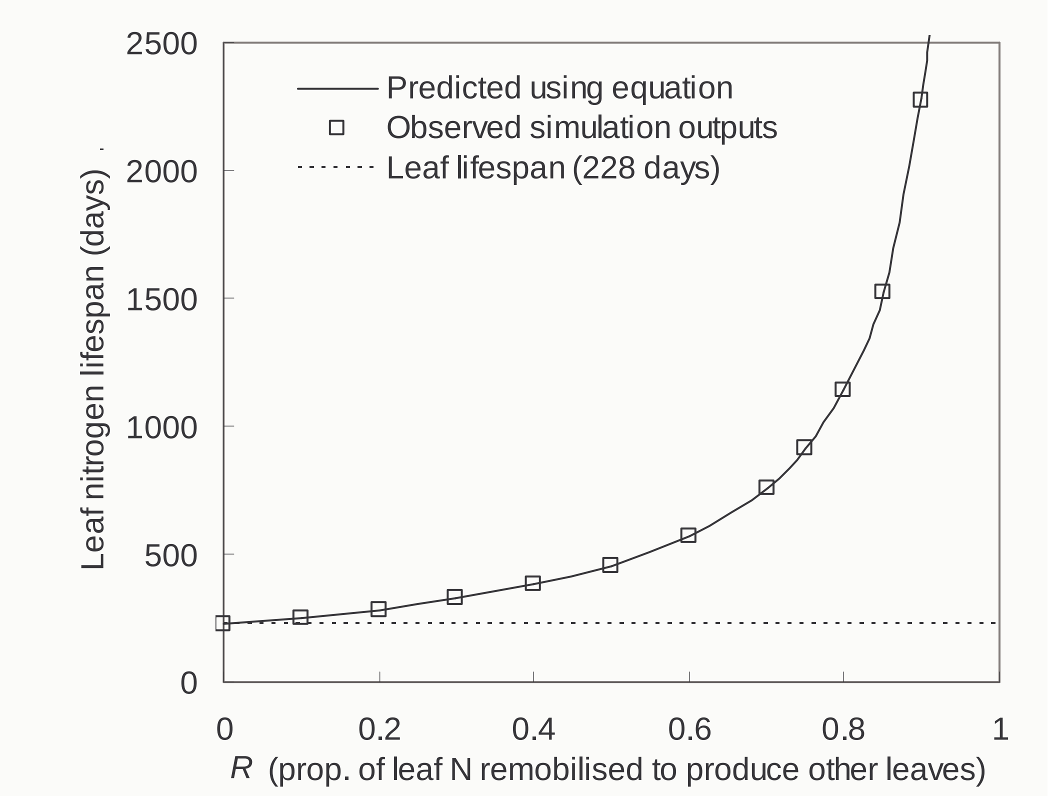


Figure 12.4: Observed and predicted values for MRT_{leaf} (mean leaf nitrogen lifespan in a shoot) found using the results from a simple iterative model of the growth of a shoot (observed) and the predictions of the equation $MRT_{leaf} = L_{leaf}/(1-R)$ (predicted). The results were obtained from the model using the parameter values of 228 days for leaf lifespan and by varying *R*. The model result is invariant to adjustment of values for leaf lifespan.

Therefore, by substituting of the new MRT_{leaf} estimation into equation (2):

$NUE_{leaf} \approx P_{Nleaf} * L_{leaf} / (1-R)$ (5)

Using this new formula, NUE_{leaf} can be now estimated from three easily measured components, P_{Nleaf}, L_{leaf} and R. Poorter and Garnier (1999) give a similar formula for whole plant *NUE*, but show no derivation. The formulation presented here allows for a straight forward parameterisation of an important component of whole plant *NUE*, that does not require complex experimentation e.g. nitrogen isotope dilution experiments. Thus, equation 5 represents an easy to parameterise formula that allows some break down of the components of leaf *NUE*.

APPENDIX 4

Estimation of nitrogen allocated to different physiological components of leaves, by measurements of gas exchange, Rubisco content, chlorophyll and total nitrogen.

A number of studies use parameters generated by gas exchange measurements to estimate leaf nitrogen partitioning. This technique is largely based upon influential work of Evans (1989), and no subsequent review of this topic exists in the literature. Estimation of leaf nitrogen partitioning by gas exchange parameter should ideally be validated against actual measurements of leaf nitrogen components. Validating these techniques was beyond the scope of this thesis, and since a number of influential authors apply these techniques to a broad variety of species the technique was adopted here. This technique has the advantage of allowing easy estimation of complex biochemical parameters, but should be regarded solely as a means of deriving interesting questions relating to nitrogen distribution, not as an accurate method for determining biochemical concentrations. To a large degree all the authors in this field have derived formulae from the data of Evans (1989), thus that paper and the paper of Poorter and Evans (1998) should be considered as the primary references for these estimations. What follows is a review of the use of these equations.

The value of each photosynthesis parameter used for the calculation of the nitrogen components is temperature dependent (e.g. Bernacchi *et al.*, 2003; Harley *et al.*, 1992b). For this reason photosynthetic parameters should be converted to a standard temperature before calculating the nitrogen components. The temperature corrections of Harley *et al*. (1992b) have been widely used and allow for straight forward comparisons with older literature (e.g. Wullschleger, 1993). The more recent equations of Bernacchi *et al*. (2003) appear robust, and simple, and were determined *in vivo*.

Estimation of N_{LHC}

The nitrogen incorporated within the light harvesting components of the photosynthetic apparatus (N_{LHC}) can be reliably estimated from measurements of chlorophyll concentration according to the work of Evans and Seeman (1989). Dependent on the plant living in a high light environment those authors found that there were 41 moles of nitrogen per mole of chlorophyll. This estimate includes nitrogen associated with chlorophyll, as well as nitrogen that made up the chlorophyll molecule itself (~4 mol N mol^{-1} Chl). Most authors use this estimate (Table 12.1).

Estimation of N_{ETR}

The total thylakoid nitrogen content (N_{THY}), including electron transport and photophosporylation and light harvesting associated enzymes and proteins, was shown by Evans (1989) to be related to measured oxygen evolutions rates (which can be estimated by $J/4$) and leaf chlorophyll contents by the equation: $N_{THY} = 0.316*J/4+33.1*Chl$ (for variations of this formula see Table 12.1). The amount of nitrogen involved in electron transport can then be estimated by subtraction of N_{LHC} from N_{THY}. The nature of the N_{THY} to J and Chl relationship results in an interesting phenomenon when considering the *PNUE* of a leaf limited by electron transport (Evans, 1989). Due to an intercept in the relationship of N_{ETR} to J there is a shift in the nitrogen cost of electron transport with increases in the absolute electron transport capacity.

Estimation of N_{PCR}

Rubisco forms a large proportion of leaf nitrogen, and along with other enzymes associated with the Calvin-Benson-Bassham cycle forms the nitrogen incorporated within the photosynthetic carbon reduction cycle (N_{PCR}). Rubisco content may be measured by the ^{14}CABP binding technique (e.g. Poorter and Evans, 1998) or by electrophoresis (e.g. Warren and Adams, 2001). These methods assume that either the Rubisco is fully activated or that there is 100% efficiency of recovery by the electrophoresis assay. $N_{Rubisco}$ is then estimated from the molecular mass of Rubisco (550kDa) with a nitrogen content of 16% by mass (Westbeek *et al.*, 1999). These calculations do not take into account the other enzymes involved in the PCR cycle, but can be estimated by the measurement that Rubisco makes up 76% of PCR protein by mass (Pons *et al.*, 1994).

An alternative method is to calculate N_{PCR} from a measured value of V_{cmax}, by assuming that Rubisco is fully activated, and has a set specific activity (Poorter and Evans, 1998; Table 12.1). Similarly, an equation is given by Pons and Westbeek (2004) but derived from Makino *et al.* (1988) and Evans and Seeman (1989):

$N_{PCR} = V_{cmax} * 359$ mol N mmol CO_2^{-1} s.

Estimation of N_{PC} and N_{other}

The total leaf nitrogen content (N_{total}) is made up of two functional components, the nitrogen incorporated within the photosynthetic apparatus (N_{PC}) and the nitrogen used in non-photosynthetic processes (N_{other}). In general, two methods can be used to estimate these fractions. Firstly, the sum of N_{PCR}, N_{ETR} and N_{LHC} gives an indication of nitrogen involved in the photosynthetic capacity, while the N_{other} can be estimated by subtraction of N_{PC} from N_{total}. The second method assumes that the breakdown of the photosynthetic apparatus in senescing leaves is coordinated with the remobilisation of nitrogen to new leaves. By measuring photosynthetic rates and leaf nitrogen contents of young fully formed leaves and senescing leaves, a relationship between these two variables can be estimated. The intercept of the relationship relates to the nitrogen incorporated in the non-photosynthetic components of the leaf. While this method is based upon the mechanics of the degradation of the photosynthetic apparatus (Hortensteiner and Feller, 2002), and repeatable linear relationships have been derived for a number of species (Evans, 1989; Field and Mooney, 1986).

Both of these methods provide estimates of the nitrogen used in the *active* photosynthetic apparatus, but do not include nitrogen incorporated in unused photosynthetic capacity. Provided that the nitrogen component estimates are relatively realistic this is not a problem, as unused photosynthetic capacity does not contribute to the photosynthetic rate used to calculate *PNUE*. If the conditions of the gas exchange measurements represent realistic environmental conditions, then unused photosynthetic capacity is functionally a component of N_{other}.

The most appropriate equations were used in Chapter 8 of this thesis. These were generally the formulae that where published in the original validating papers (e.g. Evans, 1989), and not subsequent derivations of older work.

Table 12.1: The formulae used by various authors for the estimation of leaf nitrogen components from gas exchange parameters and chlorophyll content.

Leaf nitrogen component (mmol m^{-2})	Calculation	Reference	Comment
N_{PCR}	$=0.16*$calc. Rubisco content$/0.764$ [a]	This study	Similar to Westbeek *et al*. (1999)
N_{LHC}	$= 41$ mol N mol Chl^{-1}		All cited studies use this calculation
N_{THY}	$= 0.316*J/4+33.1*Chl$ [b]		Same as Poorter and Evans (1998)
N_{ETR}	$= N_{THY} - N_{LHC}$		Same as Poorter and Evans (1998)
N_{PC}	$= N_{PCR}+N_{ETR}+N_{LHC}$		
N_{PCR}	$= V_{cmax}*359$ mol N mmol CO_2^{-1} s	Pons and Westbeek (2004)	derived from Evans and Seeman (1989) and Makino (1988)
N_{P+E}	$= J_{max}*47.8$ mol N mol $e\text{-}^{-1}$ s		N incorporated in pigments (P) + electron transport (E)
N_{LH}	$= 41$ mol N mol Chl^{-1}		For high light plants from Evans and Seeman (1989)
N_{PC}	$= N_{PCR}+N_{P+E}$		
N_{P+E}	$= ((J/4/Chl)*0.316+33.1)*Chl$ [b] $= J/4*0.316+33.1*Chl$	Poorter and Evans (1998)	Thylakoid N in components with N incorporated in pigments (P) & electron transport +phosphorylation (E)
N_P	$= 41$ mol N mol Chl^{-1}		For high light plants from Evans and Seeman (1989)
N_E	$= N_{P+E} - N_P$		
N_{LH}	$= 41$ mol N mol Chl^{-1}	Westbeek *et al*. (1999)	
N_{ET}	$= 0.058$ mmol $N*J_{max}$		derived from Pons *et al*. (1994)
N_{PCR}	$= 0.16*$Measured Rubisco content$/0.764$		Assumes that 76.4% of PCR enzymes are Rubisco (Evans and Seeman, 1989), and that 16% of protein is N

[a] Rubisco content (mg m^{-2}) $= (V_{cmax}/1000)*(550000/5375)$, where Rubisco has a molecular mass of 550 kDa and specific activity of 5375.

[b] This formula was derived from Evans (1989) data on four species (specifically Figure 3).

APPENDIX 5

Theoretical additions to the competition model of low biomass

The basic equation of the competition model of low biomass is:

$$R = \frac{P * X}{Z}$$

This equation is probably representative of the growth of a multi-stemmed single plant, or stand of plants of the same species. Extending similar logic to a system with numerous plants, and assuming that all available nutrients are taken up, this equation becomes:

$$X = \frac{Z_1 * R_1}{P_1} + \frac{Z_2 * R_2}{P_2} + \ldots + \frac{Z_i * R_i}{P_i}$$

where, X is the amount of limiting resource available as a constant rate (g resource m^{-2} of dune surface day^{-1}), Z_i is the biomass of the ith individual (g dry mass m^{-2} of dune surface), P_i is the unit of biomass produced per unit resource for the ith individual (g dry weight g^{-1} resource), and R_i is the rate of growth per gram of biomass present for each individual (g dry weight g^{-1} dry weight day^{-1}).

The addition of terms representing many plants results in equations which are difficult to solve analytically. For instance, by assuming that all plants have similar P_i, solving for R_1 in the previous equation, gives a non-linear solution:

$$R_1 = \frac{P * X - Z_2 * R_2 - \ldots - Z_i * R_i}{Z_1}$$

These equations were not explored further. Never-the-less they could be solved iteratively, however the outcome is not expected to differ substantially from the proposed competition model of low biomass. More complex models would include differential species abilities to compete for the nutrient pool.

Similarly, not all plants have the same resource conversion rate (P_i). Resource conversion rate relates to the resource use efficiency of the plant, and has been discussed in Chapter 8. The addition of species or individual specific resource conversion rates to the basic model would be easy, and does not affect the outcome of the simple model.

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