# Photoinhibition under Drought and High Light Loads in New Zealand's Divaricate Shrubs

A Thesis
submitted in partial fulfilment
of the requirements for the Degree
of
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by

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Dedicated to my father,

Ralph Bernd Schneiderheinze
(10/01/1942, 09/11/1999).

Thank you for all the love and encouragement.

## ABSTRACT

A question that has plagued New Zealand botanists for many years is the occurrence of the divaricate growth form in several different plant families, and what selection pressure could have led to such parallel evolution. One prominent theory is that the divaricate habit is an adaptation to climatic extremes. This study aims to test if the 'self-shading' growth form of divaricates protects their internal leaves from photoinhibition under physiological drought and high irradiance. By being able to forego the costs of maintaining photoprotective mechanisms, they should have greater carbon gain than their non-divaricate congeners under these conditions.

To test if divaricates are protected from the detrimental effects of photoinhibition, the water potentials, pigment and vitamin E concentrations, and photosynthetic rates of two divaricates species in their natural habitat were measured. Additionally, these parameters were recorded for the same divaricate species and their non-divaricate congeners under glasshouse conditions. In the field there were clear differences in several key parameters between divaricates under different levels of irradiance and water availability, and in most cases there was clear evidence of photoinbition. In the glasshouse, the maximum photosynthetic rates were significantly higher in divaricate leaves than in non-divaricate leaves, but there were no clear differences in the avoidance of photoinhibition between divaricates and non-divaricates. Interestingly, more pronounced responses to the different treatments were observed between genera than between the growth forms in the field and glasshouse experiments.

The presence of photoinhibition and photoprotective mechanisms in divaricate leaves does not support the theory that the divaricate habit evolved as a physiological response to extreme climate conditions. The absence of a strong difference in the amount of photoinhibition between divariacte and non-divaricate congeners mean that high irradiance and drought stress are unlikely to have been a key factor in the evolution of the divaricate habit. That the within genus physiologies are more similar than within the growth forms would indicate that the divaricate habit possibly evolved after the evolution of the physiological responses of the genera.

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# 1. INTRODUCTION

## 1.1 Overview

Divaricate plants, defined as much-branched and small-leaved shrubs or tree juveniles with a wide branching angle (Kelly, 1994) appear to be adapted to exist under extreme climatic conditions, such as mountain areas or salt marshes (McGlone & Webb, 1981). In New Zealand the percentage of divaricate plant forms is much higher than in many other parts of the world. About 60 species in 20 different genera, 10% of the woody species in New Zealand's flora, are divaricate plants (Tomlinson, 1978).

The overall premise of this research was that the combination of physiological drought, high irradiances and high temperatures initiates stress reactions with different intensities between divaricate and non-divaricate plants. I compared two divaricate shrubs with two non-divaricate species under different water conditions in the field and under experimental conditions in the glasshouse. Also the divaricate species *Corokia cotoneaster* and *Coprosma propinqua* growing on a dry N-facing slope and in a streambed at Cass were used in a field trial. This research provides increased knowledge of environmental reactions of divaricate shrubs. It gave us an opportunity to test some of the possible processes and pathways that may have led to the evolution of divaricate shrubs. This will therefore increase our knowledge of the evolution of the New Zealand flora. Observing the responses of divaricate shrubs or tree juveniles under water stress and several light conditions will help to explain their reactions in extreme environments. The purpose of my research was to provide more information about the hypothesis that the divaricate habit maximises photosynthetic production by minimising the damage of high light loads and drought on photosynthesis. In that context, the effect of architecture versus photochemical strategies on the ability to tolerate certain physiological conditions in two genera with divaricate and non-divaricate congeners was determined. It provides answers to the possible evolutionary developments in the structure and habitat of these species.

## 1.2 Introduction

The biogeographic formation of New Zealand is characterised by its early separation from the Godwana land mass and the summation of mountain formations and faulting processes, frequent occurences of volcanism and repeated changes between glacial periods and periods of arid climate (Wardle, 1991). New Zealand's colonisation by vascular plants was almost exclusively via the long-distance transport of seeds. The main colonisation period is assumed to be between the end of the Miocene and beginning of the Pleistocene periods. New Zealand and southern Australia did not only have the same latitude, 50-40°S, but also the same warm-temperate to subtropical climate during the early Miocene. The flora was similar for both of the main land masses (Pole, 1994). In the middle Miocene, temperatures increased in Australia, the climate became more arid and monsoonal. New Zealand's climate did not change until cooling in the Pliocene-Pleistocene transition. Many taxa of tropical and subtropical plants became extinct in New Zealand during this time (Mildenhall, 1980). This time period was also marked by mountain formation and volcanism. Alpine and dryland habitats were created as well as new offshore islands. The colonisation by tall woody taxa as well as the prevalence of seeds with long-distance dispersal mechanisms are related to these geographic and climatic changes (McGlone *et al*., 2001).

Herbaceous plants form around 32% of New Zealand's native flora, but around 78% of them are non-endemics (Mildenhall, 1980). Most endemic plants to New Zealand are woody plants (McGlone *et al*., 2001.). Depending on their distribution, 55% of the woody flora are endemic in the upper North Island, 17% in the lower North Island and 24% in the upper South Island. The lower South Island has only a few endemic species, whereas on the Three Kings 85% of the endemic species are woody plants (McGlone, 1985). Wardle (1991) defined about 50 of the woody endemic species in 16 families as 'divaricates'. The high percentage of endemic species is partly caused by the high degree of isolation of species into distinctive habitats with harsh climates. The plant endemism is strongly related to the species but not family level, and the amount of generic endemism is small. Wardle (1963) and Burrows (1965) also argue that recent disruptions by glacial periods and rapid climate change affected the dispersibility of plants and the degree of endemism. Offshore islands north of New

Zealand represent the same pattern of endemism, with woody rather than herbaceous plants more often being endemic. McGlone *et al*. (2001) list endemic vascular plants by habitat types and find a 98% endemism in forest and scrub habitats and 93% endemism in alpine areas. By grouping the endemic species as plant types, 100% endemism is found for trees and 81% for shrubs in the wetlands of New Zealand (Johnson & Brooke, 1989).

Tall mountains with diverse topography, steep climate gradients and strong regional habitats, as found in particular at the South Island, provide optimal environments for specialised trees and shrubs. Large populations can be maintained in small areas and over a variety of community structures. The genus *Carmichaelia* is a good example. The shrubs are characterised by photosynthetic stems and a xeromorphic growth form. Adaptations to drought and high radiation loads as well as cold and exposed sites allow this genus a patchy distribution in small areas of specific habitats scattered across the landscape (Heenan, 1997). McGlone & Webb (1981) also assign divaricate and semi-divaricate shrubs as adapted to wind-exposed, cold and dry environments.

The term “divaricate” plant in New Zealand defines loosely a group of some 60 species of shrubs, small trees, and tree juveniles that characteristically have small well-separated leaves and often widely branched, interlacing stems (Figure 1.1). The interlaced stems are of different age and order. This growth form has developed independently in at least 26 taxa in New Zealand (Wilson & Galloway, 1993). Microphylly (*sensu* Tomlinson, 1978), which is more commonly found in young heteroblastic species, as well as a trend towards short shoots and rarity of terminal flowers, are common for woody plants with a divaricate habit. Flowers are often lateral situated on short shoots, therefore the architecturally important axes of the shrubs are not determined by flower buds (Tomlinson, 1978).

Kelly (1994) published a summary of architectural definitions of divaricates and a multitude of measurements regarding shoot architecture traits comparing divaricate and non-divaricate growth forms. The interlacing of branches is caused by reduced apical growth associated with a continued growth of lateral branches. Straight branches can grow at widely divergent angles, often 90° or more, or reduced angles (<90?) bending downwards or sideways in directions away from the main stems.

Outer branches have longer internodes with very few and small leaves, a trend which increases with the degree of divarication (Greenwood & Atkinson, 1989). Species like *Corokia cotoneaster* have a high number of short-shoots, transformations between long shoots and short shoots are seen and specification between both is sometimes limited (Tomlinson, 1978). Strong plagiotropy (expressing rhythmic growth and therefore an effective spacing of branches with the branch angles of 90?; Tomlinson, 1978) is initially assumed for *Coprosma* species. Terminal growing points would branch after flowering, but that is not found in mature divaricate shrubs (Tomlinson, 1978). Often the stems of divaricating plants are very tough and therefore difficult to break, compared to those of their non-divaricating relatives (Greenwood & Atkinson, 1989; Bond *et al*., 2004). Cockayne (1912) describes divaricate branches as thin and wiry. The bifurcation ratio is small but in the range of most trees (Tomlinson, 1978). In heteroblastic species the leaf area can increase up to one hundred-fold with reaching adolescence. Some divaricates like *Aristotelia fruticosa* define their branching pattern by died-back axes. On the other hand, *Discaria toumatou* has spines on the lateral axes (Tomlinson, 1978).


Figure 1.1: Growth form of *Corokia cotoneaster* at Cass.

Most of the divaricate taxa have large-leaved relatives with wide branch angles (Philipson, 1963). Divaricating plants are also found in other countries, notably in xeric environments. They occur spineless in New Zealand, although *Coprosma quadrifida* appears with short spiny branchlets and reduced numbers of leaves on the outer branches in Tasmania and Eastern Australia (Greenwood & Atkinson, 1977). Tucker (1974) listed 53 divaricating species for California and Arizona, of which 44 had spiny branches or leaves. Depending on definition, 10% of New Zealand's woody flora are characterised as divaricating plants, and widely distributed throughout New Zealand (McGlone & Clarkson, 1993). Greenwood & Atkinson (1977) listed 54 species of plants native to New Zealand considered to be divaricating. These species belong to 20 genera; represent 16 families of angiosperms and 1 of gymnosperms (Greenwood & Atkinson, 1989). No country apart from New Zealand has anything close to a tenth of the woody flora as spineless, small-leaved divaricating shrubs. As Greenwood and Atkinson (1977) and Tomlinson (1978) have stated, New Zealand has an unusually high concentration of divaricating plants, and nowhere else do they play such an important ecological role in the vegetation. Divaricate shrubs are found in a wide variety of habitats, such as coastal areas, marshlands, wetlands, sub-alpine and alpine as well as mountain habitats. Several species are found in open land, others in forest and forest margins. In these habitats, they withstand harsh conditions such as frost combined with high light loads, drought, wind, salt concentrations, high altitudes etc. (Wardle, 1963).

Attempts to explain the evolution of divaricate shrub plants in New Zealand have generated two main theories, the moa-browsing theory and the climate theory (see below). As Tomlinson (1978) argues, the branching of divaricates can be caused by sequential branching, meaning genetically determined, or reiterative branching, and therefore environmentally caused. Environmental factors could be climate influences as well as browsing animals. The evolution of New Zealand's flora is unique, because it is marked by a close relationship between long-distance dispersal of seeds and the co-evolution between plants and a mammal-free fauna. Divaricate plants only reach such high frequency in New Zealand and are represented in many different genera and families. Therefore, they are not only an important component in the ecosystem, but it would be of major importance to investigate factors which lead to such prominence in New Zealand.

### 1.2.1 Moa Browsing

Greenwood & Atkinson proposed in 1977 that the divaricating shrub form developed as a protection against moa-browsing. They pointed out that there are several divaricating plant species, which are often, or always, found on the mainland. But those species have non-divaricating populations on offshore islands, which lacked moa in the past. Before Polynesian colonization about 1000 years ago, flightless birds were prominent in the avifauna of New Zealand. Ratite birds might have been present in New Zealand since the Cretaceous (Fleming, 1975). Together with a few species of smaller birds, moa were the only browsing vertebrates in New Zealand prior to the arrival of man (Greenwood & Atkinson, 1977). The highly interlaced branches with wide branch angles as well as small leaves are argued to restrict or prevent browsing, which could go hand in hand with the change in leaf size and shape in mature shrubs and trees, which reached over a height moa could access. Divaricating plants might also recover quickly from damage because of their multiple growing points.

This hypothesis has had some acceptance because of the unusually high concentration of divaricate plants in New Zealand and their importance in ecological systems. It became less popular as a result of the discovery of subfossil moa gizzards containing twigs, leaves and seeds from a number of divaricate plants (Burrows, 1980a). It is assumed, that the moa sheared the twigs off with sharp-edged bills and strong facial musculature and ground them up in their large gizzards with up to 500 gizzard stones (Burrows, 1980b). Burrows *et al.* (1981) found up to 90% of the volume of the plant material in the gizzard was presented by short pieces of twigs of small diameter. Leaves, fruits and seeds were present, but barely reached 5% of the volume.

Bond *et al*. (2004) presented divaricate shrubs to African and Australian ratite birds on the premise that their feeding patterns would be similar to that of the extinct moa. They hypothesised that the divaricate growth form would prevent or minimize browsing of those birds on leaves and twigs. Bond *et al*. (2004) showed that leaf size is not a singular criterion to prevent browsing. The tensile strength as well as elasticity of branches were as important as the distances between leaves and the actual leaf sizes. The results were verified by feeding heteroblastic species of different ages to the same animals. However, these findings do not take into account the difference in

build between moa, emu and ostrich. The mandible of a moa is much stronger built and probably had more powerful tendons and muscles attached (Holdaway, pers. comm.). So the findings of less biomass removal on divaricate shrubs by emus and ostriches do not necessarily show a preventative mechanism against moa feeding. Additionally, moa gizzards contained large numbers of gizzard stones to help to break down roughage and high percentages of twigs were found in those gizzards (Burrows, 1980a+b). Gizzards of emu and ostrich also contain stones for better digestion (Bond *et al*., 2004).

New Zealand also has a high proportion of trees (200 tree species; Cockayne, 1912) with heteroblastic forms (Darrow *et al*., 2001). Heteroblastic plants show juvenile forms that differ from the adult tree form; therefore different vegetative phenotypes are displayed during ontogeny (Day, 1998). The transition of divaricating juveniles to non-divaricating adult plants often occurs above the reach of the tallest moa and therefore is often argued as a protection against moa-browsing (Greenwood & Atkinson, 1977 and 1989; Mitchell, 1980). On the contrary, it is also hypothesised as a response to changed light environment (see next section).

McQueen (2000) compared divaricates in New Zealand with divaricates in Patagonia, because there are many southern genera in common between New Zealand and Chile. In Patagonia he found a lower number of species with wide-angle branches and lesser interlacing of branches, but also an increase in divarication with decreasing precipation. While divaricates are common in New Zealand forests (33 species of 53 are able to survive in a forest), in Patagonia divaricates are only found in open country. All except two divaricate species are spiny in Patagonia. In New Zealand, only *Discaria toumatou* is spiny in dry habitats, but spineless and with leaves in moist areas (Cockayne, 1967). Such a difference also suggests that the prominence of spines of Patagonian divaricates and of many other shrubs is a response to coevolution with indigenous browsing mammals. The two non-spiny Patagonian species are apparently non-palatable as are the plants of the Andean forest and adjacent scrubs. McQueen (2000) summarises that New Zealand's divaricates are today limited to semi-arid areas, which were forested in the pre-human past. Therefore the divaricating growth habit is seen as a response to moa-browsing but, as in Patagonia, this adaptation also enables shrubs to survive in harsh habitats (McQueen, 2000).

The limitation of browsing on spiny shrubs by large mammals was shown by Cooper & Owen-Smith in 1986. Spines restricted the mammals in bite size and bite rate. Cooper & Ginnett (1998) also showed that spines are a defence against small mammals by reducing their mobility within the canopy and their efficiency of feeding. Gutierrez *et al*. (1997) determined herbivory by small mammals on the vegetation in a Chilean Mediterranean community and in semiarid sites. This study showed that small mammals can significantly change the plant community, directly by browsing and changes in seed distribution, but also indirectly by physical disturbance and chemical modifications of the soil.

McGlone & Clarkson published in 1993 a theory speculating about a lower risk of herbivory attack by modifying plant appearance, such as dark leaf colours and dead looking branches. It is assumed as a form of mimicry to unpalatable or dead plants and plant parts, respectively. However, McGlone & Clarkson (1993) acknowledge that darker leaf colours are possible adaptations to balance heat and protect from ultra-violet radiation or are an answer to frost damage (see next section).

### 1.2.2 Environmental Conditions

That so many unrelated plant families show the same parallel trend towards divarication could indicate environmental conditions peculiar to New Zealand. Diels (1897) was the first to attribute the origin of divaricate shrubs to climatic conditions. He interpreted the structure of the branches as an adaptation to windy and xeric climates. The branch structure was suggested to reduce transpiration and to keep the moisture in the middle of the shrub where the majority of the leaves are. Cockayne (1912) considered the divaricate habit as a xerophytic growth form resulting from adaptation processes to an earlier “steppe-climate period”, when conditions were more windy and drier than at present. Rattenbury (1962) considered the divaricate habit as an adaptation to drier or cooler conditions in the Pleistocene. He suggested that the compact nature of the growth form, with leaves confined mostly to the inner shoots, act as an effective windbreak. Wardle (1963) pointed out that there was scarcely any development of a distinctive xeric flora adapted to areas of New Zealand which experience dry climates at present. He suggested that the divaricate growth form was an adaptation to still-existing conditions such as those that occur in drier forests or

shrub communities. Greenwood and Atkinson (1989) disregarded drought and desiccation as evolutionary forces for the divaricating habit, because approximately 30% of the divaricate species are more or less restricted to areas with apparently adequate water supply. Greenwood & Atkinson (1977) pointed out that divaricating species might be restrained by competition with their non-divaricate congeners. Fewer divaricate species are found in areas of higher rainfall, but high densities are reached in drier habitats. Greenwood & Atkinson (1977) also draw attention to the increase in divarication with increasing sun exposure. For example, *Pittosporum divaricatum* features only semi-divaricate habits within the forest but divaricate habits in open habitats. Greenwood & Atkinson (1977) assumed that, through shading, divarication is less pronounced due to insufficient carbohydrate production. Day (1998) hypothesised that the mature stage of divaricate shrubs was lost, because the light capturing capability as normally seen in shade grown species was no longer required in open habitats and became too expensive in structural respects.

McQueen (2000) suggested that divarication evolved as a microclimatic shield and evolved to aid leaves in light harvesting. Lloyd (1985) argues that heteroblasty occurs in 11 of 67 divaricate species. Obviously, the juvenile and divaricate form loosens after the plants have deep, well-established root systems and their foliage is well above the height of damaging ground frost. The divaricating juvenile form may enable forest trees to act as colonisers in the forest margin (McGlone & Webb, 1981). Divaricate growth forms could have functioned as wind protection and refugia areas of possible pollinators.

McGlone & Webb (1981) argued that divaricating plants are adapted to occur in non-forest and forest margin habitats, which emerged during glacial periods of the Pleistocene. The main function of the divaricating form would be to protect growing points and leaves from wind abrasion, desiccation and frost damage. Also, a favourable microclimate would be maintained inside the shrub and this may permit higher rates of photosynthesis during periods of unfavourable environment. The densely branched structure may reduce transpiration of the leaves by providing a relatively moist interior to the bush. This adaptation could enable divaricating plants to thrive in areas marginal for forest (McGlone & Webb, 1981). This has been interpreted as showing that New Zealand has not evolved a true mountain vegetation

and/ or as showing that environmental conditions in the New Zealand mountains are somehow different from those prevailing in the North Temperate Zone (Wardle, 1963). It is suggested that this vegetation arose during the harsh, near-treeless glacial periods of the Pleistocene.

The last Glaciation which ended 14,000 years ago, gave much colder temperatures, with an annual temperature of around 6?C or even lower. Now, annual temperatures vary between 10?C in the South Island and 16?C in the North Island. New Zealand's landscape was covered with shrubs or grassland; the climate became more variable with stronger winds, and more frequent drought and frost events. In general, New Zealand trees are more frost-sensitive than tree species grown in more continental climate, as frost normally only occurs at night (McGlone & Webb, 1981), and might be expected to have juvenile forms that are more frost resistant than their mature forms (Darrow *et al.*, 2001). Divaricating small-leaved juvenile forms are slow-growing despite the fact that growth in thickness is sacrificed in favour of growth in length, but their xeromorphy enables them to survive on drier sites. The development of more or less mesomorphic foliage in the adult trees is thought to be connected with the development of larger, deeper and more efficient root systems (Wardle, 1963).

### 1.2.3 Alternative Hypotheses

A non-adaptive explanation for the divaricate flora was proposed by Went (1971), who suggested that a particular chromosome segment carrying the genes controlling divarication was transferred asexually between families. There is little evidence so far to support this idea. Tucker (1974) pointed out the difficulty of assuming that any given taxon would carry all the genes controlling the divaricate habit on one segment of one chromosome. Greenwood & Atkinson's (1977) examination of these plants shows that the interlaced branch system has evolved in more than one way. Thus, assuming Went was correct; one would have to invoke the transfer of several different chromosome segments between unrelated families. It is important to differentiate between so called "sequential branching", which is an architectural model and "reiterative branching" caused by environmental damage like storm or browsing animals (Tomlinson, 1978; Hallé *et.al.*, 1978).

### 1.2.4 Empirical Research to Date

#### Anatomy and Morphology

The theory that the divaricating habit developed as a response to past climates was published in several research papers. Diels (1897) and Cockayne (1912) began with descriptions of morphology and their possible relationship to drier habits. Diels referred to some divaricates as descendants of a forest flora and accentuated their xeromorphic characteristics, which he associated with a reduction in transpiration. He also noted that there were hardly any reductions in leaf size and number with anatomical changes in leaves of New Zealand's plants of xeric habitats, whereas it is well described for Australian species in dry areas (Diels, 1897). Transpiration reduction via reduction in leaf size is only ultimately successful if the total leaf area per plant also is reduced. Small leaves have a thin boundary layer and therefore lose more water per unit leaf area than larger and thicker leaves (Grace, 1983). Cockayne related the divaricate growth form primarily to a wind adaptation, but also to a xeromorphic adaptation to low soil moisture. Maximov (1931) was the first who attempted to prove reduced transpiration experimentally. He found low rates of cuticular transpiration for plants grown under drought stress, but the same xeromorphic plants showed higher transpiration rates than mesomorphic plants under well-watered conditions.

#### Water Loss

By comparing the water relations of a divaricate shrub, some heteroblastic and several homoblastic species in New Zealand, Darrow *et al.* (2002) suggested that the divaricating habit produces a microclimate that characterises the divaricate as a water spender. They found that *Coprosma rotundifolia* had relatively high rates of water loss whereas heteroblastic trees tend to conserve water more in their mature than in their juvenile stages. Farquhar *et al.* (1980) linked more negative $d^{13}C$ values to plants with open stomata and justified it with the discrimination against heavier $^{13}C$ under unlimited carbon dioxide supply. Darrow *et al.* (2002) also investigated the relationship of water loss and $d^{13}C$ values. They found heteroblastic species with high water contents had less negative values of $d^{13}C$. Darrow *et al.* (2002) observed that in young heteroblastic and divaricate forms, the losses of water content were greater than

in their mature forms. The divaricate *Coprosma propinqua* demonstrated higher transpiration rates than a hybrid of *C. propinqua* and *C. robusta*. Their data also showed that species with the largest leaves experienced the lowest water lost rates per unit dry weight and leaf area. In an investigation of juvenile and adult desert shrubs, Donovan & Ehleringer (1992) showed that the juvenile forms had a greater rate of photosynthesis but also greater water loss, carbon discrimination and poorer water use efficiency than mature plants of the same species.

Species of the American Continent

New Zealand has a remarkably high number of divaricate species in numerous families and very different habitats. But other parts of the world also have divaricate shrub species, mostly growing in arid and semi-arid environments. Two sub-shrub species (*Hymenoclea salsola* and *Ambrosia dumosa*, both Asteraceae) growing in a warm desert in western North America were analyzed by Comstock (2000). He examined the relationship between hydraulic architecture, gas exchange and the responses to environmental conditions these species grow in. Populations from the south (semi-arid) showed higher leaf-specific hydraulic conductance than populations of both species from the north (intermountain), as well as plants growing at higher temperatures but not as a response to the relative humidity. Comstock (2000) found no correlation between hydraulic conductance and root: shoot ratio, but a negative correlation of hydraulic conductance to total leaf biomass. A strong response of stomatal closure to decreasing ambient humidity was registered for both species, which limited transpiration rates at higher leaf-to-air vapour pressure deficits. The main difference between the observed species was the biomass allocation pattern. In a preferred growing habitat, *H. sasola* invests greatly in allocation into a tap root, and leaf-bearing twigs, which function dually as organs of transport and photosynthesis. In the southern populations, where the leaf-specific hydraulic conductance was highest, twigs contributed around half of the photosynthetic surface. Interestingly, no change in the whole-canopy gas exchange rate per unit canopy biomass was seen, but Comstock & Ehleringer (1988) found a much lower photosynthetic rate in twigs compared to leaves of *H. sasola*. *A. dumosa* had greater allocation into leaves under preferred microclimates. Both species in Comstock's study had a lack of apical dominance and a limited branch lifespan. Frequent renewal of the canopy occurred via basal suckers.

In 1994, Franco *et al.* published gas exchange data of the xerophytic *Larrea tridentata* (creosote bush), an evergreen desert shrub growing in the arid southwest of the United States, with respect to soil and plant water characteristics during the growing season. Maximal net photosynthesis was found together with sufficient water supply and least negative predawn xylem water potentials in early summer. Predawn xylem water potential also influenced stomatal conductance, which overall seemed to determine rates of carbon gain and water loss in *L. tridentata*. The plants maintained a positive carbon balance possibly by using soil water from deeper layers. Remaining active in periods of drought helped the plant to react rapidly to sudden rain. Mooney *et al.* (1978) measured a seasonal difference in light saturated photosynthesis depending on temperature for the same species. Stomatal conductance was found to be scarcely affected by the temperature change in creosote bush.

Frost Tolerance

The advantage of a divaricate growth form may lie in creating a sheltered interior with lower wind speeds, higher humidity and slightly higher temperatures (McGlone & Webb, 1981). These conditions would bring higher photosynthetic rates as well as greater stomatal opening, as found by Darrow *et al.* (2002). McGlone & Webb (1981) published the idea that the outer branches of divaricate shrubs have a self-shading function. They assumed that this comes with a high cost to the plants because the inner leaves receive a reduced quantity of sunlight. McGlone & Webb (1981) also considered the divaricating structure of leafless branches on the outside as a "frost-screen" to protect the inner leaves from the damaging effects of frost. They observed divaricate shrubs with frozen leaves on the outside, whereas in the interior the leaves were frost-free. Therefore, the inner leaves were protected against frost instead of frost tolerant.

Kelly & Ogle's (1990) measurements of several climate parameters in the interior and exterior of divaricate shrubs could not prove the assumption of a sheltered environment in the inside of divaricate shrubs during their winter measurements. The temperature range showed that there is only sometimes an effect of position on temperature within divaricate plants. However, this effect was inconsistent and small, even contradicting the hypothesis that the interior should experience higher

temperatures due to its sheltered position. A weak relationship between wind speed and frost was recorded (Kelly & Ogle's climate data from May and June 1989; see also Howell *et al*., 2002). Also a small difference in specific humidity between the interior and exterior of divaricate shrubs was found. Frost had a highly significant effect on more outer leaves than leaves from the inside of divaricate shrubs. Bannister & Lee (1989) found that temperatures below -9?C caused leaf damage in *C. propinqua*.

Kelly & Ogle (1990) presented a temperature difference of 0.5°C or less between young outer and inner parts of *Coprosma propinqua* and were also able to demonstrate that those plants had more frost-damaged leaves on the exterior than inside the shrub. Darrow *et al.* (2001) investigated the frost resistance of heteroblastic trees of different ages. Because of the morphological change between juvenile and adult trees, a change in the acclimatisation to cold air was hypothesised. Juvenile forms, which were closer to the ground, and therefore to cold air, were expected to be more frost resistant. Darrow *et al.* (2001) found that there was no uniformity in the frost resistance of juvenile heteroblastic trees, and populations of different environments differed depending on the frost the species experienced in their habitat. Bannister & Lee (1989) investigated the frost resistance of the fruits and leaves of 8 *Coprosma* species by exposing them to cold in a freezer for 8 hours and reporting a change in leaf and fruit colour. They observed different levels of freezing resistance, but showed that the non-divaricate *C. robusta* was the least frost resistant. Bannister *et al.* (1995) compared several *Pittosporum* species and found the greatest frost resistance in the divaricate *P. obcordatum*, which had a greater capacity for frost hardening than the observed tree species. In comparing seedlings and mature plants of *Pittosporum*, the homoblastic *P. eugenioides* seedlings appeared to have the greatest frost. Dwyer *et al.* (1995) showed a decrease of frost resistance in saplings of *P. eugenioides* by application of gibberellins. Horrell *et al.* (1990) caused a reversion in mature heteroblastic trees of New Zealand to the juvenile form with an application of gibberellin.

Howell *et al.* (2002) studied the influence of frost events on three species of divaricate shrubs (*Aristotelia fruticosa*, *Coprosma propinqua* and *Corokia cotoneaster*). The

self-shading growth form was assumed to protect leaves in the interior from cold-induced photoinhibition. Photoinhibition demonstrates reversible and irreversible damage to the photosystem II by excessive excitation energy (see Section 3.1). By pruning the outer shield of ‘leafless’ stems and exposing them to the winter climate, short- and long-term decreases in maximum photosynthetic capacity and photochemical efficiency were observed. Protecting the artificially exposed leaves with a shade cloth screen reduced photoinhibition and therefore maximized carbon fixation. The species investigated in this study varied markedly in the extent to which they experienced photoinhibition and in the extent of recovery.

## 1.3 General Aims

This thesis will increase the knowledge about plant adaptations to the combination of high light loads and drought in summer and also will provide a comparison between divaricate and non-divaricate congeners. As described in Section 1.2, the evolution of divaricate shrubs and their non-divaricate congeners in New Zealand is still hotly debated. My study will test whether there is any evidence for the climatic theory of the evolution of the divaricate growth form and determine if this is a likely explanation for the evolution of this morphological type. I proposed that in dry conditions the self-shaded leaves of divaricate shrubs are less likely to be influenced by the potentially photoinhibitory effects of high photon flux densities (PFDs) than non-divaricate plants. Thus, my investigation was based on the premise that the combination of drought, high PFDs and high temperature initiates stress reactions with different intensities between divaricate and non-divaricate plants.

An overall hypothesis guiding this research was that the self-shading growth form of divaricate shrubs is a protection against high PFDs for their internal leaves in particular during drought. When the temperatures are very high and soil water potential is very low, it is particularly important to be able to photosynthesise with high water use efficiency.

Divaricate shrubs have very small leaves, which should reduce the transpiration surface. I hypothesised that the stomatal closure of divaricate shrubs is much less sensitive to drought conditions than that of non-divaricate shrubs. The internal $CO_2$

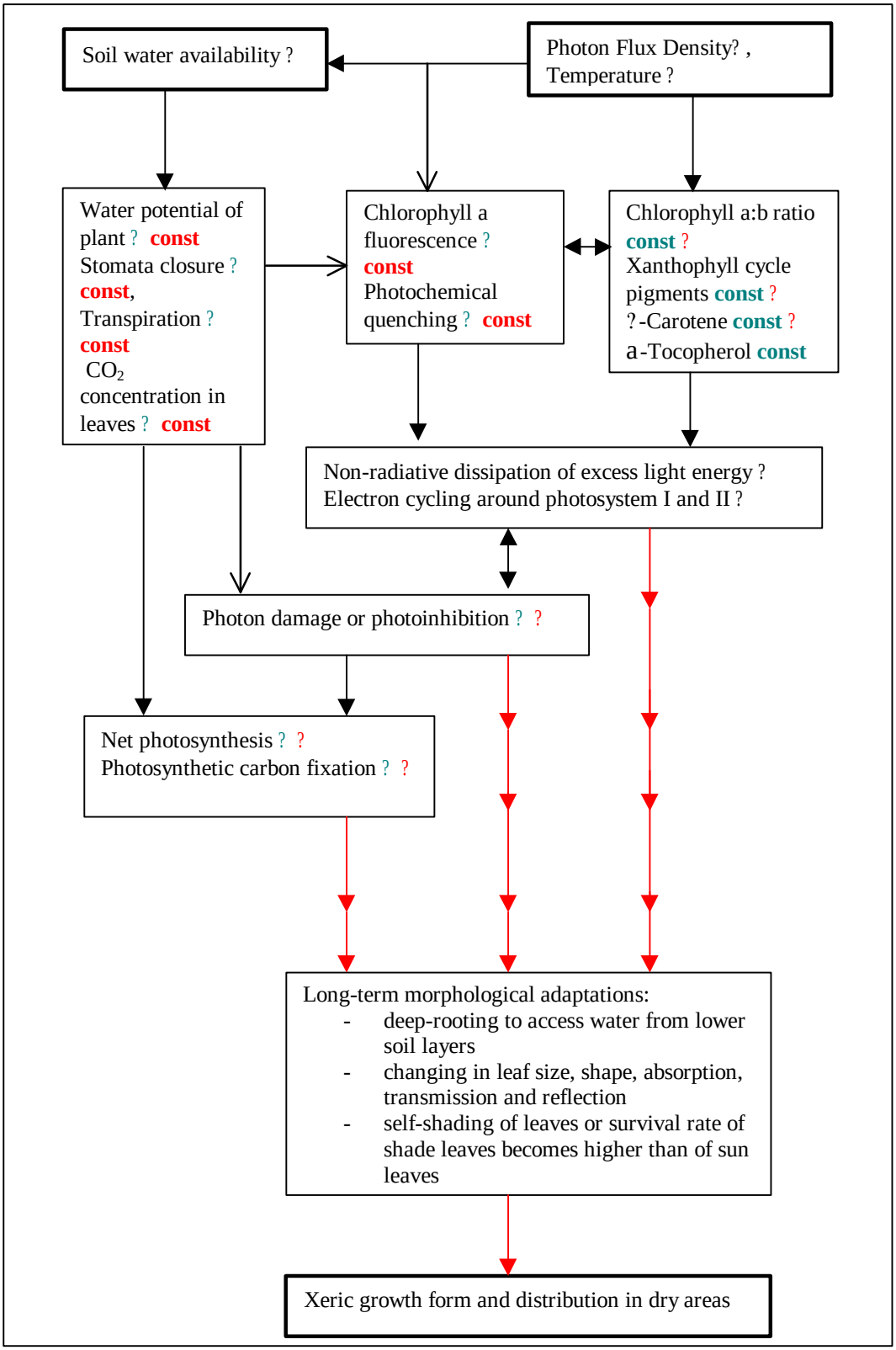
concentration should therefore be constant over short periods of water shortage. Under dry conditions the divaricate shrubs are hypothesised to be able to keep the photosynthetic rate constant.

Divaricate and non-divaricate leaves could also be different in their morphological adaptation (leaf size, shape, thickness and boundary layer) and their spectral characters (absorption, transmission and reflection). Their physiological reactions are also likely to be very different. My proposed conceptual model in Figure 1 shows the typical reactions in photosynthetically active plants under dry conditions and high irradiances. Established mechanistic relations found in the literature are shown with full coloured arrow lines; hypothetical relationships have dashed connections.

Lower soil water potentials also influence photosynthetic light reactions. I hypothesised that in dry conditions shaded leaves and leaves of divaricate shrubs (self-shaded) show a lesser reduction in their ratio of variable fluorescence to maximal fluorescence ratio ($F_v/F_m$ ratio) than sun leaves. The electron flux at the inner side of photosystem II, the water oxidation site, can be inhibited. The chlorophyll a-fluorescence decreases because of the change in the redox-states of quencher Q. $F_v$ and $F_m$ are heavily quenched under water stress, so the activity of photosystem II can be deactivated.

In the summer, along with reduced water availability, higher PFDs and temperatures are also stress factors for the plants. An increase in photoprotective pigment contents, such as xanthophyll cycle pigments, and a decrease in pigments in the epoxidated state show a reaction to excessive irradiance. Additionally, an increase in the contents of antioxidants, such as ascorbate, glutathione and ? -tocopherol, indicates a protective reaction against high PFDs. I hypothesised that divaricate shrubs, with their self-shaded leaves should have lower contents of photoprotective pigments or antioxidants than their congeneric non-divaricates.

Figure 1.2: Conceptual model of plant responses to summer conditions.




Symbols: **colour**… hypotheses for divaricate plants, **colour**… hypotheses for non-divaricate plants, ? … low amount or descrease, ? … high amount or increase, **const**… no change or constant, → … as found in the literature, ⟶ … hypothesised in this study

## 1.4 Thesis outline

To investigate the combination of drought stress and high PFDs on divaricate shrubs in summer conditions and to relate their acclimatisation to the non-divaricate congeners, my study included three major experimental parts.

In Section 2.1, the experimental trial and field conditions are described. All the plant species selected were grown under different water availabilities in the field and in the glasshouse trial. In the field, two genera were used to estimate shoot water potentials of divaricate shrubs in different habitats (Section 2.2). In the glasshouse, all shoot water potentials of the investigated divaricate shrubs were evaluated and compared with the leaf water potential of non-divaricates.

Environmental stress conditions can cause photoinhibition in leaves. To investigate the different acclimatisation spans of divaricate and non-divaricate leaves to drought and high light, the fluorescence of these leaves was measured with a Mini-Pam and the $F_v/F_m$ ratios compared (Section 3.1). Photoprotective pigments such as carotenoids and antioxidants like a-tocopherol prevent the damaging effects of reactive oxygen species, which are increasingly synthesized under high PFD in the chloroplasts. The different concentrations of photoprotective pigments and a-tocopherol in divaricate and non-divaricate leaves are presented in Section 3.2.

The gas exchange of leaves is highly dependent on the environmental conditions in which the plants are grown. Stomata are very sensitive to changes in the water status and $CO_2$ and $O_2$ concentrations of leaves and the whole plant. Therefore, photosynthetic rates and daytime respiration values of divaricate and non-divaricate leaves were measured with a LICOR 6400 system, and results from the glasshouse trial were compared with the field experiment (Chapter 4).

All findings are summarised in Chapter 5 and discussed in detail with relation to different adaptations of growth forms to stress conditions found in summer.

In 2003, additional measurements of all methods described above were conducted between divaricate, hybrid and non-divaricate *Corokia* plants (Appendix A1). These

comparisons became necessary as the plants which were purchased as *C. cotoneaster* in 2002 were identified as morphological hybrids between *C. cotoneaster* and *C. buddleioides*. It was essential to determine if the hybrid plants would express a physiological acclimatisation similar to their divaricate congeners, and therefore whether they were suitable as divaricate model plants.

# 2. EXPERIMENTAL DESIGN

## 2.1 Glasshouse and Field Set Up

### 2.1.1 Choice of Species

The genera *Corokia* and *Coprosma* were chosen to represent divaricate and non-divaricate species from the Canterbury region. Divaricate shrubs of both genera are found in alpine and sub-alpine areas in Canterbury, as well as along the coastline and the Port Hills. The ubiquity of these plants in the local area provided natural populations for field studies as well as the purchase of nursery specimens for glasshouse trials. In both the field and the glasshouse, *Corokia cotoneaster* and *Coprosma propinqua* were used as representatives of the divaricate growth form. In addition, non-divaricate *Corokia buddleioides* and *Coprosma robusta* were used in the glasshouse set up. An intermediate hybrid of *Corokia buddleioides* x *cotoneaster* was accidentally purchased and used in the first year of the glasshouse trial (Section 2.1.2). The outstanding morphological differences in leaf forms between divaricate, non-divaricate and intermediate hybrid leaves of *Corokia* are shown in Figure 2.1. The leaves of the divaricate and non-divaricate *Coprosma* plants are shown in Figure 2.2.

The genus *Coprosma* is closely related to *Coffea*, both representatives of the family Rubiaceae, found in the tropics and subtropics with some extension to temperate regions. More than 50 species of *Coprosma* are found in New Zealand and nearly 30 of them show the divaricate growth form (Wilson & Galloway, 1993). Allan (1961) describes over 90 *Coprosma* species in the Southern Pacific with 45 endemic in New Zealand. Most of the divaricate *Coprosmas* are found in Canterbury and Westland. Opposite leaves and branches as well as stipules on young shoots are a common characteristic. Small, unisexual flowers on different plants are pollinated by wind (Wilson & Galloway, 1993). The authors also point out that the divaricate species are presented in a huge growth variety in the field within a species, especially by comparison of sun and shade grown plants (e.g. open habitat versus forest vegetation) different degrees of divarication are found.

*C. propinqua* can reaches 3 to 6 m in height (Allan, 1961), but is sometimes found with a depressed or prostrate growth. Normally, branches are highly interlaced with wide branching angles, hairless with dark grey bark (Wilson & Galloway, 1993). Leaves are 7 to 16 mm long and 2 to 5 mm wide and hairless (Allan, 1961). Natural hybrids between *C. propinqua* and *C. robusta* are found and the intermediate growth form varies highly (Wilson & Galloway, 1993). *C. robusta* is a large-leaved non-divaricate which grows up to 6 m tall. The branches and branchlets are spreading evenly. The leaf size varies between 7 and 12 cm length and 3 to 5 cm width (Allan, 1961). *C. robusta* is found in forest habitats as well as in scrubland, in particular on alluvial soils. The distribution of *C. propinqua* is much broader; this divaricate *Coprosma* is found in costal areas, scrubland, forest, swamps as well as bogs, lowland and rocky and gravel habitats throughout New Zealand.

Allan (1961) assigned *Corokia* to the family of Cornaceae, Wilson & Galloway (1993) to the Escalloniaceae. Cornaceae includes around 15 genera, which are mostly distributed in the northern hemisphere. The Escalloniaceae are distributed in the southern hemisphere, including around 17 genera.

*Corokia cotoneaster* is a divaricate shrub with prominent zigzag branches, which are highly interlaced. Young branches appear nearly white, due to a cover of the tomentum. Older branches have dark bark (Wilson & Galloway, 1993). The alternate leaves vary in size, between 2 and 15 mm length and 2 to 10 mm width (Allan, 1961), and are spoon-shaped. Shaded leaves are usually larger than sun-exposed ones (Wilson & Galloway, 1993). When *C. cotoneaster* and *C. buddleioides* grow in the same habitat in New Zealand, hybrids appear. *C. cotoneaster* and *C. buddleioides* grow up to 3 m heigh, both are much-branched (Allan, 1961). *C. buddleioides* is a non-divaricate, leaves reach 5-15 cm of length and are 1-3 cm wide. Contrary to the divaricate congener, *C. buddleioides* leaves are broad-lanceolate. Non-divaricate *Corokia* spp. are distributed throughout the coastline and in the lowland forest and forest margins. Divaricate *Corokia* spp. are found in lowland scrubland, river-flats and rocky habitats.

Figure 2.1: Shoot Structure of *Corokia cotoneaster* (left), *Corokia* hybrid and *C. buddleioides* (right). Shoots are between 12 and 14 cm long.

Figure 2.2: Shoot Structure of *Coprosma propinqua* (left) and *C. robusta* (right). The shoot of *C. propinqua* is approximately 6 cm, the shoot of *C. robusta* about 14 cm long.

## 2.1.2 Field Set Up

Shoot water potential, leaf chlorophyll fluorescence and leaf gas exchange of the divaricate species *C. cotoneaster* and *C. propinqua* were measured in natural and manipulated field conditions at the Cass field site, 600m above sea level (Figure 2.3 and 2.4).


Figure 2.3: Map of New Zealand (http://www.maptown.com/geos/newzealand.html, edited)

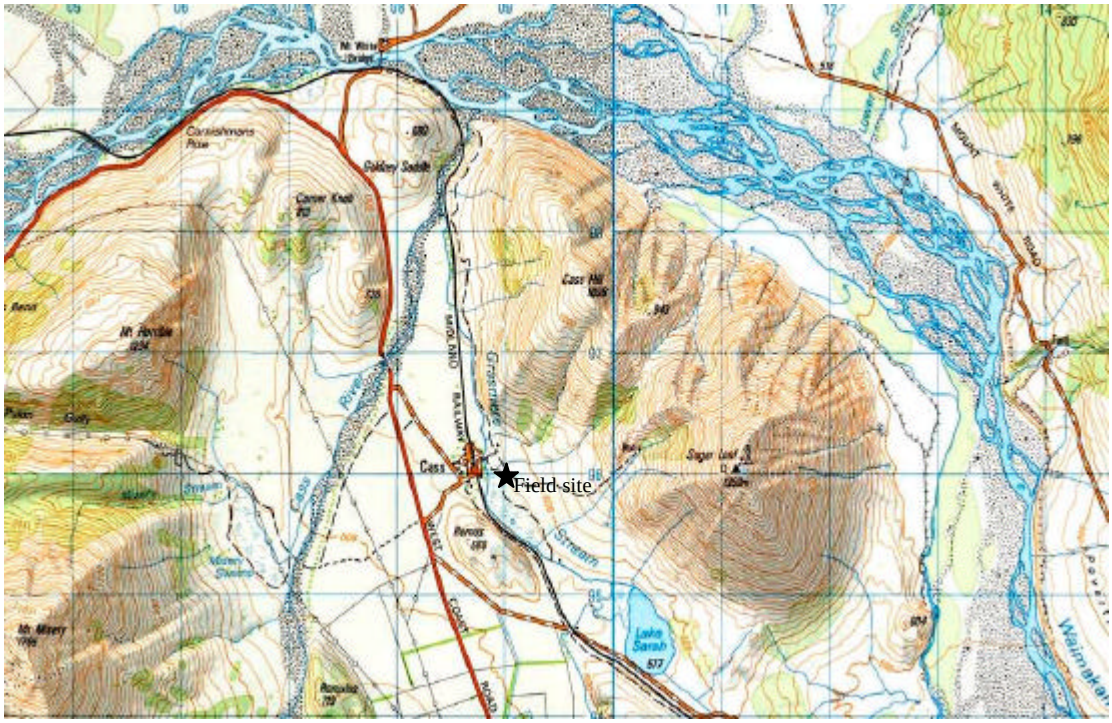



Figure 2.4: Map of the Location of the Field Site at Cass (43? 02’ S, 171? 45’ E, map: nzms 260 series, K 34, edited).

At Cass these two species of divaricate shrubs grow naturally under varying conditions of water availability. Both *C. cotoneaster* and *C. propinqua* grow on a N-facing slope and in a streambed. The N-facing slope is relatively dry and exposed to high PFDs. Plants in the streambed experience greater soil moisture availability than plants growing on the N-facing slope. Light levels were also manipulated in these field plants. Four plants of each species on the N-facing slope and in the streambed were allowed to grow under natural light, four others of each species experienced increased PFDs on inner leaves by canopy manipulation. To expose inner leaves to the PFD of the exterior; outer branches were bent forwards and fixed with string and pegs close to the ground. Additionally four shrubs of each species were covered by shade cloth suspended above the canopy to provide a 25% reduction in sunlight (Figure 2.5).

The divaricate shrubs at the Cass field station were evaluated and adapted to their experimental set up 3 months before the first summer measurements started in 2001/02.

Schematic of growing conditions in the field:

| | | |
|---|---|---|
| Exposed to Light (EL) Streambed | Sun Light (HL) Streambed | Shaded from Light (LL) Streambed |
| Exposed to Light (EL) N-facing Slope* | Sun Light (HL) N-facing Slope* | Shaded from Light (LL) N-facing Slope* |

* below half the plants plastics covered the ground in 2002/03

Following a wetter than average summer in 2001/02, in summer of 2002/03 plastic covers on the ground were installed for two plants of each species on the N-facing slope to increase drought stress by intercepting rainfall for those shrubs (Figure 2.6). These plastic sheets were 2 x 3 m rectangles, pegged tightly to the ground.

Shoot water potential and leaf chlorophyll fluorescence were measured predawn, noon and evening in November 2001 + January 2002 and January + February/ March 2002/03 (referred as summer 2001/02 and 2002/03). Gas exchange measurements

were performed during the day for summer 2002/03 only. During both summer seasons leaves for biochemical analysis (Section 3.2) were collected.

Figure 2.5: Photo showing experimental field set up at Cass in 2001/02. Photo was made by approaching the field site from NNW.

Figure 2.6: Photo showing experimental field set up at Cass in 2002/03. Manipulation of light environment using shade cloth and rainfall input using plastic sheeting can be seen in the mid- ground. Photo was made by approaching the field site from the NE.

### 2.1.3 Glasshouse Experiment

For the glasshouse experiments, *Coprosma propinqua* and *Coprosma robusta* were sourced from the Motukarara Nursery (Ridge Rd, Christchurch). Both species were from cuttings taken from plants on Banks Peninsula. *Corokia buddleioides* was purchased from Trees for Canterbury (261 Opawa Rd, Christchurch). Putative *Corokia cotoneaster* plants came from Ardmore Nurseries, Auckland (230 Clevedon-Takanini Rd), but were determined as *C. cotoneaster* x *C. buddleioides* hybrids. All these plants were purchased in winter 2001. In 2003 true *C. cotoneaster* were sourced from Wai-ora Nursery (48 Watsons Rd, Christchurch), and these plants were grown for at least six months under the treatment conditions prior to measurements being recorded. All plants were grown in 20 litre pots filled with standard University of Canterbury potting mix with slow release fertiliser. It is a pre-made all bark potting mix with 3 to 4 month and 8 to 9 month "Nutricote", pH balanced with elements of nitrogen, phosphorus and potassium. The temperature in the glasshouse was set for 21ºC; it varied in summer between 10-30ºC and in winter between 8-23ºC. When necessary, plants were sprayed to kill scale insects using a mineral oil with an insecticide (" Attack").

Two treatments were used in a factorial design in the glasshouse study, to give a total of four treatment combinations. The light regime was manipulated by either leaving the pots exposed to full light on the bench (sun light) or by covering the plants with shade cloth suspended on wooden frames, reducing available light by 25% (shaded) to 75% of natural light. The watering regime consisted of two different treatments: the water stressed plants received 200 ml every second day (15% of field capacity, data not shown) and the well-watered plants receiving 500 ml (90% of field capacity) every second day.

The plants for the glasshouse trial were purchased in winter 2001 and adapted to the experimental conditions described below for 3 to 4 months before the first measurements were carried out.

Schematic of growing conditions in the glasshouse:

| | |
|---|---|
| Sun Light (HL)<br>Well-Watered | Sun Light (HL)<br>Water Stressed |
| Shaded (LL)<br>Well-Watered | Shaded (LL)<br>Water Stressed |

Shoot and leaf water potential and leaf chlorophyll fluorescence were measured predawn, noon and evening during summer in 2002. After the initial analysis of these data the rate of watering of the water stressed plants was decreased to 200 millilitres every third day and measurements recorded following a 2 months acclimatisation period. Gas exchange measurements were performed during the day in the summer of 2003 only. Measurements for all *Corokia* taxa were recorded using the above methods during 2003, after the correct *C. cotoneaster* plants had been sourced. During both summers (referred as 2002 and 2003) leaves for biochemical analysis (Section 3.2) were collected from the glasshouse plants.

### 2.1.4 Climate Data

The University of Canterbury Cass field station is situated 22 km east of the main divine of the Southern Alps (43? 02’ S, 171? 45’ E). The area is dominated by the open grasslands of the Waimakairi basin. Situated 105 km west of Christchurch, subalpine and alpine habitats with abundant divaricate species are found. In this area the winters are usually frosty and even in summer periods of snow cover can occur. Around 300 m west of the field site is a weather station, which recorded meteorological parameters such as soil moisture (CS615 Water Content Reflectometer, Campbell Scientific) at different soil depths, wind speeds (Pulse Output Anemometer A101, Vector Instruments), rainfall (Hydrological Services TB3 raingauge) and photon flux density (LICOR Li190SB quantum sensor) at appropriate time intervals, and recorded them to a computer system into the department. The averages for PFD, wind speed, air temperature, ground temperature, relative humidity in the air and soil moisture for the years 2001/02 and 2002/03 are shown in Figures 2.7 and 2.8.

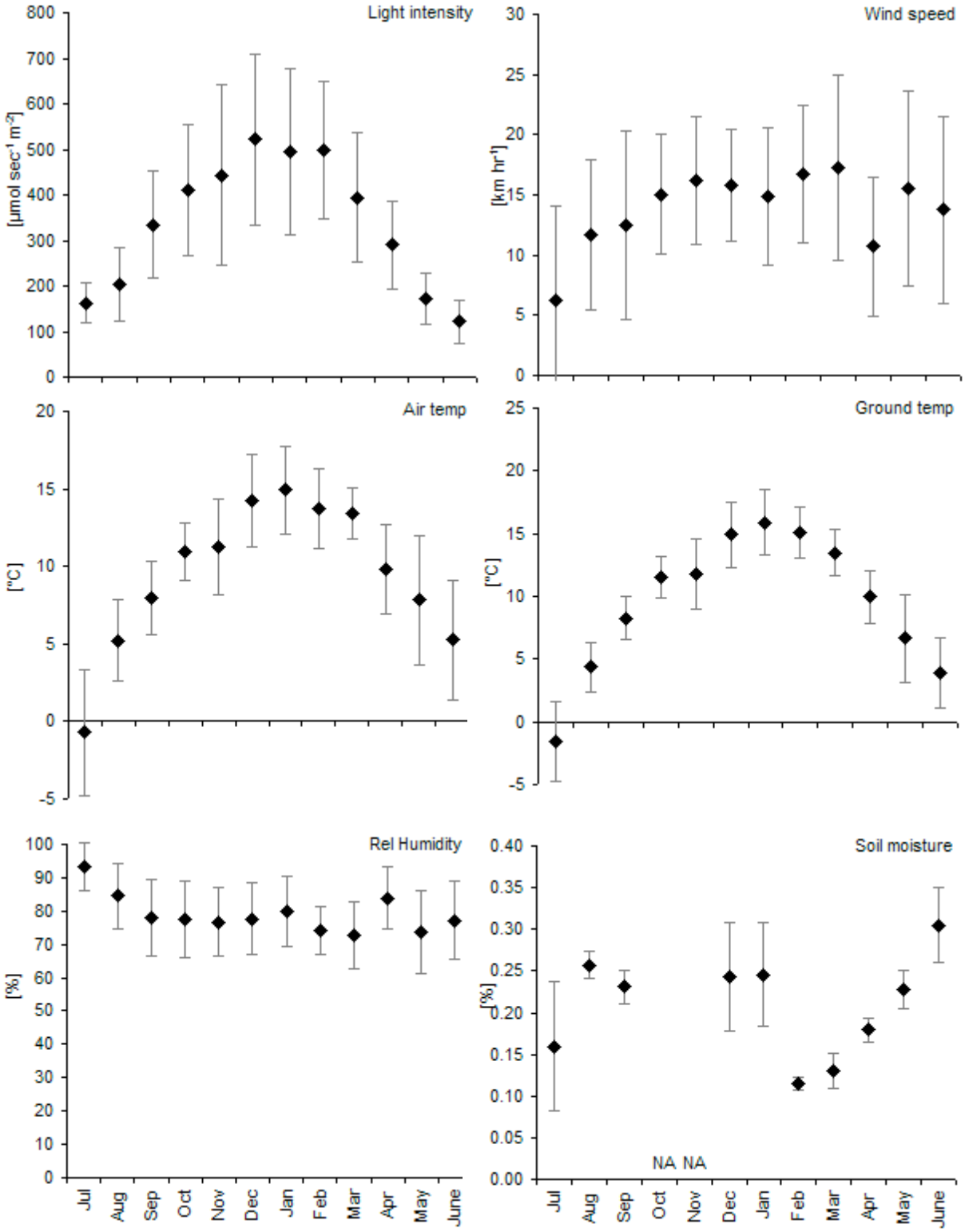



Figure 2.7: Means for PFD (light intensity), wind speed, air temperature (Air temp), ground temperature (Ground temp), relative humidity in air (Rel humidity) and soil moisture at Cass in July to December 2001 and January to July 2002. Means are averages over month from hourly measurements. All measurements were done from a climate weather station on a 14 m tall tower, except ground temperature, which was measured just above ground and soil moisture, measured at 10 cm depth. Data for October and November 2001 are missing for the soil moisture due to technical difficulties (marked as 'NA').

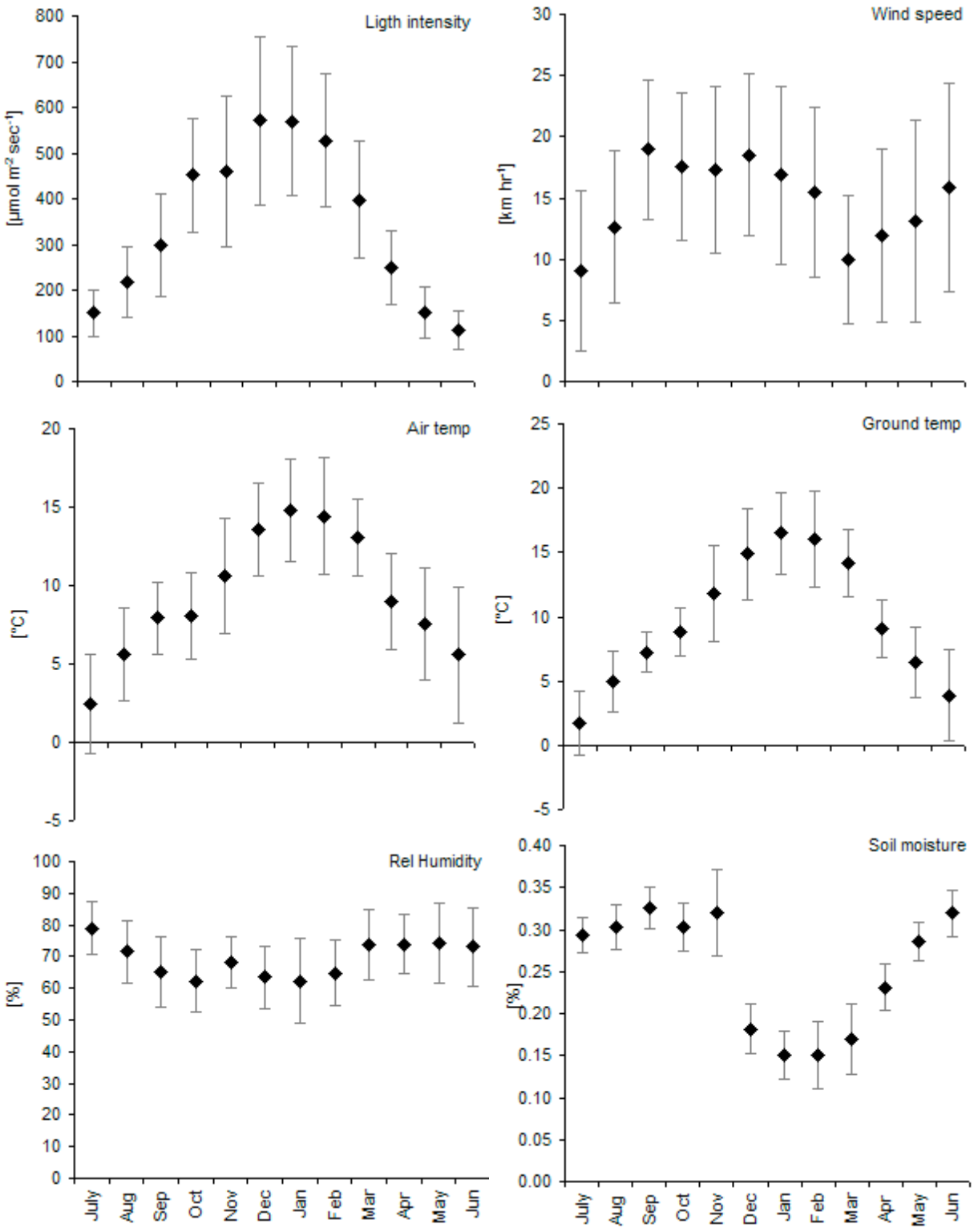



Figure 2.8: Means for PFD (light intensity), wind speed, air temperature (Air temp), ground temperature (Ground temp), relative humidity in air (Rel humidity) and soil moisture at Cass in July to December 2002 and January to July 2003. Means are averages over month from hourly measurements. All measurements were done from a climate weather station on a 14 m tall tower, except ground temperature, which was measured just above ground and soil moisture, measured at 10 cm depth.

The two summer seasons varied considerably from each other. PFDs for November 2001 and January 2002 varied between 440 and 500 µmol $sec^{-1}$ $m^{-2}$, whereas they were between 400 and 570 µmol $sec^{-1}$ $m^{-2}$ in the period from January to March 2003. The relative humidity in the air in the season of 2001/02 was around 80 %, in the season of 2002/03 averaged at 67%. The air temperature differed only by 1 °C between both seasons. The ground temperature in the season of 2001/02 averaged for November to January at 14°C. In season 2002/03 the average ground temperature of January to March was 16°C. The soil moisture at a depth of 10 cm was in season 2001/02 at 0.25 %, in 2002/03 at 0.15 % on average. Summarising those data, the season of 2001/02 was slightly colder and moister than the season of 2002/03.

### 2.1.5 Statistics

All analyses were performed using S-Plus version 4.5 (Mathsoft, Cambridge, MA, USA). The difference in responses between treatments was evaluated using a two way ANOVA with factors such as 'Month' (November01 + January 02 in season 2001/02, January03 + February/ March03 in season 2002/03), 'Light level' (inner leaves exposed, sun light, shaded), 'Water availability' (streambed, N-facing slope) and 'Genus' (*Corokia*, *Coprosma*) as factors for the field trials in 2001/02 and 2002/03. The factors for the glasshouse trial in 2002 were 'Month' (March, June), 'Light level' (sun light; shaded), 'Water availability' (well-watered, water stressed), 'Genus' (*Corokia, Coprosma*), 'Habit' (divaricate, non-divaricate) and in 2003 'Light level' (sun light, shaded), 'Water availability' (well-watered, water stressed), 'Habit' (divaricate, hybrid, non-divaricate). The glasshouse trial included divaricate *Corokia cotoneaster* and *Coprosma propinqua* as well as non-divaricate *Corokia buddleioides* and *Coprosma robusta*. The field study included only *C. cotoneaster* and *C. propinqua.* All interactions of the factors for the field trial and the glasshouse experiment are presented too. Due to the normal distribution of the response variable, no transformation was employed. For significant factors where there were greater than two levels of the treatment, a multiple comparison test was used to determine between which levels of the treatment the significance lay. All presented graphs present result means with the standard deviation; 'NA' indicates data were missing and '#' indicates an insufficient number of data ($n<3$).

## 2.2 Water Potentials

This project aims to determine the photosynthetic reactions of divaricate shrubs to drought conditions, compared to their non-divaricate congeners. In this section I present data on water potentials to ensure that the various field and glasshouse treatments did result in different water status of the experimental plants.

### 2.2.1 Introduction

Water stress affects leaf water potential, net photosynthesis, and leaf conductance due to stomatal closure during drought conditions. The internal $CO_2$ concentration is thus also lower during drought. Drought stress can be found in plants with, for example, intense evaporation, in saline or frozen soils. The accumulation of solutes in leaves or roots has the effect of maintaining tissue turgor (Willert *et al.*, 1995).

At a given leaf temperature, the partial pressure of water vapour inside a leaf is in equilibrium with the saturated vapour pressure of the atmosphere (Farquhar *et al.*, 1978). The resistance to water loss is the total of stomatal and cuticular resistances. As the stomatal resistance changes, the rate of transpiration also varies, which in turn affects the leaf temperature through the energy balance of the leaf (Farquhar *et al.*, 1980a).

Divaricate shrubs have very small leaves, which are situated in the inner parts of the shrub. The outer branch canopy is assumed to reduce radiation loads ('self-shading') and to keep the moisture in the interior of the shrub, which reduces transpiration (Kelly & Ogle, 1990 and McGlone & Webb, 1981). Therefore, divaricate shrubs that may be adapted to drought conditions should be able to maintain their stomata open and photosynthesize during the summer. If turgor maintenance is possible over a wide range of soil water potentials, this would allow the plant to remain photosynthetically active, and therefore maintain a positive carbon balance during summer drought.

Aim of the Study

The aim of this section was to ensure a difference in the water availability between plants in the streambed and the N-facing slope in the field set up (Section 2.1.2) and the well-watered and water stressed plants in the glasshouse (Section 2.1.3). Lower water potentials and therefore significant treatment effects were hypothesised between the different months. The measurements of water potentials in plants under different light and water treatments were made to ensure the different responses of all plants to high light versus shaded conditions as well as to test if lower water potential values were found in plants with reduced water supply. The genera were hypothesised to show similar responses to the light and water treatments, whereas the different growth forms were hypothesised to vary substantially in response. The 'self-shading' divaricate growth form was hypothesised to reduce water loss and to maintain a stable water status in divaricate plants during the day (Chapter 1) and therefore I tested if divaricate shoots have less negative water potentials than non-divaricate leaves. Diurnal changes in water potential were expected to be more prominent in non-divaricate leaves due to higher transpirational water loss.

### 2.2.2 Materials and Methods

Water potential was measured for leaves of non-divaricate plants. Due to the small leaf size, divaricate plants had shoot water potential recorded (Chapter 2.1). Shoot water potential was calculated from at least 3 photosynthetic leaves on a branchlet not longer than 20 mm. All water potential measurements were carried out with a Scholander pressure chamber (PMS Instruments Co., Corvallis, Oregon, USA). To avoid water loss after the collection of either leaves or shoots, samples were stored in plastic bags with moist filter paper and kept on ice. All measurements were performed within 10 minutes of collection.

In spring 2002/03, plastic covers were installed in the field on the ground under some of the plants grown on the N-facing slope. Plants from the two genera and all three light level treatments were used. These plastic covers were intended to reduce the available precipitation in a 2 x 3 m area surrounding the shrub. The plastic cover did not influence the water potentials of the leaves significantly and therefore data are not shown. The plants with ground covers were used in the presented data in the same

way as plants without ground covers. Gravimetric measurements to determine soil moisture (by weight of unit water per unit dried soil sample) did not show any significant differences between plants with or without ground covers on the N-facing slope (data not shown). This may have been because the summer had very low rainfall (Section 2.1.4).

### 2.2.3 Results

#### Cass Field Experiment 2001/02

Four shrubs of each species were measured in each treatment combination during summer, following pre-treatment measurements carried out in spring. The water availability was significantly influenced by the location of the plants, either in the streambed with supposed high water availability or on the N-faced slope with supposed lower water availability. An overview for the measurements recorded in spring and summer of 2001/02 is given in Figure 2.9 for *C. cotoneaster* and in Figure 2.10 for *C. propinqua*. In the light and watering treatments, both species showed a diurnal adaptation to changing water availability.

*C. cotoneaster* (Figure 2.9) shows even less negative water potentials in January than in spring, especially predawn and in the evening. Only at noon was mild drought stress experienced and all plants displayed a fast recovery in the evening. The water potentials were lower for plants that grew in the streambed compared to plants on the supposedly drier N-facing slope. The recovery to the predawn water potential values of shaded plants was slower than for plants under normal light or exposed conditions. *C. propinqua* (Figure 2.10) did not exhibit a uniform response to increased rainfall in January. Interestingly, in January the water potentials at noon and in the evening for exposed plants grown in the streambed were less negative than for plants from the same location but grown under normal light or shade cloth.

All measurements of water potential predawn, noon and evening show significant effects of water availability and genus (Table 2.1). Of interest was the lack of a significant month* light level interaction (the changes in water potential between spring and summer in the different light treatments) for the water potential measurements taken at noon. Predawn and evening measurements did show a

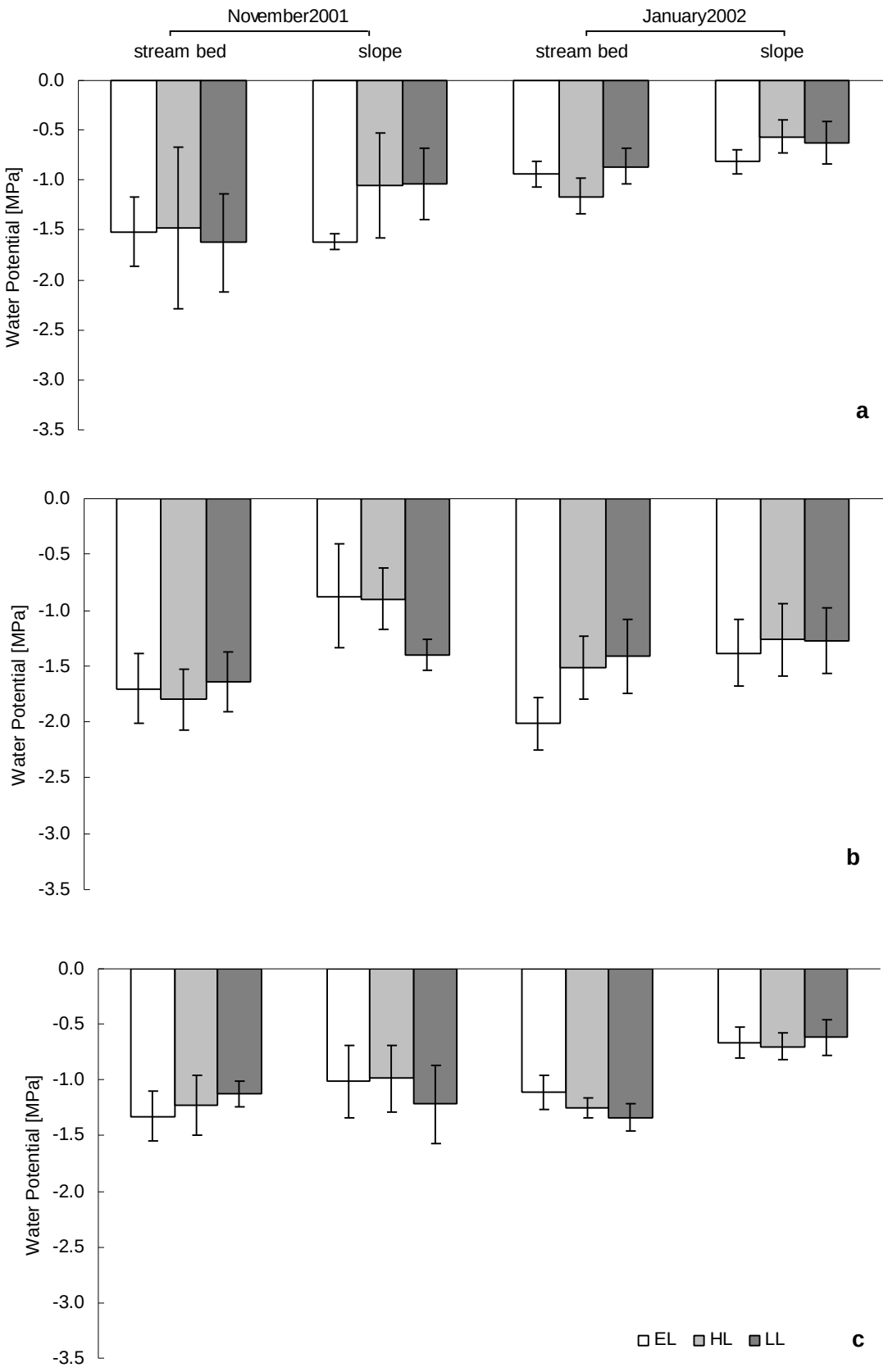



Figure 2.9: Water potential for *Corokia cotoneaster* shoots at Cass at (a) predawn, (b) noon and (c) evening measurements taken during summer 2001/02 in a streambed and on a N-facing slope and under three different light treatments (EL = inner canopy exposed, HL = sun light, LL = shaded) [n = 4].

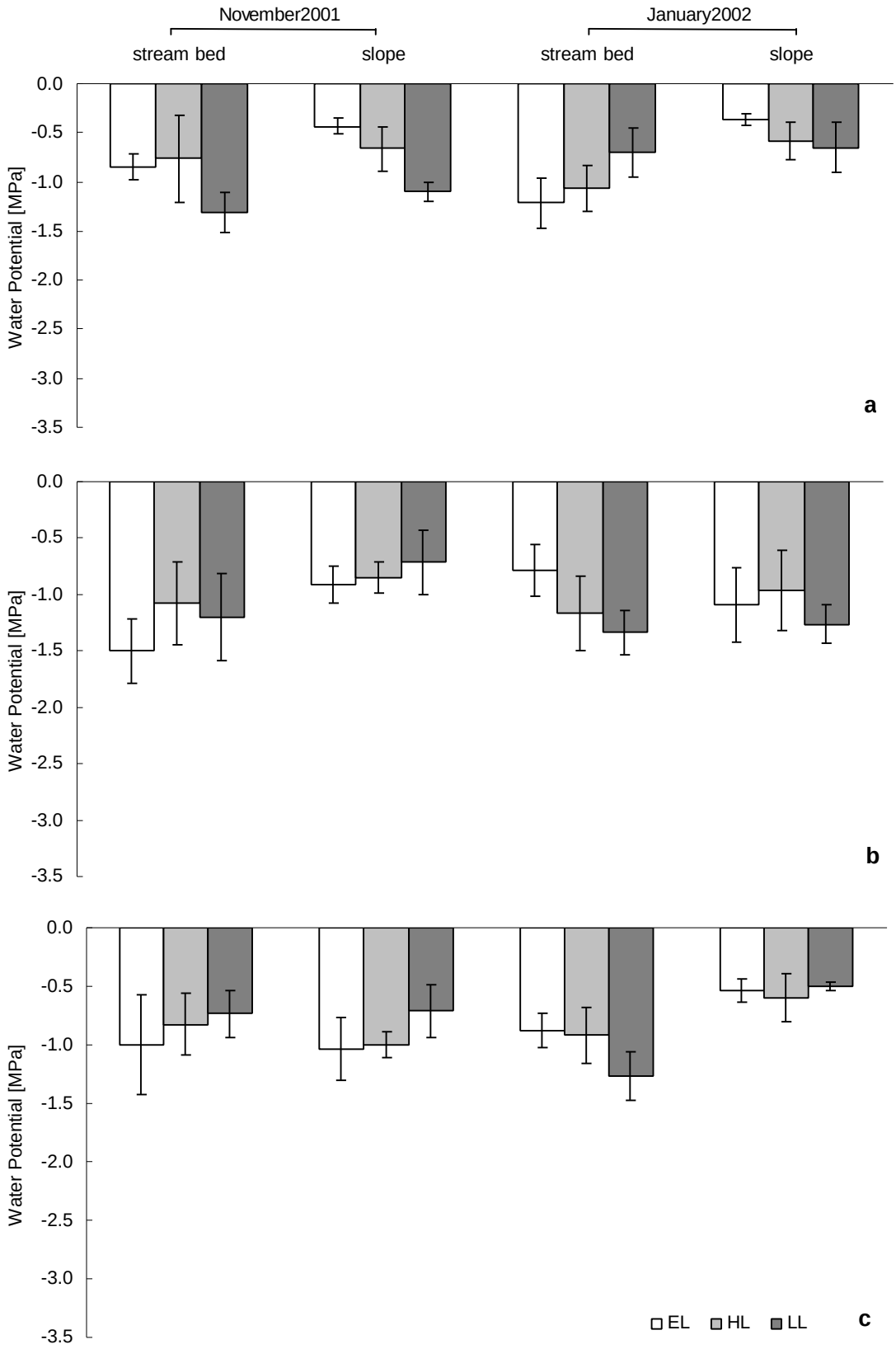



Figure 2.10: Water potential for *Coprosma propinqua* shoots at Cass at (a) predawn, (b) noon and (c) evening measurements taken during summer 2001/02 in a streambed and on a N-facing slope and under three different light treatments (EL = inner canopy exposed, HL = sun light, LL = shaded) [n = 4].

Table 2.1: Analysis of variance table for shoot water potential at the Cass field site, (a) predawn, (b) noon and (c) evening measurements taken during November 2001 and January 2002. Month, light level (natural daylight, shaded and exposed plants), water availability (streambed and N-facing slope) and genus (*Corokia cotoneaster* and *Coprosma propinqua*) as treatments [significant results in bold].

| a | Df | Sum of Sq | Mean Sq | F Value | Pr(F) |
|---|---|---|---|---|---|
| **Month** | 1 | 2.436684 | 2.436684 | 29.95 | **0.000** |
| Light level | 2 | 0.292122 | 0.146061 | 1.80 | 0.175 |
| **Water availability** | 1 | 2.728043 | 2.728043 | 33.53 | **0.000** |
| **Genus** | 1 | 2.077709 | 2.077709 | 25.53 | **0.000** |
| **Month*Light level** | 2 | 0.700409 | 0.350205 | 4.30 | **0.018** |
| Month*Water availability | 1 | 0.035091 | 0.035091 | 0.43 | 0.514 |
| Light level*Water availability | 2 | 0.073916 | 0.036958 | 0.45 | 0.637 |
| **Month*Genus** | 1 | 1.249252 | 1.249252 | 15.35 | **0.000** |
| **Light level*Genus** | 2 | 0.785997 | 0.392998 | 4.83 | **0.011** |
| Water availability*Genus | 1 | 0.007416 | 0.007416 | 0.09 | 0.764 |
| Month*Light level*Water availability | 2 | 0.325384 | 0.162692 | 1.99 | 0.144 |
| **Month*Light level*Genus** | 2 | 0.918886 | 0.459443 | 5.65 | **0.006** |
| Month*Water availability*Genus | 1 | 0.172597 | 0.172597 | 2.12 | 0.150 |
| **Light level*Water availability*Genus** | 2 | 0.544606 | 0.272303 | 3.35 | **0.042** |
| Month*Light level*Water availability*Genus | 2 | 0.048893 | 0.024446 | 0.30 | 0.742 |
| Residuals | 62 | 5.045000 | 0.081371 | | |

| b | Df | Sum of Sq | Mean Sq | F Value | Pr(F) |
|---|---|---|---|---|---|
| Month | 1 | 0.127223 | 0.127223 | 1.51 | 0.224 |
| Light level | 2 | 0.156507 | 0.078253 | 0.93 | 0.401 |
| **Water availability** | 1 | 3.086576 | 3.086576 | 36.61 | **0.000** |
| **Genus** | 1 | 2.352765 | 2.352765 | 27.91 | **0.000** |
| Month*Light level | 2 | 0.012833 | 0.006417 | 0.08 | 0.927 |
| **Month*Water availability** | 1 | 1.042390 | 1.042390 | 12.36 | **0.001** |
| Light level*Water availability | 2 | 0.038926 | 0.019463 | 0.23 | 0.795 |
| Month*Genus | 1 | 0.044421 | 0.044421 | 0.53 | 0.471 |
| Light level*Genus | 2 | 0.107281 | 0.053640 | 0.64 | 0.533 |
| **Water availability*Genus** | 1 | 0.446062 | 0.446062 | 5.29 | **0.025** |
| Month*Light level*Water availability | 2 | 0.059580 | 0.029790 | 0.35 | 0.704 |
| **Month*Light level*Genus** | 2 | 1.091435 | 0.545718 | 6.47 | **0.003** |
| Month*Water availability*Genus | 1 | 0.021766 | 0.021766 | 0.26 | 0.613 |
| Light level*Water availability*Genus | 2 | 0.453127 | 0.226563 | 2.69 | 0.076 |
| Month*Light level*Water availability*Genus | 2 | 0.449219 | 0.224610 | 2.66 | 0.078 |
| Residuals | 61 | 5.143125 | 0.084314 | | |

| c | Df | Sum of Sq | Mean Sq | F Value | Pr(F) |
|---|---|---|---|---|---|
| **Month** | 1 | 0.587603 | 0.587603 | 11.86 | **0.001** |
| Light level | 2 | 0.008066 | 0.004033 | 0.08 | 0.922 |
| **Water availability** | 1 | 1.698342 | 1.698342 | 34.28 | **0.000** |
| **Genus** | 1 | 1.109264 | 1.109264 | 22.39 | **0.000** |
| **Month*Light level** | 2 | 0.314587 | 0.157294 | 3.17 | **0.048** |
| **Month*Water availability** | 1 | 1.179363 | 1.179363 | 23.80 | **0.000** |
| Light level*Water availability | 2 | 0.053790 | 0.026895 | 0.54 | 0.584 |
| Month*Genus | 1 | 0.041782 | 0.041782 | 0.84 | 0.362 |
| Light level*Genus | 2 | 0.051308 | 0.025654 | 0.52 | 0.598 |
| Water availability*Genus | 1 | 0.168296 | 0.168296 | 3.40 | 0.070 |
| Month*Light level*Water availability | 2 | 0.220417 | 0.110209 | 2.22 | 0.116 |
| Month*Light level*Genus | 2 | 0.163327 | 0.081663 | 1.65 | 0.200 |
| Month*Water availability*Genus | 1 | 0.021039 | 0.021039 | 0.42 | 0.517 |
| Light level*Water availability*Genus | 2 | 0.167971 | 0.083985 | 1.70 | 0.192 |
| Month*Light level*Water | 2 | 0.024165 | 0.012083 | 0.24 | 0.784 |

significant difference in the month* light level interaction. This may have been due to the weather conditions during January, which were unusually cold, cloudy and wet (climate data for 2001-2003 are presented in Section 2.1.4).

Cass Field Experiment 2002/03

Due to the unusually wet summer of 2001/02, the water potential data from the field site at Cass show that the shrubs experienced more drought stress in November than in January. Therefore, all measurements were repeated in January and March 2003. The summer of 2002/03 was warmer and humidity and soil moisture were lower than in 2001/02 (Section 2.1.4). As shown in Figures 2.11 and 2.12, diurnal water potentials of *C. cotoneaster* and *C. propinqua* show adaptation. Compared to the wet summer of 2001/02, the measurements at noon in the dry summer of 2002/03 produced lower water potential values for all plants. As was found in 2001/02, plants in the supposedly drier locations showed less negative water potentials than the plants grown in the streambed (Figure 2.11 and 2.12). Plants with exposed inner shoots showed the slowest recovery rate; the evening water potential values were still much lower than the predawn values. *C. cotoneaster* plants with exposed inner parts had the lowest water potentials at noon and recovered slowly in the evening. *C. propinqua* showed a similar pattern even if it was not as pronounced.

Table 2.2 summarises the results of the Analysis of Variance. There were significant to highly significant differences between the plants growing in the streambed and those on the N-facing slope. The change to the drier and hotter season is particularly evident at noon, with significant differences between spring and summer and between light level and water availability. Different strategies to cope with these environmental conditions are indicated by significant effects of genus and month* light level* genus.

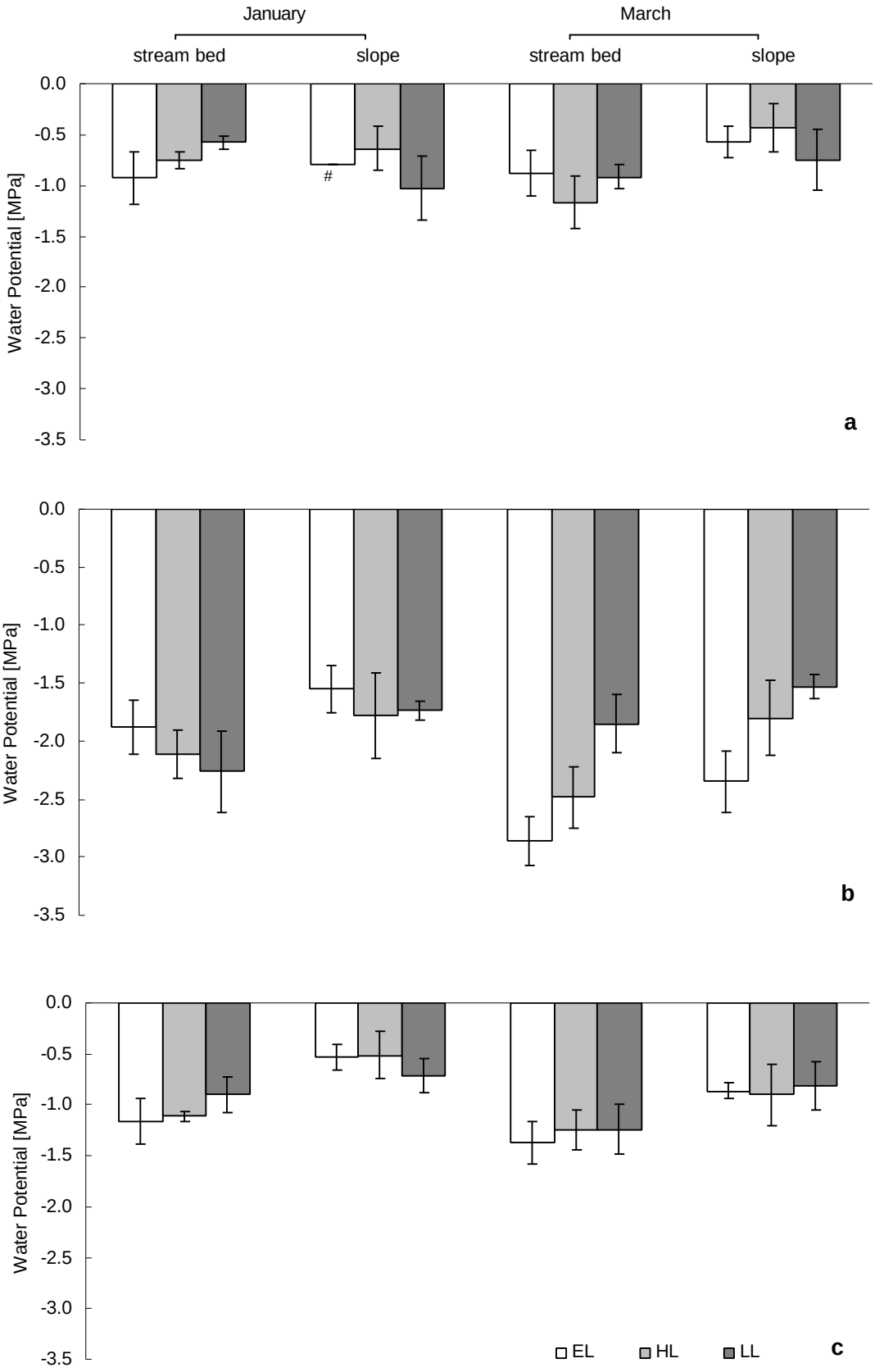



Figure 2.11: Water potential measurements for *Corokia cotoneaster* at (a) predawn, (b) noon and (c) evening in summer 2002/03 at Cass in a streambed and on a N-facing slope and under 3 different light treatments (EL = inner canopy exposed, HL = sun light, LL = shaded) [n = 4].

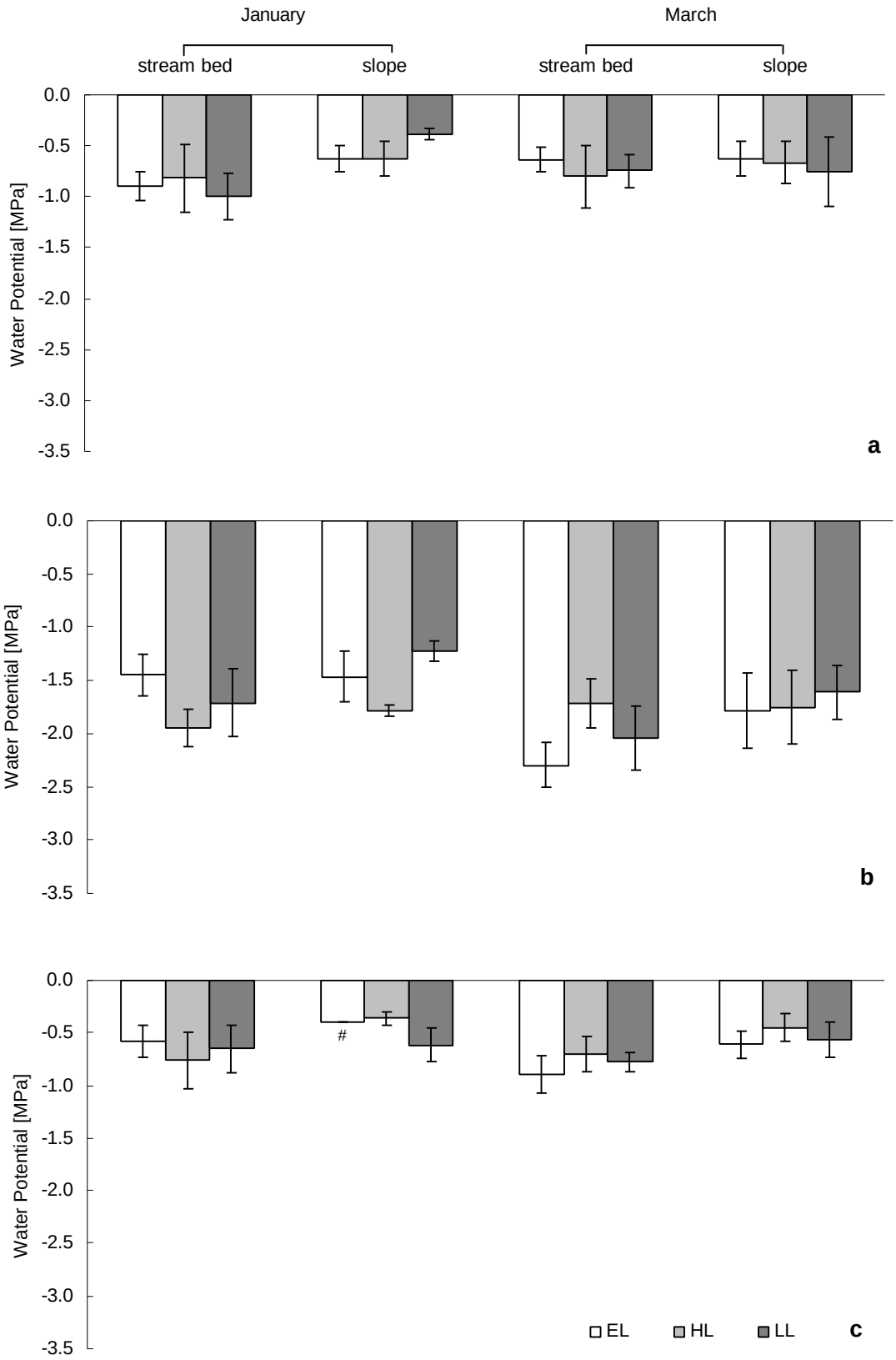



Figure 2.12: Water potential measurements for *Coprosma propinqua* at (a) predawn, (b) noon and (c) evening in summer 2002/03 at Cass in a streambed and on a N-facing slope and under 3 different light treatments (EL = inner canopy exposed, HL = sun light, LL = shaded) [n = 4].

Table 2.2: Analysis of variance table for shoot water potential at the Cass field site, (a) predawn, (b) noon and (c) evening measurements taken during January and March 2003. Month, light level (natural daylight, shaded and exposed plants), water availability (streambed and N-facing slope) and genus (*Corokia cotoneaster* and *Coprosma propinqua*) as treatments [significant results in bold].

| a | Df | Sum of Sq | Mean Sq | F Value | Pr(F) |
|---|---|---|---|---|---|
| Month | 1 | 0.014821 | 0.0148209 | 0.32 | 0.572 |
| Light level | 2 | 0.002897 | 0.0014486 | 0.03 | 0.969 |
| **Water availability** | 1 | 0.800142 | 0.8001415 | 17.41 | **0.000** |
| Genus | 1 | 0.073643 | 0.0736430 | 1.60 | 0.211 |
| Month*Light level | 2 | 0.171034 | 0.0855171 | 1.86 | 0.165 |
| Month*Water availability | 1 | 0.019770 | 0.0197696 | 0.43 | 0.515 |
| Light level*Water availability | 2 | 0.147613 | 0.0738065 | 1.61 | 0.210 |
| Month*Genus | 1 | 0.016825 | 0.0168248 | 0.37 | 0.548 |
| Light level*Genus | 2 | 0.011266 | 0.0056330 | 0.12 | 0.885 |
| Water availability*Genus | 1 | 0.000204 | 0.0002040 | 0.01 | 0.947 |
| Month*Light level*Water availability | 2 | 0.121691 | 0.0608453 | 1.32 | 0.274 |
| Month*Light level*Genus | 2 | 0.014356 | 0.0071778 | 0.16 | 0.856 |
| **Month*Water availability*Genus** | 1 | 0.710656 | 0.7106558 | 15.46 | **0.000** |
| **Light level*Water availability*Genus** | 2 | 0.397837 | 0.1989183 | 4.33 | **0.018** |
| Month*Light level*Water availability*Genus | 2 | 0.127800 | 0.0638999 | 1.39 | 0.257 |
| Residuals | 57 | 2.620250 | 0.0459693 | | |

| b | Df | Sum of Sq | Mean Sq | F Value | Pr(F) |
|---|---|---|---|---|---|
| **Month** | 1 | 1.303508 | 1.303508 | 20.81 | **0.000** |
| **Light level** | 2 | 0.654268 | 0.327134 | 5.22 | **0.008** |
| **Water availability** | 1 | 2.508929 | 2.508929 | 40.06 | **0.000** |
| **Genus** | 1 | 1.779657 | 1.779657 | 28.41 | **0.000** |
| **Month*Light level** | 2 | 2.161469 | 1.080734 | 17.25 | **0.000** |
| Month*Water availability | 1 | 0.045896 | 0.045896 | 0.73 | 0.396 |
| Light level*Water availability | 2 | 0.069126 | 0.034563 | 0.55 | 0.579 |
| Month*Genus | 1 | 0.021528 | 0.021528 | 0.34 | 0.560 |
| Light level*Genus | 2 | 0.147056 | 0.073528 | 1.17 | 0.316 |
| Water availability*Genus | 1 | 0.143064 | 0.143064 | 2.28 | 0.136 |
| Month*Light level*Water availability | 2 | 0.225157 | 0.112578 | 1.80 | 0.175 |
| **Month*Light level*Genus** | 2 | 1.206347 | 0.603173 | 9.63 | **0.000** |
| Month*Water availability*Genus | 1 | 0.000586 | 0.000586 | 0.01 | 0.923 |
| Light level*Water availability*Genus | 2 | 0.197100 | 0.098550 | 1.57 | 0.216 |
| Month*Light level*Water availability*Genus | 2 | 0.179705 | 0.089852 | 1.43 | 0.247 |
| Residuals | 58 | 3.632850 | 0.062635 | | |

| c | Df | Sum of Sq | Mean Sq | F Value | Pr(F) |
|---|---|---|---|---|---|
| **Month** | 1 | 0.706126 | 0.706126 | 21.61 | **0.000** |
| Light level | 2 | 0.035412 | 0.017706 | 0.54 | 0.584 |
| **Water availability** | 1 | 2.564452 | 2.564452 | 78.49 | **0.000** |
| **Genus** | 1 | 2.587972 | 2.587972 | 79.21 | **0.000** |
| Month*Light level | 2 | 0.088286 | 0.044143 | 1.35 | 0.266 |
| Month*Water availability | 1 | 0.001785 | 0.001785 | 0.05 | 0.816 |
| Light level*Water availability | 2 | 0.141493 | 0.070747 | 2.17 | 0.123 |
| Month*Genus | 1 | 0.110839 | 0.110839 | 3.39 | 0.070 |
| Light level*Genus | 2 | 0.054163 | 0.027082 | 0.83 | 0.441 |
| **Water availability*Genus** | 1 | 0.282972 | 0.282972 | 8.66 | **0.005** |
| Month*Light level*Water availability | 2 | 0.170027 | 0.085014 | 2.60 | 0.082 |
| Month*Light level*Genus | 2 | 0.063670 | 0.031835 | 0.97 | 0.383 |
| Month*Water availability*Genus | 1 | 0.012256 | 0.012256 | 0.38 | 0.542 |
| Light level*Water availability*Genus | 2 | 0.038912 | 0.019456 | 0.60 | 0.554 |
| Month*Light level*Water availability*Genus | 2 | 0.019364 | 0.009682 | 0.30 | 0.745 |
| Residuals | 68 | 2.221833 | 0.032674 | | |

Glasshouse 2002

As mentioned in Section 2.2.1, the glasshouse trial included divaricate and non-divaricate congeners with two light and watering levels. All species established a diurnal pattern of water potential under different water and light conditions. The drought stress was increased after the measurements in March, but the water potential values were not greatly lower in June than in March (Figure 2.13 and 2.14).

*C. buddleioides* (non-divaricate) showed a lower water potential than *C. cotoneaster* (divaricate) for both levels of water availability (Figure 2.13). All *Corokia* plants grown under high light conditions had more negative water potentials than shaded *Corokia* spp. in both well-watered and water stress treatments, especially at noon. In contrast to the field results, the genus *Coprosm*a exhibited significantly lower water potentials for all measurements in a day. *Coprosma* leaves and shoots had lower water potentials in June, but the response to different light regimes was not uniform (Figure 2.14). In March divaricate and non-divaricate *Coprosma* spp. expressed different reaction patterns to drought during the day; in June the difference was less obvious. In March, water stressed *Coprosma* shoots and leaves had lower water potentials than shoots without predawn water limitation. During the day water potentials decreased proportionally and in the evening, water stressed plants expressed nearly twice as low water potential values as well-watered shoots and leaves. Generally, it was similar in June. But by comparison of divaricate *Coprosma* shoots under high light in June values were not as low as in March, whereas the non-divarcate *Coprosma* leaves under high light expressed the lowest water potential values in June.

For all measurements during March and June, the effects of water availability, genus, water availability* genus and genus* habit were significant or highly significant (Table 2.3). The water potential was significantly lower in plants under lower water availability. In contrast to my hypothesis, habit did not show a consistent effect and had only a significant effect on the evening measurements. The adaptation to different water availabilities for the two genera is demonstrated by the significant effect of the genus* habit interaction, all *Coprosma* species show low water potentials and divaricate *Coprosma* shoots reached the lowest water potential values (Figure 2.14).

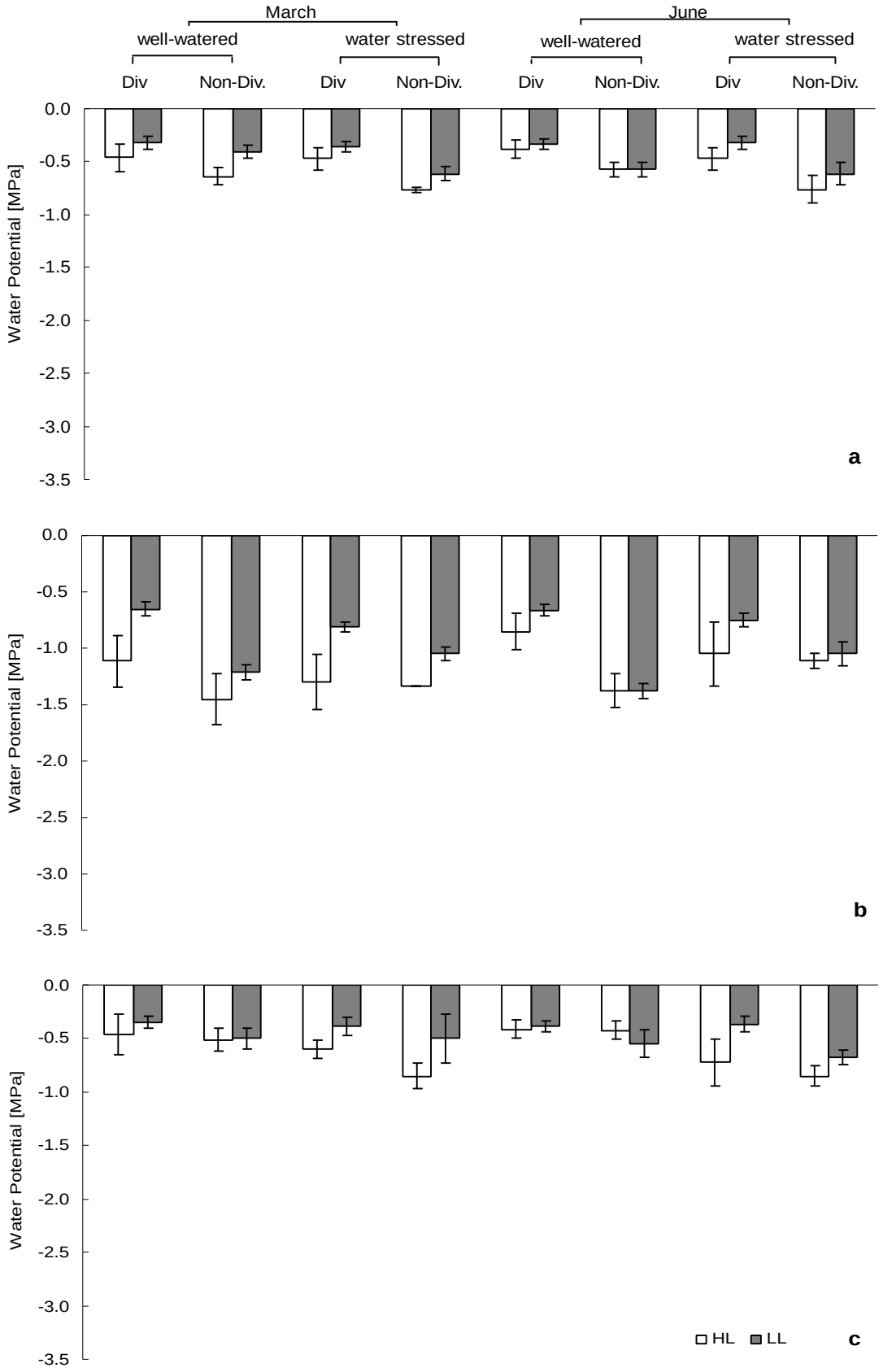



Figure 2.13: Water potential for *Corokia cotoneaster* (Div) and *C. buddleioides* (Non-Div), measurements taken at (a) predawn, (b) noon and (c) evening in the glasshouse, 2002. Under well-watered and water stressed conditions and two different light treatments (HL = sun light, LL = shaded) [n = 4].

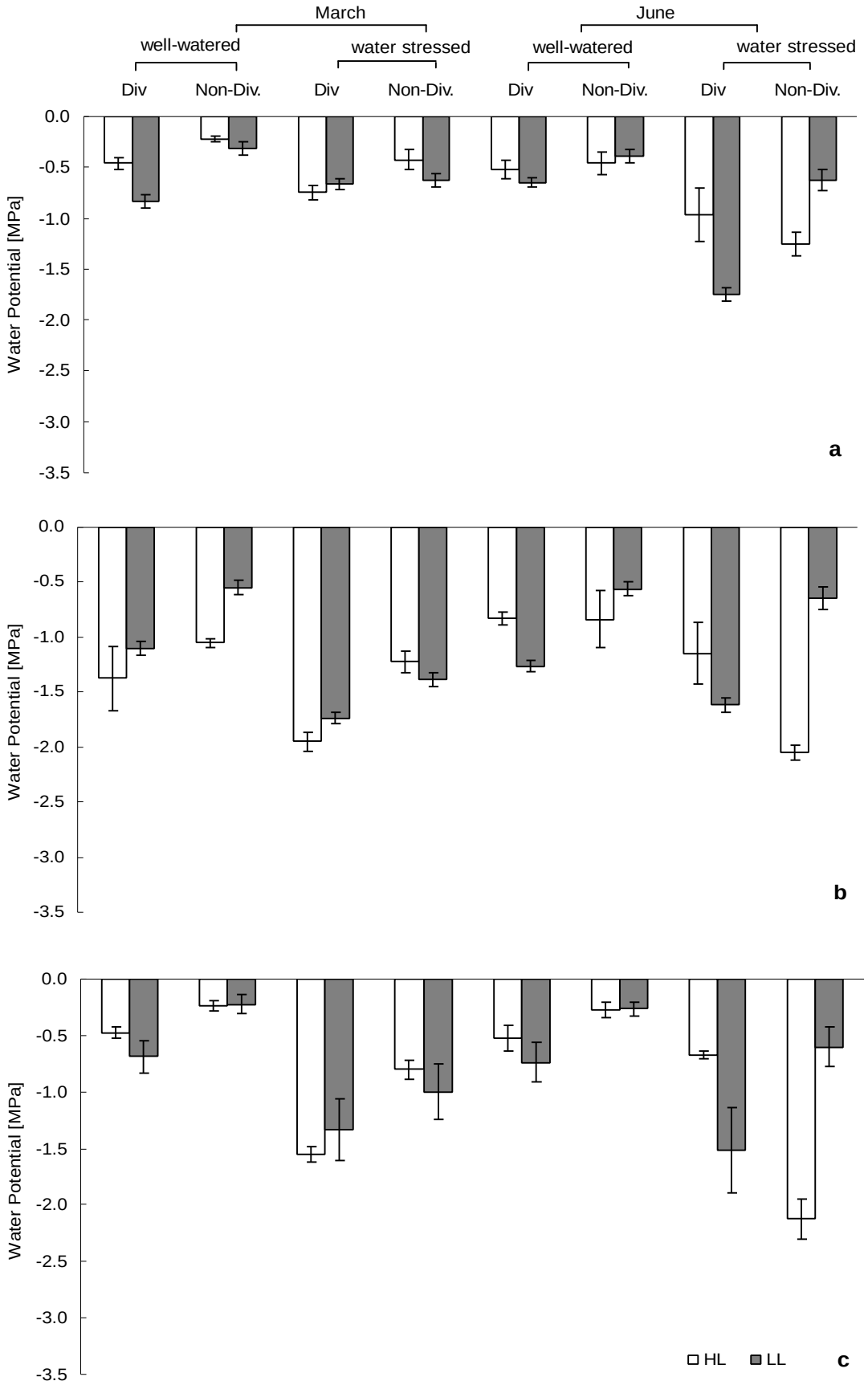



Figure 2.14: Water potential for *Coprosma robusta* (Div) and *C. propinqua* (Non-Div), measurements taken at (a) predawn, (b) noon and (c) evening in the glasshouse, 2002. Under well-watered and water stressed conditions and two different light treatments (HL = sun light, LL = shaded) [n = 4].

Table 2.3: Analysis of variance table for water potential in the glasshouse, (a) predawn, (b) noon and (c) evening measurements during March and June 2002. Month, light level (100% vs 75% of natural daylight), water availability (200 mls every 2 days and 50 mls every 3 days) and genus and habit (*Corokia cotoneaster*, *Coprosma propinqua*, *Corokia buddleioide* and *Coprosma robusta*) as treatments. Measurements recorded from shoots of *Corokia cotoneaster* and *Coprosma propinqua*; leaves of *Corokia buddleioides* and *Coprosma robusta.* [significant results in bold].

| a | Df | Sum of Sq | Mean Sq | F Value | Pr(F) |
|---|---|---|---|---|---|
| **Month** | 1 | 0.527508 | 0.527508 | 36.33 | **0.000** |
| Light level | 1 | 0.008809 | 0.008809 | 0.61 | 0.438 |
| **Water availability** | 1 | 1.524261 | 1.524261 | 104.97 | **0.000** |
| **Genus** | 1 | 0.729505 | 0.729505 | 50.24 | **0.000** |
| Habit | 1 | 0.033662 | 0.033662 | 2.32 | 0.131 |
| Month*Light level | 1 | 0.004252 | 0.004252 | 0.29 | 0.590 |
| **Month*Water availability** | 1 | 0.340145 | 0.340145 | 23.42 | **0.000** |
| Light level*Water availability | 1 | 0.001317 | 0.001317 | 0.09 | 0.764 |
| **Month*Genus** | 1 | 0.620564 | 0.620564 | 42.74 | **0.000** |
| **Light level*Genus** | 1 | 0.478082 | 0.478082 | 32.92 | **0.000** |
| **Water availability*Genus** | 1 | 0.684390 | 0.684390 | 47.13 | **0.000** |
| Month*Habit | 1 | 0.004212 | 0.004212 | 0.29 | 0.592 |
| **Light level*Habit** | 1 | 0.307980 | 0.307980 | 21.21 | **0.000** |
| Water availability*Habit | 1 | 0.001930 | 0.001930 | 0.13 | 0.716 |
| **Genus*Habit** | 1 | 2.168133 | 2.168133 | 149.31 | **0.000** |
| Month*Light level*Water availability | 1 | 0.001759 | 0.001759 | 0.12 | 0.729 |
| Month*Light level*Genus | 1 | 0.035262 | 0.035262 | 2.43 | 0.123 |
| **Month*Water availability*Genus** | 1 | 0.428730 | 0.428730 | 29.52 | **0.000** |
| Light level*Water availability*Genus | 1 | 0.001827 | 0.001827 | 0.13 | 0.727 |
| **Month*Light level*Habit** | 1 | 0.188247 | 0.188247 | 12.96 | **0.001** |
| **Month*Water availability*Habit** | 1 | 0.178689 | 0.178689 | 12.31 | **0.001** |
| Light level*Water availability*Habit | 1 | 0.052910 | 0.052910 | 3.64 | 0.060 |
| Month*Genus*Habit | 1 | 0.027549 | 0.027549 | 1.90 | 0.172 |
| **Light level*Genus*Habit** | 1 | 0.264410 | 0.264410 | 18.21 | **0.000** |
| Water availability*Genus*Habit | 1 | 0.050946 | 0.050946 | 3.51 | 0.064 |
| **Month*Light level*Water availability* Genus** | 1 | 0.068117 | 0.068117 | 4.69 | **0.033** |
| **Month*Light level*Water availability* Habit** | 1 | 0.333457 | 0.333457 | 22.96 | **0.000** |
| **Month*Light level*Genus*Habit** | 1 | 0.333414 | 0.333414 | 22.96 | **0.000** |
| **Month*Water availability*Genus*Habit** | 1 | 0.080992 | 0.080992 | 5.58 | **0.020** |
| Light level*Water availability*Genus*Habit | 1 | 0.049697 | 0.049697 | 3.42 | 0.068 |
| **Month*Light level*Water availability* Genus*Habit** | 1 | 0.314650 | 0.314650 | 21.67 | **0.000** |
| Residuals | 87 | 1.263333 | 0.014521 | | |

| b | Df | Sum of Sq | Mean Sq | F Value | Pr(F) |
|---|---|---|---|---|---|
| **Month** | 1 | 0.532023 | 0.532023 | 15.91 | **0.000** |
| **Light level** | 1 | 1.298317 | 1.298317 | 38.83 | **0.000** |
| **Water availability** | 1 | 1.257647 | 1.257647 | 37.61 | **0.000** |
| **Genus** | 1 | 0.260419 | 0.260419 | 7.79 | **0.007** |
| Habit | 1 | 0.001759 | 0.001759 | 0.05 | 0.819 |
| **Month*Light level** | 1 | 0.181725 | 0.181725 | 5.43 | **0.022** |
| Month*Water availability | 1 | 0.083534 | 0.083534 | 2.50 | 0.118 |
| Light level*Water availability | 1 | 0.002445 | 0.002445 | 0.07 | 0.788 |
| Month*Genus | 1 | 0.093152 | 0.093152 | 2.79 | 0.099 |
| Light level*Genus | 1 | 0.034376 | 0.034376 | 1.03 | 0.314 |
| **Water availability*Genus** | 1 | 1.940009 | 1.940009 | 58.01 | **0.000** |
| **Month*Habit** | 1 | 0.231223 | 0.231223 | 6.91 | **0.010** |
| Light level*Habit | 1 | 0.106252 | 0.106252 | 3.18 | 0.079 |
| **Water availability*Habit** | 1 | 0.215867 | 0.215867 | 6.46 | **0.013** |
| **Genus*Habit** | 1 | 3.945732 | 3.945732 | 117.99 | **0.000** |
| **Month*Light level*Water availability** | 1 | 0.311839 | 0.311839 | 9.32 | **0.003** |
| Month*Light level*Genus | 1 | 0.072271 | 0.072271 | 2.16 | 0.146 |
| Month*Water availability*Genus | 1 | 0.000006 | 0.000006 | 0.00 | 0.989 |
| Light level*Water availability*Genus | 1 | 0.000144 | 0.000144 | 0.00 | 0.948 |
| **Month*Light level*Habit** | 1 | 0.490484 | 0.490484 | 14.67 | **0.000** |
| Month*Water availability*Habit | 1 | 0.000210 | 0.000210 | 0.01 | 0.937 |
| Light level*Water availability*Habit | 1 | 0.002780 | 0.002780 | 0.08 | 0.774 |
| Month*Genus*Habit | 1 | 0.014518 | 0.014518 | 0.43 | 0.512 |
| **Light level*Genus*Habit** | 1 | 1.079224 | 1.079224 | 32.27 | **0.000** |
| **Water availability*Genus*Habit** | 1 | 0.212841 | 0.212841 | 6.36 | **0.014** |
| **Month*Light level*Water availability*Genus** | 1 | 0.286443 | 0.286443 | 8.57 | **0.005** |
| **Month*Light level*Water availability*Habit** | 1 | 0.217701 | 0.217701 | 6.51 | **0.013** |
| **Month*Light level*Genus*Habit** | 1 | 0.662931 | 0.662931 | 19.82 | **0.000** |
| Month*Water availability*Genus*Habit | 1 | 0.114312 | 0.114312 | 3.42 | 0.068 |
| Light level*Water availability*Genus*Habit | 1 | 0.040802 | 0.040802 | 1.22 | 0.273 |
| **Month*Light level*Water availability*Genus*Habit** | 1 | 0.333613 | 0.333613 | 9.98 | **0.002** |
| Residuals | 78 | 2.608333 | 0.033440 | | |

| c | Df | Sum of Sq | Mean Sq | F Value | Pr(F) |
|---|---|---|---|---|---|
| Month | 1 | 0.021130 | 0.021130 | 1.05 | 0.308 |
| Light level | 1 | 0.029571 | 0.029571 | 1.47 | 0.229 |
| **Water availability** | 1 | 4.875477 | 4.875477 | 242.79 | **0.000** |
| **Genus** | 1 | 1.483476 | 1.483476 | 73.88 | **0.000** |
| **Habit** | 1 | 0.128455 | 0.128455 | 6.40 | **0.013** |
| Month*Light level | 1 | 0.001738 | 0.001738 | 0.09 | 0.769 |
| Month*Water availability | 1 | 0.022197 | 0.022197 | 1.11 | 0.296 |
| **Light level*Water availability** | 1 | 0.357883 | 0.357883 | 17.82 | **0.000** |
| Month*Genus | 1 | 0.000219 | 0.000219 | 0.01 | 0.917 |
| **Light level*Genus** | 1 | 0.147055 | 0.147055 | 7.32 | **0.008** |
| **Water availability*Genus** | 1 | 2.322045 | 2.322045 | 115.63 | **0.000** |
| Month*Habit | 1 | 0.074059 | 0.074059 | 3.69 | 0.058 |
| **Light level*Habit** | 1 | 0.256190 | 0.256190 | 12.76 | **0.001** |
| **Water availability*Habit** | 1 | 0.157926 | 0.157926 | 7.86 | **0.006** |
| **Genus*Habit** | 1 | 1.405273 | 1.405273 | 69.98 | **0.000** |
| Month*Light level*Water availability | 1 | 0.075190 | 0.075190 | 3.74 | 0.056 |
| **Month*Light level*Genus** | 1 | 0.111061 | 0.111061 | 5.53 | **0.021** |
| Month*Water availability*Genus | 1 | 0.005440 | 0.005440 | 0.27 | 0.604 |
| Light level*Water availability*Genus | 1 | 0.000885 | 0.000885 | 0.04 | 0.834 |
| **Month*Light level*Habit** | 1 | 0.224801 | 0.224801 | 11.19 | **0.001** |
| **Month*Water availability*Habit** | 1 | 0.119326 | 0.119326 | 5.94 | **0.017** |
| **Light level*Water availability*Habit** | 1 | 0.145258 | 0.145258 | 7.23 | **0.009** |
| **Month*Genus*Habit** | 1 | 0.110319 | 0.110319 | 5.49 | **0.021** |
| **Light level*Genus*Habit** | 1 | 0.597980 | 0.597980 | 29.78 | **0.000** |
| Water availability*Genus*Habit | 1 | 0.010425 | 0.010425 | 0.52 | 0.473 |
| Month*Light level*Water availability*Genus | 1 | 0.048488 | 0.048488 | 2.41 | 0.124 |
| **Month*Light level* Water availability*Habit** | 1 | 0.371207 | 0.371207 | 18.49 | **0.000** |
| **Month*Light level*Genus*Habit** | 1 | 0.788261 | 0.788261 | 39.25 | **0.000** |
| **Month*Water availability*Genus*Habit** | 1 | 0.208856 | 0.208856 | 10.40 | **0.002** |
| **Light level*Water availability*Genus* Habit** | 1 | 0.153332 | 0.153332 | 7.64 | **0.007** |
| **Month*Light level*Water availability* Genus*Habit** | 1 | 1.017593 | 1.017593 | 50.67 | **0.000** |
| Residuals | 85 | 1.706875 | 0.020081 | | |

Summary

The measured water potential values reflected different adaptations of the plants to given treatments. Water availabilities can directly influence the water status of the plants, but variations in light levels can also be important.

In the field, the lowest values for water potential were recorded in the plants grown in the streambed; which was in contrast to the hypothesis that it would be the habitat with the better water supply. Possible reasons for this finding will be discussed in following sections. The finding that the streambed plants had lower water potentials than the plants on the N-facing slope will change the conceptual model in Section 1.3 (Figure 5.1). I hypothesised that the values for fluorescence, pigment and a-tocopherol concentrations and gas exchange parameters in the streambed plants would show less sings of stress than for the plants on the N-facing slope (Figure 1.2). Because the plants on the N-facing slope had less negative water potentials than those in the streambed, this hypothesis does not hold and the findings should be interpreted with this in mind. Evaluating the two genera, *Corokia* expressed lower water potential values than *Coprosma*. Interestingly and in contrast to my hypothesis, the two genera responded significantly differently in their water potential values to the light and water treatments.

In the experimental trial, plants under water limitation showed the lowest water potentials. Plants that were not shaded also had lower water potentials than shaded plants, except for some *Coprosma* plants. Also, non-divaricate *Corokia* leaves had lower water potential readings than divaricate *Corokia* shoots. In contrast to my hypothesis, habit had only significant effects on water potential measurements in the evening. No consistent effect of the divaricate growth form on the water status was found and *Coprosma* shoots often showed responses contrary my hypothesis (see Section 2.2.1). The two genera responded differently to the water treatment, shown in the genus* habit interaction, in contrast to my hypothesis.

### 2.2.4 Discussion

In summer, along with reduced water availability, high PFDs and temperatures are important stress factors for plants. An overall hypothesis guiding this research was that divaricate shrubs use their 'self-shading' growth form to protect their internal leaves against high radiation loads during drought. They also have very small leaves, which reduce the transpiration surface. When the temperatures are very high and soil water potential is very low, it is particularly important to be able to photosynthesize with high water use efficiency. Stomata respond to several environmental conditions, for example $CO_2$ concentration, PFDs, and water potential differences between soil and atmosphere. At midday plants are most vulnerable to water loss. The plants investigated showed diurnal patterns, due to the stomatal reaction protecting the leaves against high water lost at noon and recovering from that water loss in the evening and during the night.

The field experiments tested the different adaptations of *C. cotoneaster* and *C. propinqua* to naturally different water availabilities and artificially varied light. A strong and significant effect of genus was found. In contrast to my hypothesis, the field plants had lower water potential values at the streambed than at the N-facing slope. This could be due to a delayed stomata closure under water-limitation in the plants of the streambed compared to plants from the more wind exposed N-facing slope, or different rooting depths or root: shoot ratios at sites developed in response to the previous history of limited water availability.

In the glasshouse trial all plants responded significantly to the water availability treatment. My hypothesis that divaricate shoots would display higher water potentials than their non-divaricate congeners due to the 'self-shading' growth and therefore that divaricates would use water more conservatively due to their crown structure could not entirely be supported via water potential measurements. Divaricate *Corokia* shoots had higher water potential values than non-divaricate leaves, but it was not true for the findings on *Coprosma* leaves and shoots. The differences between the different genera were greater than any differences between the habits. Crown structure and leaf shape varied remarkably between the two genera (Chapter 2.1.1). Not only are the branches of *Coprosma* thinner in diameter and in younger stages hairy, but the leaves are more

lanceolate, whereas *Corokia* has thick zigzag branches and spoon-shaped leaves. Small differences in morphological adaptations like this can have remarkable effects in physiological reactions. Jones & Rawson (1979) have shown that in addition to physiological processes being differently sensitive to leaf water deficits, plants are also differently sensitive to the rate of development of leaf water deficits. Studies have demonstrated the occurrence of osmotic adjustment due to solute accumulation in bulk leaf tissue in response to slowly developing leaf water deficits (Hsiao *et al*. 1976, Jones & Turner 1978). This suggested that this process may be responsible for the lower sensitivity of leaf conductance to leaf water potential in field-grown plants compared to small container-grown plants (Kanemasu & Tanner 1969, Thomas *et al*. 1976).

Water stress, like many other environmental and edaphic stresses, can predispose the primary photosynthetic reactions of chlorophyll-bearing tissues to damage by excess light. Drought avoidance is defined as the extent to which high plant water potentials are maintained in the presence of environmental drought (Hall & Schulze, 1980). Therefore, the findings in the water potential differences between given water availabilities and light levels as well as the comparison of divaricate and non-divaricate shrubs is continued with relation to the findings in the fluorescence measurements (Section 3.1), pigment and antioxidant concentrations (Section 3.2) as well as the response measured in the gas exchange of the leaves investigated (Chapter 4).

# 3. PHOTOCHEMISTRY

## 3.1 Fluorescence

To test the hypothesis that divaricates avoid photoinhibition by 'self-shading' their leaves, the $F_v/F_m$ ratios of divaricate leaves in the field, and divaricate and non-divaricate leaves in the glasshouse, were recorded during summer.

### 3.1.1 Introduction

During summer months illumination with natural sun light can cause light stress in plants, because it easily reaches over-saturating intensities. Unshaded and/ or unprotected plants show decreased rates of photosynthesis, accompanied by decreases in quantum yield (Ögren & Rosenqvist, 1991). Exposure over longer periods of time can cause serious damage, which can lead to the death of the leaf. This phenomenon of stress is called photoinhibition and can be found in sun plants as well as in shade plants, which suddenly experience increases in sunlight (Chow, 1994; Lovelock *et al.*, 1994). Water stress also increases photoinhibition. Variable water potentials in plants influence their photosynthetic light reactions, leading to an inactivation of photosystem II (PSII) and therefore to a reduction in the ratio of variable fluorescence to maximum fluorescence ($F_v/F_m$) (Leipner, 2004).

Photoinhibiton is defined as a deterioration of the photosynthetic functioning of photosystem II due to excessive light (Osmond, 1994). Photoinhibition is the destructive consequence of oversaturation of the photosynthetic apparatus by light, caused by light absorption by chlorophyll. All stress factors which lead to the inhibition of the biochemical process of photosynthesis increase photoinhibition massively, for example stomatal closure during drought conditions or deactivation of enzymes by heat or cold (Mohr & Schopfer, 1995).

Direct photodamage to PSII or photoprotection can cause photoinhibition. Photoprotection is a diversion of excessive excitation energy away from PSII, with the excitation energy mostly being thermally dissipated (Osmond, 1994). If photochemistry and heat dissipation are low, chlorophyll fluorescence is high and

reflects changes in photochemical efficiency and heat dissipation (Fracheboud, 2001). Such fluorescence induction kinetics are used as an indicator of possible damage to or obstruction of, the whole photosynthesis apparatus by environmental stress (Ball *et al*., 1994).

Mechanism

Two mechanisms can attenuate the photoinhibitory decline in the potential quantum yield of the $CO_2$ uptake caused by a reduced photochemical efficiency in PSII. Firstly [I], over-excitation of PSII reaction centers can be prevented by reduced absorption or by additional thermal dissipation of excitation energy, in particular in alliance with an active xanthophyll cycle. Second [II], a cycle of inactive and damaged PSII reaction centers, which can be repaired and re-activated (Long *et al*., 1994).

**I.** Thermal energy dissipation from PSII antennae and/or reaction centers is the fastest response to excessive light in leaves, which coincides with the development of a trans-thylakoid ? pH gradient. A decline in quantum efficiency of PSII has also been associated with changes in the trans-thylakoid ? pH gradient. The mechanism itself is still uncertain, but it may include an aggregation of the light harvesting complexes of PSII (LHC II) to increase the heat dissipation of the antenna. The pH change might be caused by the reversible inactivation of PSII reaction centers, including a loss of calcium and increased thermal dissipation in the reaction centers (Long *et al*., 1994). Also it is possible that this may act synergistically, with an increase in activity of the xanthophyll cycle, with zeaxanthin increasingly dissipating heat (Long *et al*., 1994; Demmig *et al*., 1987). In this mechanism di-epoxide violaxanthin converts to epoxide-free zeaxanthin via mono-epoxide antheraxanthin using a pH optimum of 5.2 and a de-epoxidase transforming ascorbate to dehydroascorbate and oxidising glutathione. Zeaxanthin can be recycled via epoxidase and NADPH usage in the dark, with a pH optimum of 7.5 (Hager, 1975, 1980). Osmond (1994) refers to this mechanism as dynamic photoinhibition, which is only present when light is excessive. It potentially affects the carbon assimilation of the leaf.

**II.** PSII reaction centres are reversibly inactivated by overexcitation. Overexcitation also produces non-functional reaction centers with damaged D1 polypeptides, resulting from reversibly inactive PSII centers or from a pool of

functional complexes. Damaged D1 can be exchanged with newly synthesized D1 by a temperature-sensitive serine protease following the migration of the PSII complex into the stomatal non-appressed thylakoids. After restoration, the PSII reaction centres migrate back and regain photochemical competence. The amount of D1 damage, the migration rate of PSII reaction centres, the decomposition of damaged D1, and the rate of synthesis of new D1 determine the extent of photoinhibition (Long *et al.*, 1994).

D1 is situated in PSII, forming a heteromer with D2, and has a rapid turnover, even in plants growing under low light. It holds donor and acceptor sites for the electron transport and has a close proximity to oxidants, which are able to form highly reactive radicals. An example is the quinone acceptor $Q_A$ in its reduced state, which transfers $P_{680}$ in a triplet excited state ($^3Chl^*$). $P_{680}$ can generate singlet oxygen radicals ($^1O_2$) by reaction with molecular oxygen, inhibiting the electron transport from the water-splitting complex to the $P_{680}$ reaction centers and therefore inactivating the electron transport. The conversion of inactive PSII reaction centers to non-functional PSII reaction centers depends on the presence of molecular oxygen, inhibiting the donation of electrons from the water-splitting complex to the PSII reaction centers, causing polypeptide damage. Inactive PSII can recover without the replacement of D1, following the accumulation of photoinhibited reaction centers in the thylakoids. Inactive PSII reaction centers also are potential quenchers of excessive light by converting absorbed energy into thermal dissipation (Long *et al.*, 1994). Here, D1 synthesis is required, except under low temperatures. The mechanism is seen as a possible protection system for remaining active PSII because of increased thermal dissipation. Long *et al.* (1994) defines it as stress-induced photoinhibition, which exhibits more prolonged reductions in quantum efficiency.

Osmond (1994) and Osmond & Grace (1995) define two types of photoinhibition: dynamic and chronic. Dynamic photoinhibition occurs in sun plants and is represented by a trans-thylakoid ? pH gradient, and high xanthophyll cycle activity and heat dissipation, as described above. It is marked by a decreased $F_v/F_m$ ratio and a decrease in $F_0$ (ground fluorescence). There are no changes in the potential quantum yield of $CO_2$. Öquist & Malmberg (1989) and Öquist *et al.* (1992) define this as stress-induced photoinhibition. Chronic photoinhibition occurs in shade plants when exposed to high

light and is defined by D1 damage and thermal dissipation. An increase in $F_v/F_m$ with a decrease in $F_0$ and a declining quantum yield of $CO_2$ occurs during this process (Osmond, 1994).

Photoinhibition can occur from very high PFDs alone, or from lower PFDs in combination with other limiting conditions such as water stress or frost. A reduction in the fluorescence kinetics at midday is defined as diurnal photoinhibition, if a full recovery occurs within the same day. It is correlated with an increased accumulation of zeaxanthin and therefore increased heat dissipation (Long *et al.*, 1994).

Diurnal (diurnal decline in maximum quantum yield in $CO_2$ uptake with complete recovery in hours, Long *et al*., 1994) and stress-induced photoinhibition (long-term decline with days till recovery, Long *et al*., 1994) overlap in their implications. Although diurnal photoinhibition is a short-term effect, it can influence the fitness and survival of species in the long-term as shown by Ball *et al.* (1991) and Ball (1993). Raven's (1989, 1993) cost-benefit analysis estimates only little energetic investment is necessary in plants for protective mechanisms and repair of D1 compared to photoinhibitory effects on the plants. Therefore, species that have an increased capacity for D1 repair should have an increased carbon gain. Sun-grown species have high xanthophyll cycle activity to avoid damage to PSII and therefore costs of decreased potential carbon gain. Long *et al*. (1994) presents two experiments to support this. In the first example, *Zea* genotypes from higher altitudes possessed a greater resistance to chilling-dependent photoinhibition than genotypes from lower altitudes. The second example showed that *Cyperus longans* had higher rates of recovery in northern than southern habits.

Through avoidance and restriction of damage, the xanthophyll cycle is a substantial mechanism for preventing photoinhibition. Often, high xanthophyll cycle activity is coupled with an increased ability to synthesize D1 under high light. Shade plants have a low capacity to dissipate heat via the xanthophyll cycle. Also, D1 repair can decline under low temperatures. *Pinus sylvestris* needles have shown a complete loss of their photosynthetic activity without the loss of functional D1 polymers when temperatures were below freezing and a incomplete recovery in spring (Ottander & Öquist, 1991).

Fluorescence Parameters

During light absorption in chlorophyll, electrons are raised from their ground state to an excited state. The energy difference between the ground state and the first level of chlorophyll molecule excitation equates to the energy of a red light photon. Therefore, in the following de-excitation, a small amount of excitation energy, 3-5% in vivo, is dissipated as red fluorescence, which is an alternative pathway to prime photochemistry and heat dissipation (Lawlor, 1990). Due to the heat dissipation being coincident with the chlorophyll fluorescence, the wavelength shifts towards infrared - the so-called Stoke's shift (Bolhar-Nordenkampf & Öquist, 1993).

Kautsky & Hirsch (1934) were the first to expose dark-adapted leaves to UV-A or blue light, and record the emitted red fluorescence. They interpreted these fluorescence kinetics as the initialisation of photosynthesis. The mechanism for this is the absorption of light by chlorophyll in the reaction centres, where the primary photochemical phase of photosynthesis is initiated. Excess radiation is re-emitted as fluorescence or heat. Shade plants can use up to 97% of the absorbed photons in photochemical processes. The residual energy is dissipated as 2.5% heat and 0.5% red fluorescence. If the amount of radiation produced by fluorescence is high, the quantum gain of photosynthesis is small. Carbon fixation requires light-activation, whereas the electron transport starts in milliseconds upon illumination. Therefore, the fluorescence yield reaches a steady state value after a transient rise in the lag phase before carbon fixation starts (Kautsky & Hirsch, 1931). This phenomenon of the fluorescence induction kinetic is called the KAUTSKY- effect. It describes a characteristic change of the measurable signal of fluorescence, which appears if dark-adapted leaves are exposed to light. An analysis of this fluorescence provides an overview of the photosynthetic electron transport processes and an assessment of the instantaneous activity condition of the photosynthetic apparatus (Leipner, 2004).

One of the most widely used fluorescence parameters is the ratio of $F_v/F_{m,}$ measured during a dark to light transition in dark-adapted leaves (Ball *et al.*, 1994). The minimal fluorescence yield, $F_0$, is observed when all reaction centres are open, and the maximal fluorescence yield $F_m$, is observed when all reaction centres are closed. The difference between $F_0$ and $F_m$ is called the variable fluorescence $F_v$ (Bolhar-Nordenkampf & Öquist, 1993).

After an application of a saturating light pulse to a dark-adapted leaf, fluorescence rises from the ground state value ($F_0$) to a maximum value ($F_m$). The first electron acceptor of the photosystem II ($Q_A$) becomes fully reduced. To determine the maximum quantum efficiency of photosystem II, the ratio of $F_v/F_m=(F_m-F_0)/F_m$ is used. Maximum quantum gain ? $_{max,}$ or maximum efficiency of transport of the initiation energy from the antenna complexes in open PSII-reaction centres (RC), is calculated as ? $_{max}=F_v/F_m=(F_m-F_0)/F_m$ (Pörs *et al.*, 2001). Healthy plant material has a $F_v/F_m$ ratio close to 0.8, independent of the plant species investigated (Ball *et al.*, 1994). With an efficiency in photosynthesis at about 80%, the $F_v/F_m$ ratio for healthy unstressed plants rarely exceed 0.85 (Demmig & Björkman, 1987). Lower values indicate a damage of the photosystem II reaction centres, so called photoinhibition, which is found in plants under stress conditions. The repair system of PSII tolerates only a certain amount of inactive PSII. When this is exceeded an irreversible inhibition of PSII can be detected as a decrease in $F_v/F_m$ (Lovelock *et al.*, 1994). Usually, $F_m$ decreases and $F_0$ increases, caused by a parting of the LHC of PSII from the PSII core. Stress- induced damage to the D1 protein or reduced repair activity on the D1 protein reduce the $F_v/F_m$ ratio too. The repair of PSII RC is temperature-dependent, and D1 protein repair is light- enhanced (Long *et al.*, 1994).

As described above high light can damage the PSII reaction centers by inactivating the D1 protein during phosphorylation, resulting in its degradation, leaving the PSII center inactive. The dark fluorescence $F_0$ increases with increasing temperature exposure. The rate of energy trapped in PSII centers is reduced (Havaux, 1993), due to physical dissociation of the light harvesting complexes from the PSII core due to heat damage (Armond *et al.*, 1980). Exposure of shade-grown plants to full sunlight results in an abrupt decrease in the chlorophyll fluorescence parameter $F_v/F_m$, which is a useful indicator of photoinhibition. Additionally, sun leaves are more sensitive in their fluorescence kinetics to drought conditions than shaded leaves. The electron flux at the inner side of photosystem II, the water oxidation site, can be inhibited under water stress. The chlorophyll a- fluorescence decreases, because of the change in the redox-states of quencher Q. $F_m$ and $F_v$ are heavily quenched under water stress, and the activity of photosystem II can be deactivated (Ludlow & Powles, 1988).

Mooney *et al*. (1978) found that when the desert shrub, *Larrea divaricata*, was subjected to water stress, the photon yield was reduced by approximately the same degree as was the light-saturated photosynthetic rate. In contrast, well-watered *L. divaricata* plants used in experiments by Lovelock *et al*. (1994), showed no tendency towards photoinhibition following 3 weeks exposure to full sunlight. Demmig *et al*. (1987) linked increased zeaxanthin concentrations and increased fluorescence parameters, such as $F_v/F_m$, under high light influence on species of *Populus*, *Hedera* and *Monstera*. Zeaxanthin acts as a protector against excessive irradiation, which is thermally dissipated. In 1989, Demmig-Adams *et al*. reported midday depression in the rate of $CO_2$ assimilation in water-stressed leaves of a sclerophyllous shrub in summer. The chlorophyll fluorescence parameter $F_v/F_m$ was decreased, whereas the zeaxanthin concentration increased. Kitajima & Butler (1975) showed quenching in $F_0$ and $F_m$ coupled with increased thermal dissipation of excitation energy via chloroplast antennae. Zeaxanthin mediates this process through de-excitation of excited chlorophyll (Demmig-Adams, 1990).

The $F_v/F_m$ ratio represents the maximum quantum yield for PSII and increases with decreasing photochemistry (Cotton, 1998). Therefore, changes in $F_v/F_m$ are well correlated with changes in the quantum efficiencies of $CO_2$ fixation or $O_2$ evolution (Ögren & Sjörström, 1990), Lamontagne *et al*. (2000) found a close linear relationship between $F_v/F_m$ and the rate of $CO_2$ assimilation in the leaf. In the experiments of Ögren & Sjöström (1990) and Ögren & Rosenqvist (1991), photoinhibitory effects shown in gas exchange measurements and expressed by a decreased quantum yield of electron transport were compared to photoinhibitory effects shown in fluorescence measurements and therefore $F_v/F_m$ ratios. Their findings suggested less photoinhibition when measured by the $F_v/F_m$ readings than by the electron transport readings. The magnitude of photoinhibition depended strongly on the average air temperature. As $F_v/F_m$ can be measured much more rapidly and easily than quantum yields of $CO_2$ fixation or $O_2$ evolution, it has emerged as an important shorthand parameter for assessment of photoinhibition in leaves (Ball *et al.*, 1994). Environmental stresses affect chlorophyll fluorescence as well as the $CO_2$ uptake and photosynthetic rate in plants growing under water deficits (Björkman et al., 1981; Björkman & Powles, 1984) or variable temperatures (Ludlow & Powles, 1988).

Aim of the Study

Leaves of divaricate shrubs are thought to be self-shaded and water-conservative (Chapter 1). Therefore their fluorescence parameters, particularly $F_v/F_m$, were expected to be less influenced by lower water availabilities than the $F_v/F_m$ ratios of leaves of non-divaricate shrubs. As seen above, shade-adapted leaves are less sensitive in their photoinhibitory response to water deficits and the higher PFDs of summer. Therefore, I wished to determine if leaves of divaricate shrubs express less signs of photoinhibition than their non-divaricate congeners in stressful light and water treatments.

In the field experiment, $F_v/F_m$ was measured in November 2001 and January 2002 as well as in January 2003 and February/ March 2003. In each year, data for both months were compared against each other. As the summer progresses, light and water stress increase and I hypothesised that 'Month' would significantly influence the measurements, I also aimed to determine if there was a decrease in $F_v/F_m$ seen in the later months. In the field and in the glasshouse experiments, plants experienced different light and water availabilities. Increased light increases photoinhibition and therefore exposed leaves were hypothesised to show lower $F_v/F_m$ ratios than shaded or 'self-shaded' leaves. Therefore, leaves in the shade and divaricate leaves were examined to determine whether their $F_v/F_m$ ratios remained constant rather than show photoinhibitory effects. Water stress also decreases the $F_v/F_m$ ratio, well-watered plants and plants which use water conservatory display no or lesser decreases in the $F_v/F_m$ ratio. In the field and in the glasshouse, plants under good water availability and divaricate plants were assumed to have $F_v/F_m$ ratios close to 0.8, whereas plants under mild drought and non-divaricate plants in particular were hypothesised to show photoinhibitory effects. Significant interactions were hypothesised for light level* water availability in the field trial, as plants with exposed leaves and under mild drought were expected to have much lower $F_v/F_m$ ratios than shaded plants with a good water supply. It was also examined if it would be true for the plants in the glasshouse experiment. Additionally I predicted that in the glasshouse the interactions of light level* habit, water availability* habit and light level* water availability* habit would present significant differences. I hypothesised that divaricates in sun light or divaricates under water stressed conditions or in the combination of both treatments display higher $F_v/F_m$ ratios than their non-divaricate congeners.

### 3.1.2 Material and Methods

Experimental plants in field and glasshouse setups were given different light and water treatments (Section 2.1.2 and 2.1.3) and $F_v/F_m$ ratios of their leaves were determined during the summer in 2001/02 and 2002/03 in the field and in 2002 in the glasshouse (Section 3.1.2) predawn, noon and evening. As described above, the $F_v/F_m$ ratio can be used as a measure of photoinhibitory effects in leaves.

To estimate photoinhibition in divaricate and non-divaricate leaves grown under different water and light treatments (Section 2.1.2 and 2.1.3), the leaf chlorophyll fluorescence was measured in a quenching analysis of modulated fluorescence by the saturation pulse method. With a Portable Chlorophyll Fluorometer (Mini-Pam, Heinz Walz GmbH, Effeltrich, Germany) rapid light curves were estimated after leaves were dark adapted for twenty minutes (Fracheboud & Leipner, 2003) predawn, noon and in the evening. Measurements were taken on divaricate leaves in the field in 2001/02 and 2002/03 and divaricate and non-divaricate shrubs under controlled glasshouse conditions (Bolhar-Nordenkampf & Öquist 1993, Schreiber *et al*., 1994) in 2002 and 2003. $F_v/F_m$ was calculated by the Software WinControl V1.60 (Heinz Walz GmbH, Effeltrich, Germany).

### 3.1.3 Results

Cass 2001/02

At the field site in summer 2001/02 measurements of the $F_v/F_m$ ratio (Section 3.1.2) were combined with measurements of water potential (Section 2.2). Figure 3.1.1 shows the results for predawn, noon and evening measurements on leaves of *Corokia cotoneaster*, Figure 3.1.2 shows it for *Coprosma propinqua* in the given treatments (Section 2.1.2).

The ANOVA analysis for the $F_v/F_m$ ratios in the field experiment in summer 2001/02 displayed significant effects for genus predawn, noon and evening (Table A2.1). *C. cotoneaster* always showed slightly higher $F_v/F_m$ values than *C. propinqua* (Figure 3.1.1 and 3.1.2). Diurnal changes in the $F_v/F_m$ ratio were obvious in *C. cotoneaster* leaves in January and in *C. propinqua* leaves in November and January. These leaves showed high $F_v/F_m$ ratios predawn, followed by a midday depression and a recovery in $F_v/F_m$ towards the predawn values in the evening (Figure 3.1.1 and 3.1.2). Predawn $F_v/F_m$ was significantly influenced by water availability (Table A2.1). The plants on the N-facing slope had lower $F_v/F_m$ ratios than plants in the streambed, even though the plants on the N-facing slope showed less negative water potentials than the plants in the streambed (Section 2.2.3). The measurements at noon and in the evening also showed highly significant effects of light level (exposed interior, sun light or shaded) and of month (November 2001 versus January 2002) (Table A2.1). For the two genera (Figure 3.1.1 and 3.1.2), the highest $F_v/F_m$ ratios were found in shaded leaves, whereas most of the exposed interior leaves had low $F_v/F_m$ ratios. Leaves of shrubs under natural light showed intermediate $F_v/F_m$ ratios compared to shaded or exposed leaves. *C. cotoneaster* leaves displayed only minor differences in the $F_v/F_m$ ratio under different water availabilities, in contrast to *C. propinqua* which expressed lower $F_v/F_m$ ratios for plants on the N-facing slope (Figure 3.1.1 and 3.1.2).

These differences were consistent but small, possibly due to heavy rainfall during that particular summer (Section 2.2.4). Therefore, in the next summer the experiment was repeated.

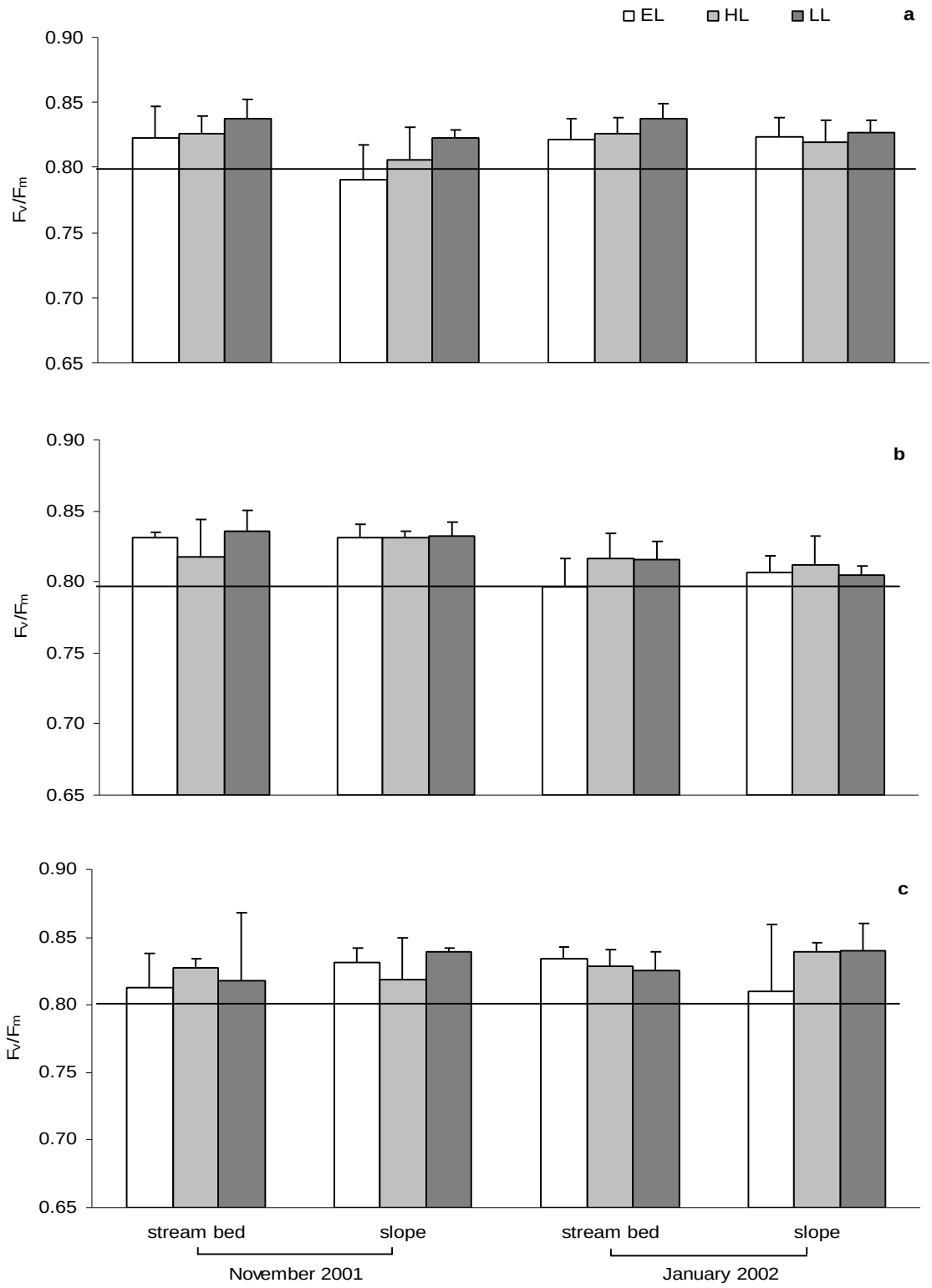



Figure 3.1.1: Photochemical efficiency of PS II ($F_v/F_m$) for *Corokia cotoneaster* shoots at (a) predawn, (b) noon and (c) evening in 2001/02 at Cass, grown in a streambed and on a N-facing slope and under 3 different light treatments (EL = inner canopy exposed, HL = sun light, LL = shaded) [n=4].

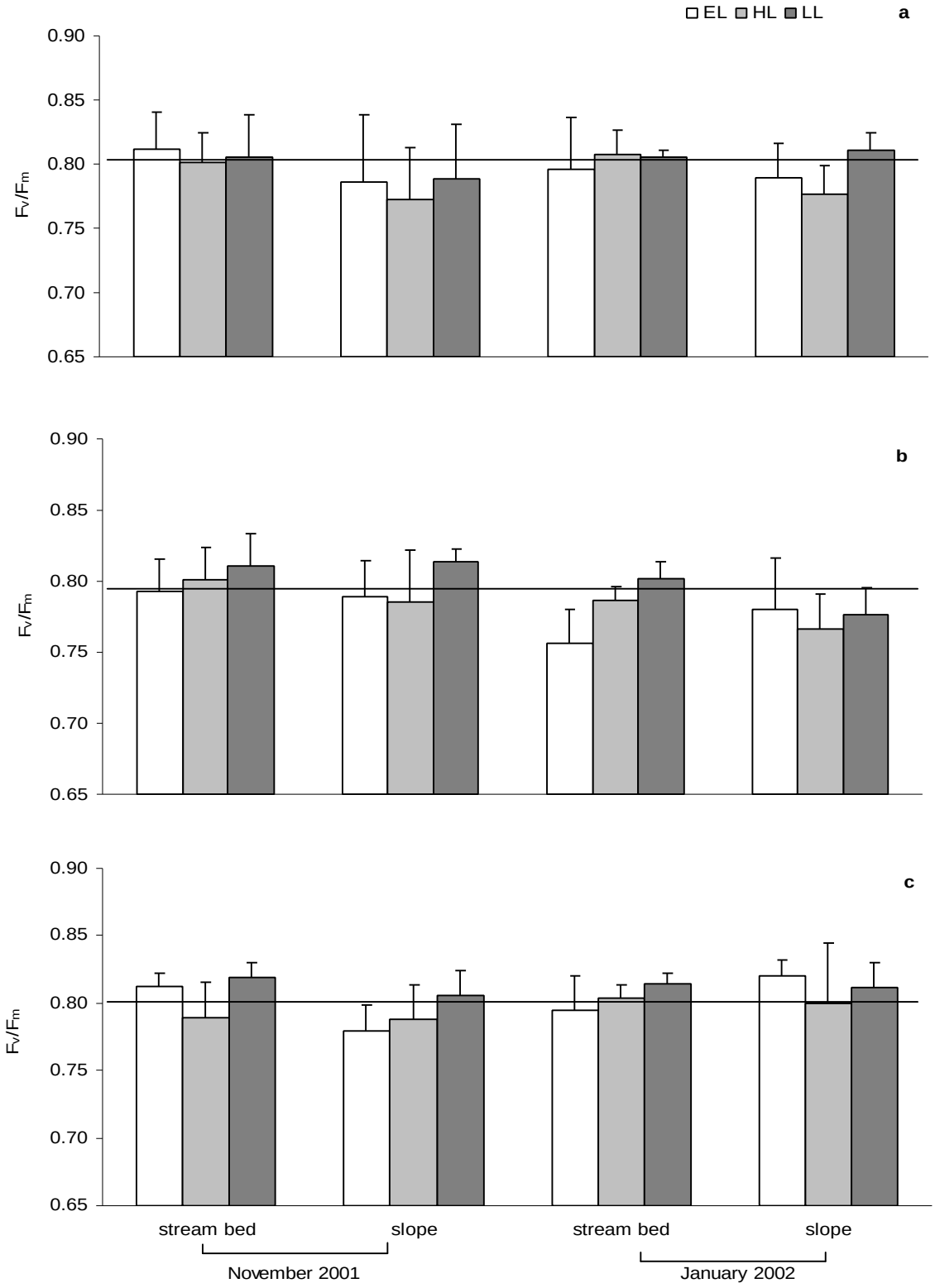



Figure 3.1.2: Photochemical efficiency of PS II ($F_v/F_m$) for *Coprosma propinqua* shoots at (a) predawn, (b) noon and (c) evening in 2001/02 at Cass, grown in a streambed and on a N-facing slope and under 3 different light treatments (EL = inner canopy exposed, HL = sun light, LL = shaded) [n=4].

Cass 2002/03

$F_v/F_m$ measurements were performed in January and February/ March of 2003. As found in the ANOVA analysis in summer 2001/02, genus had a significant to highly significant effect on the $F_v/F_m$ readings predawn, noon and in the evening (Table A2.2). In this drier summer (Section 2.1.4), *C. cotoneaster* and *C. propinqua* showed more prominent diurnal changes in the $F_v/F_m$ ratios in all treatments (Figure 3.1.3 and 3.1.4) than in the previous year. The measurements at noon and in the evening also displayed significant effects on light level and water availability (Table A2.2). Only shaded *C. propinqua* leaves did not show a big difference between the noon values versus predawn and evening (Figure 3.1.4). *C. propinqua* plants on the N-facing slope were not able to recover the predawn values of $F_v/F_m$ in the February/ March 2003 measurements (Figure 3.1.4). As seen in the previous year, the $F_v/F_m$ ratios were mostly lowest in exposed leaves and highest in shaded leaves of the two genera (Figure 3.1.3 and 3.1.4). Leaves in the streambed had lower $F_v/F_m$ ratios in January, but leaves on the N-facing slope had lower $F_v/F_m$ values in February/ March 2003 for all light treatments and in the two genera (Figure 3.1.3 and 3.1.4). A significant effect on month was only found for the measurements at noon (Table A2.2).

Comparing both field seasons

Comparing both seasons (Figure 3.1.1-3.1.4), the $F_v/F_m$ ratios were influenced by the treatments given to the plants as well as by genus. A difference between genera was not hypothesised, but *C. cotoneaster* displayed higher $F_v/F_m$ values than *C. propinqua* in spring and summer (Figure 3.1.1-3.1.4). As hypothesised, $F_v/F_m$ was slightly but significantly higher in both seasons for plants growing under the shade cloths (Table A2.1 and A2.2). At noon, plants with exposed leaves had the lowest $F_v/F_m$ values (Figure 3.1.1-3.1.4). Water availability had significant effects on predawn measurements in 2001/02 and on noon and evening measurements in 2002/03 (Table A2.1 and A2.2). Season of 2001/02 showed higher soil moisture and relative humidity than season 2002/03 (Figure 2.7 and 2.8). Predawn water potentials were similar in both measurement periods, but water potentials at noon were more negative in season 2002/03 (Section 2.2.3). Evening water potentials of the shoots in 2001/02 were slightly more negative than the ones in 2002/03. These differences in the moisture in the air and soil and therefore the water availability for

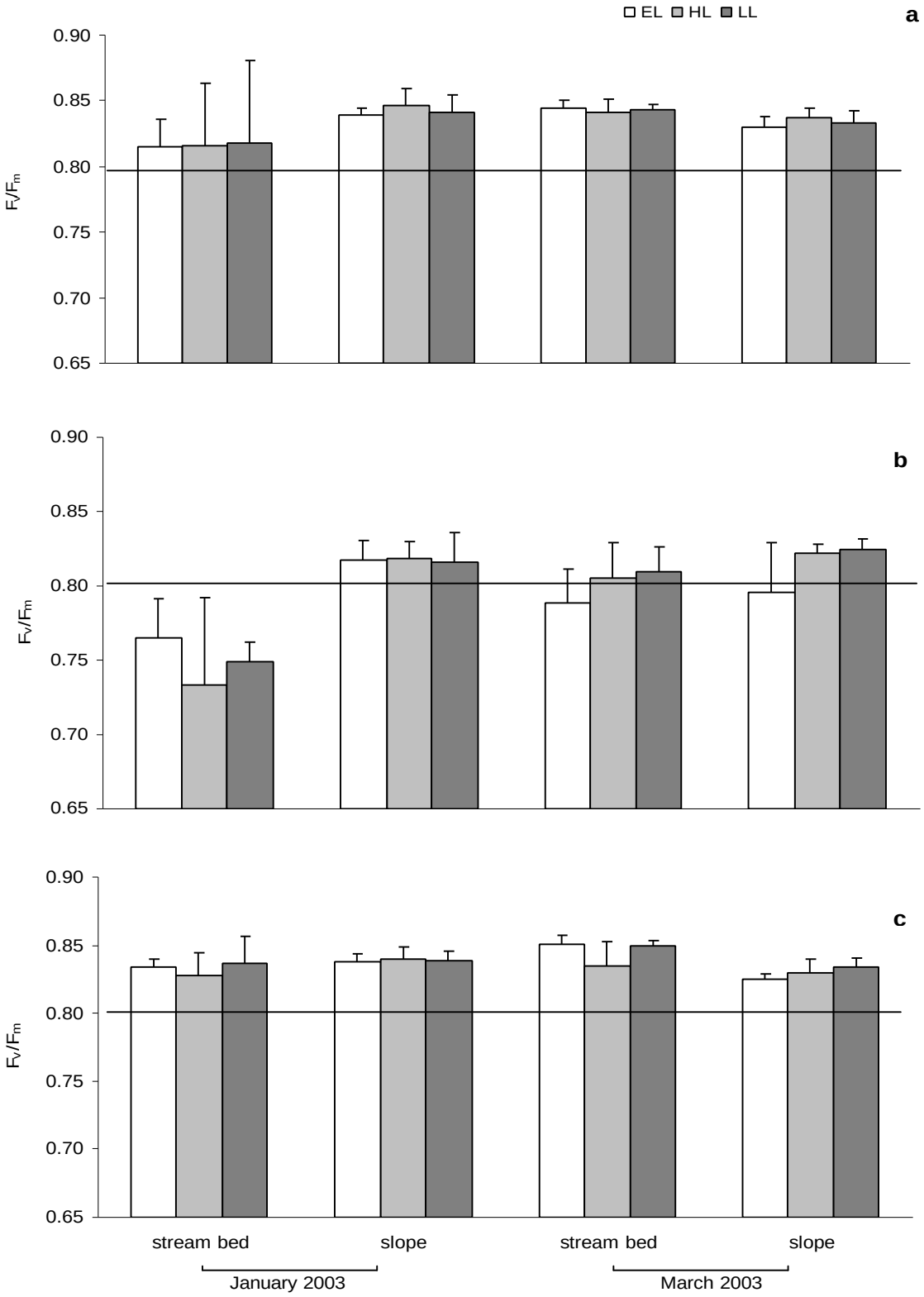



Figure 3.1.3: Photochemical efficiency of PSII ($F_v/F_m$) for *Corokia cotoneaster* at (a) predawn, (b) noon and (c) evening in 2002/03 at Cass, grown in a streambed and on a N-facing slope and in 3 different light treatments (EL = inner canopy exposed, HL = sun light, LL = shaded) [n=4].

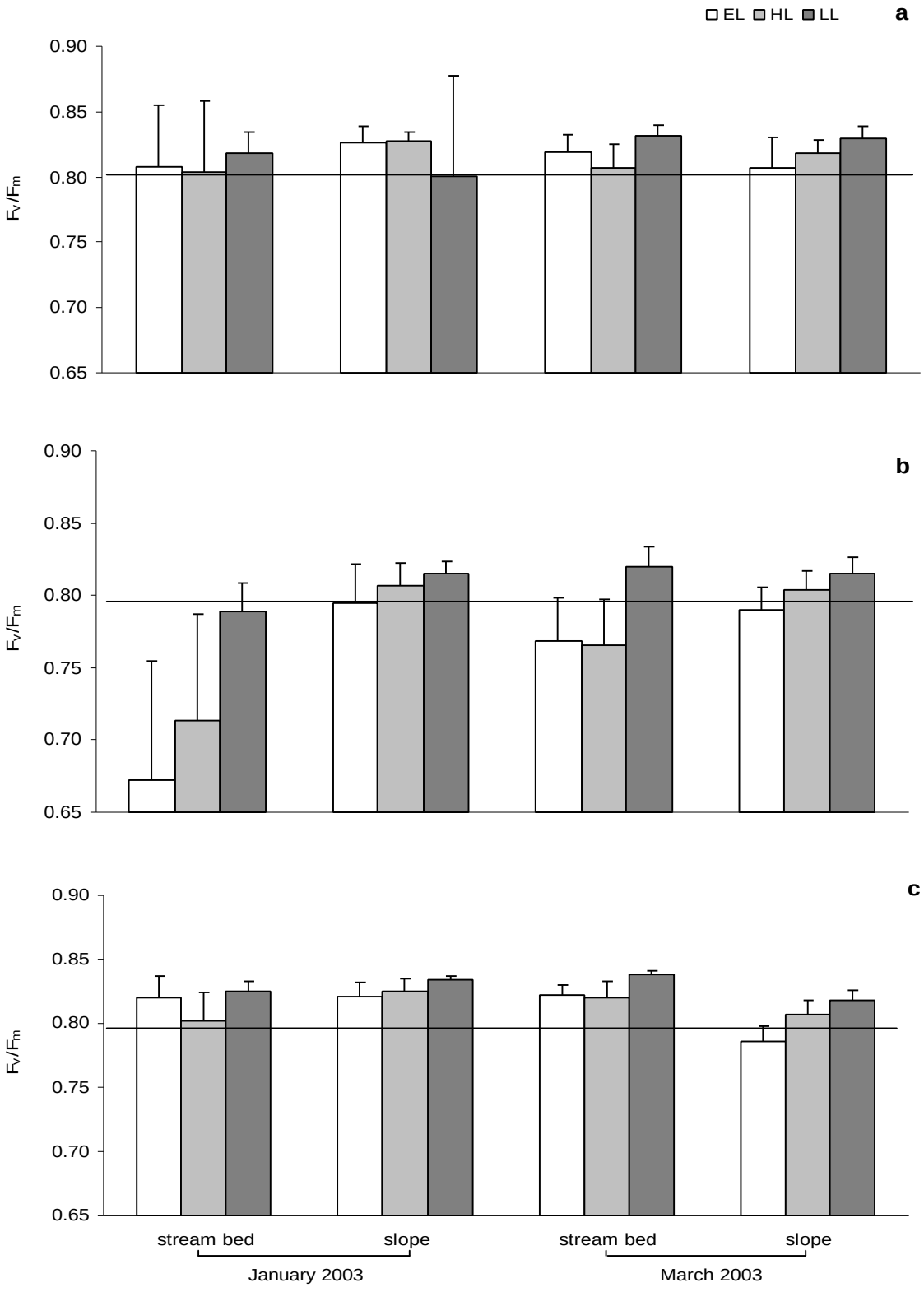



Figure 3.1.4: Photochemical efficiency of PSII ($F_v/F_m$) for *Coprosma propinqua* at (a) predawn, (b) noon and (c) evening in 2003 at Cass, grown in a streambed and on a N-facing slope and in 3 different light treatments (EL = inner canopy exposed, HL = sun light, LL = shaded) [n=4].

the plants could also have affected the $F_v/F_m$ ratios in the leaves and therefore could account for the different responses seen in 2001/02 and 2002/03.

Glasshouse 2002

Chlorophyll fluorescence was measured in the glasshouse in March and June in 2002. The level of drought stress was increased from March to June for those plants growing under suppressed water availability (Section 2.1.3). The $F_v/F_m$ ratio was determined at the same time as water potential was measured (Section 2.2). The results for the predawn, noon and evening measurements for divaricate and non-divaricate *Corokia* spp. are shown in Figure 3.1.5, and for divaricate and non-divaricate *Coprosma* spp. in Figure 3.1.6. Due to the increased drought treatment in June (Section 2.1.3), month exhibited a highly significant effect on $F_v/F_m$ ratios predawn, noon and in the evening (Table A2.3). Also, a significant effect of genus on $F_v/F_m$ was found for all measurements (Table A2.3). In the field and glasshouse measurements, a genus effect was not hypothesised, but a more prominent difference between the divaricate and non-divaricate growth form was to be tested. But in both experiments, *Corokia* showed significantly different responses than *Coprosma*.

In contrast to the field measurements, diurnal changes were not as prominent in the glasshouse plants. Mostly, the midday depression in the $F_v/F_m$ ratio were very small and some leaves even expressed slightly higher values at noon than predawn or in the evening (Figure 3.1.5 and 3.1.6). This is in contrast to my hypothesis that the change in light intensity would at least cause photoinhibitory effects at noon, particularly in non-divaricate leaves. In the evening, highly significant effects of habit were found (Table A2.3). This indicates different recovery rates from photoinhibitory effects in the different growth forms. A significant effect of genus* habit was found in the evening measurements, whereas significant interactions of water availability* genus* habit were displayed predawn, noon and in the evening (Table A2.3). When comparing divaricate and non-divaricate leaves of *Corokia*, different patterns in the fluorescence response to the treatments were displayed (Figure 3.1.5 and 3.1.6). Well-watered divaricate *Corokia* (*C. cotoneaster*) leaves had slightly lower $F_v/F_m$ ratios than nondivaricate *Corokia* (*C. buddleioides*) leaves, except for high light measurements in March (Figure 3.1.5). In contrast, I hypothesised that the divaricate growth form would display higher $F_v/F_m$ ratios (Section 3.1.1).

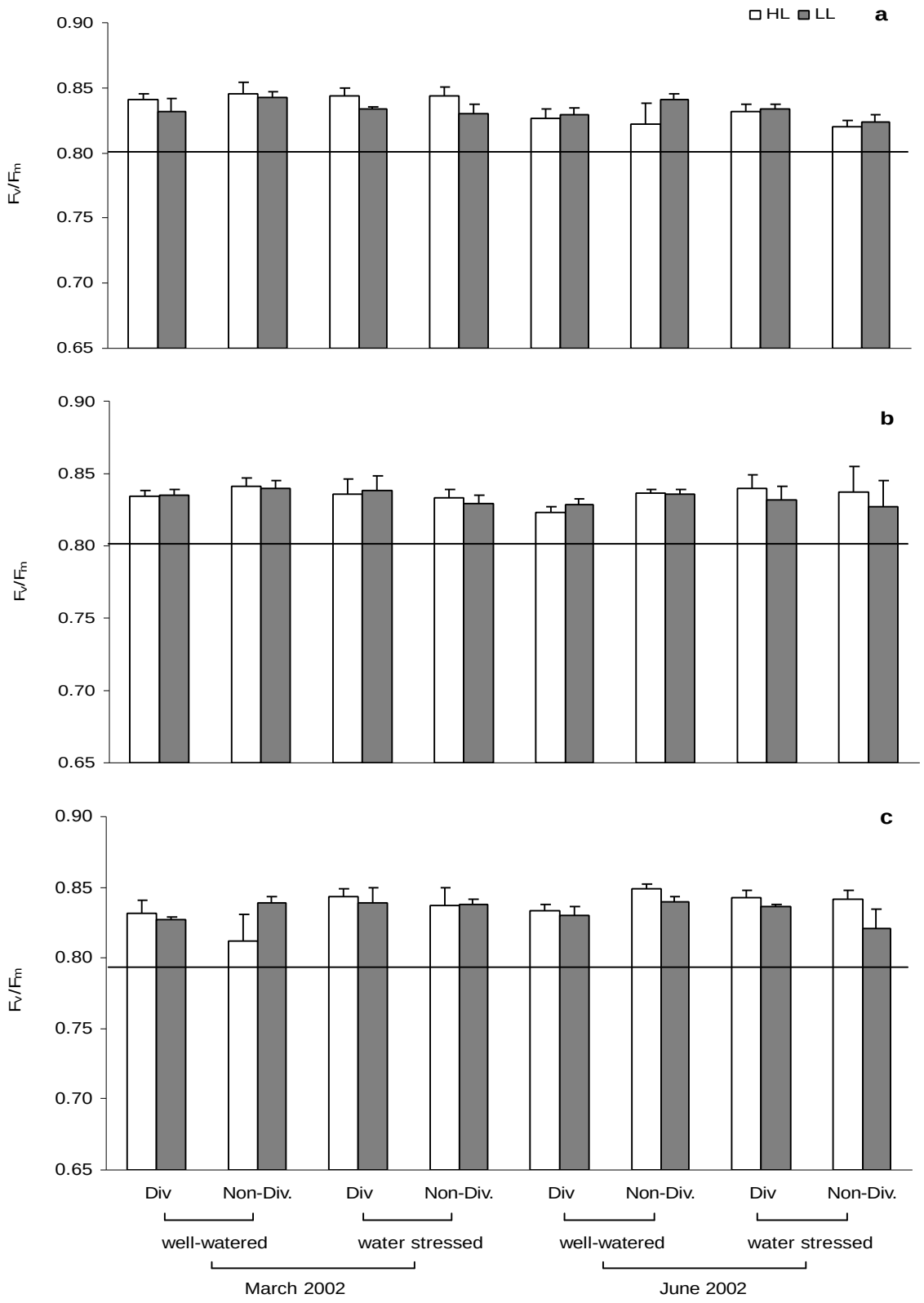



Figure 3.1.5: Photochemical efficiency of PS II ($F_v/F_m$) in the Glasshouse at (a) predawn, (b) noon and (c) evening in 2002 for *Corokia cotoneaster* (Div) and *Corokia buddleioides* (Non-Div) in well-watered and water stressed conditions and two different light treatments (HL = sun light, LL = shaded) [n=4].

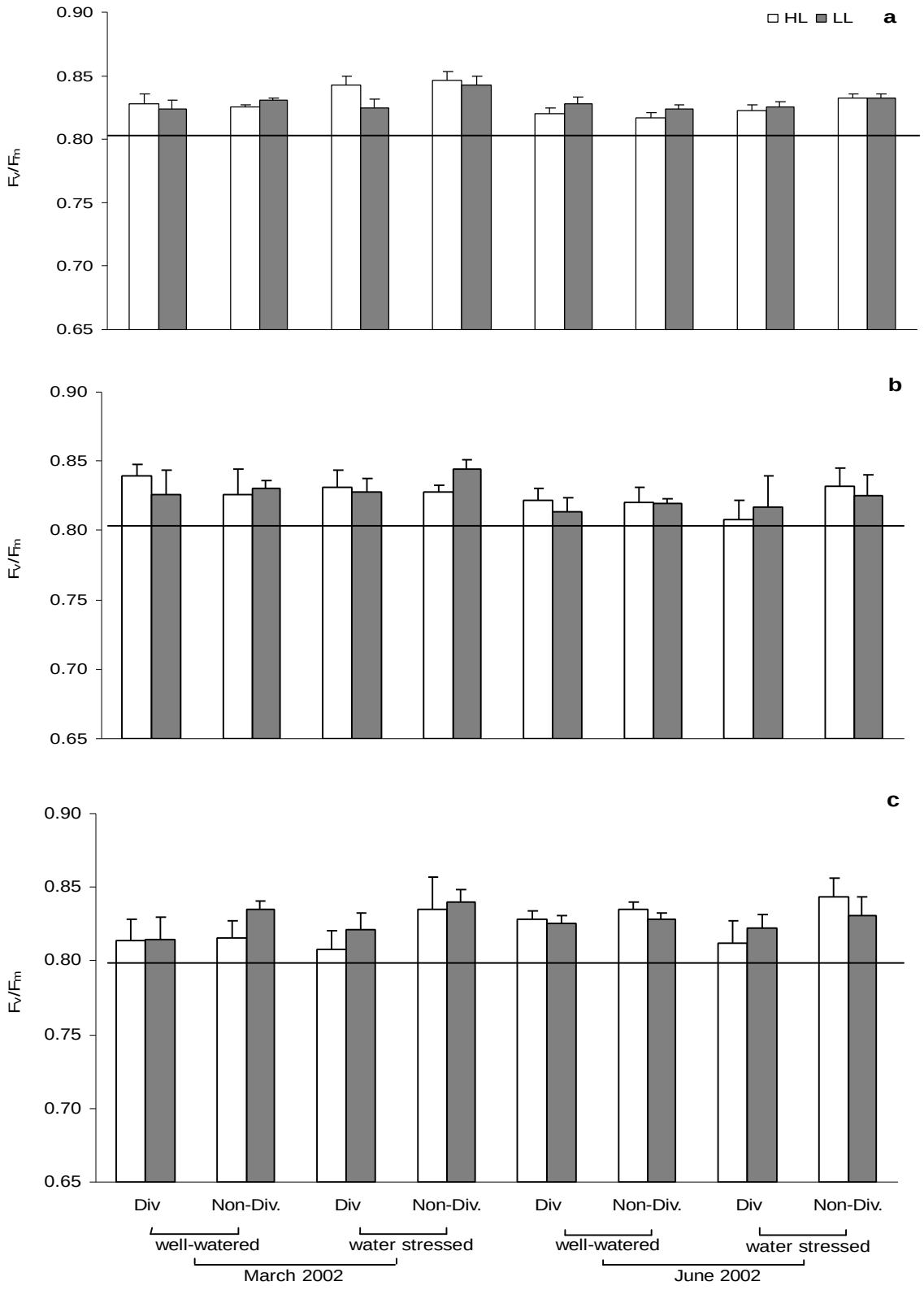



Figure 3.1.6: Photochemical efficiency of PS II ($F_v/F_m$) in the Glasshouse at (a) predawn, (b) noon and (c) evening in 2002 for *Coprosma propinqua* (Div) and *Coprosma robusta* (Non-Div) in well-watered and water stressed conditions and two different light treatments (HL = sun light, LL = shaded) [n=4].

Water stressed divaricate and non-divaricate *Corokia* leaves had higher $F_v/F_m$ values under high light than in the shade (Figure 3.1.5). I hypothesised in Section 3.1.1 that divaricate plants would use water more conservatively than their non-divaricate congeners and therefore display higher $F_v/F_m$ ratios. Only divaricate *Corokia* leaves under water stress generally expressed higher $F_v/F_m$ ratios than their non-divaricate congeners (Figure 3.1.5). These results were found predawn, noon and in the evening in March and June 2002 (Figure 3.1.5). Divaricate *Coprosma* leaves showed similar or lower $F_v/F_m$ ratios when compared with their non-divaricate congener leaves in the same water and light treatment in March and June (Figure 3.1.6), contrary to my hypothesis. In particular in March, shaded non-divaricate *Coprosma* leaves had higher $F_v/F_m$ ratios than divaricate *Coprosma* leaves (Figure 3.1.6). All these effects were displayed by small but consistent differences in the $F_v/F_m$ ratios.

### 3.1.4 Discussion

The fluorescence parameter and the $F_v/F_m$ ratio in particular, were observed for divaricate shrubs in the field (Section 2.1.2 and 3.1.2) as well as for divaricate and non-divaricate shrubs in the glasshouse (Section 2.1.3 and 3.1.2). Overall, $F_v/F_m$ showed very small but consistent treatment effects. Summarizing the results for $F_v/F_m$ measurements, it seems that the genus had a more important impact on $F_v/F_m$ than the growth habit, in contrast to my hypothesis (Section 3.1.1). Divaricate leaves were hypothesised to maintain high $F_v/F_m$ ratios during summer drought due to their self-shading and water conserving growth form. A less pronounced diurnal photoinhibition (Long *et al*., 1994) was also hypothesised to be seen in divaricate than non-divaricate leaves. In this study, diurnal changes in $F_v/F_m$ were only seen in the field experiment, where only divaricate leaves were measured. Even under increased drought stress (Section 2.2.3) the non-divaricate plants in the glasshouse did not show the hypothesised depression of $F_v/F_m$ during the course of the day.

Higher $F_v/F_m$ values for shaded than exposed leaves or leaves under natural light were found in the field in 2001/02 and 2002/03. In the glasshouse, divaricate and non-divaricate leaves of *Coprosma* showed lower $F_v/F_m$ ratios in the shade than under natural light. *Corokia* leaves in the glasshouse did not display a uniform response to shading. Sometimes the $F_v/F_m$ ratios were higher under natural light than in the shade. A reason for the different response to the light treatment could be the different age of the plants used in the field and in the glasshouse. The field plants were fully mature shrubs - grown to

an average height of 1.5 m - with their typical growth form fully developed. In the glasshouse, relative young plants had to be used and the divaricate shrubs had not yet developed their 'shelf-shading' growth form. Also, the glasshouse plants might been affected in their physiological reactions by high numbers of aphids (Section 2.1.3).

The water availability had significant effects on predawn $F_v/F_m$ in 2001/02 and on $F_v/F_m$ at noon and in the evening in 2002/03. In 2001/02, lower predawn values of $F_v/F_m$ were shown in plants on the N-facing slope than in the streambed. In contrast in 2002/03, the streambed plants displayed lower $F_v/F_m$ values than plants on the N-facing slope. The summer of 2001/02 was unusually wet, whereas the summer of 2002/03 was drier (Section 2.1.4). That could have affected not only the water potential measurements as seen in Section 2.2.4, but also the outcome of the fluorescence parameter. In the glasshouse, *Coprosma* plants under water stress showed higher predawn $F_v/F_m$ values than the well-watered plants. The drought stress applied in the field and in the glasshouse was not sufficient to cause wilting, but it was distinct enough from the environment of the well-watered plants to produce significant results in the interaction terms (water availability* genus* habit). Taken together, the field experiments of this study support the hypothesis that $F_v/F_m$ decreases under drought conditions and over-saturating light exposure. The comparison of the divaricate versus non-divaricate habit in the glasshouse was inconclusive. More detailed measurements in the glasshouse, comparing divaricate and non-divaricate species in light and drought conditions such as used in the field, generating the more negative water potentials that were found in the field might have better tested this hypothesis. Also, a comparison of divaricate and non-divaricate plants growing in their natural habitats and in more extreme climatic conditions, for example in salt marshland gradients, would benefit my study as it could present a more detailed picture of photoinhibitory effects and their recovery in divaricate and non-divaricate plants during the day, the season and during the year.

Howell *et al.* (2002) investigated frost- induced photoinhibition in divaricate *Aristotelia*, *Coprosma* and *Corokia* species in the same field area at Cass as my study. Inner parts of their shrubs were exposed to the outer micro-climate by clipping the outer branches away. The investigated leaves showed a reduction in $F_v/F_m$ during frost events, whereas neither the control leaves nor artificially shaded inner leaves of

clipped shrubs showed a similar reduction in $F_v/F_m$. Howell *et al*. (2002) also found that *Corokia* and *Aristotelia* exhibited a larger decline in $F_v/F_m$ values after frost events and slower recovery of $F_v/F_m$ values than *Coprosma*. The summer measurements in my study did not find similar results for drought-induced photoinhibition. In the field, *Coprosma propinqua* plants experienced similar midday depressions to *Corokia cotoneaster* in 2001/02 and 2002/03. Only in 2002/03, *C. propinqua* plants grown on the N-facing slope displayed a slower recovery rate for $F_v/F_m$ values than *C. propinqua* plants grown in the streambed or any *C. cotoneaster* plants. In the glasshouse, divaricate and non-divaricate leaves did not show signs of diurnal photoinhibition.

Howell *et al*. (2002) also found incomplete recovery from diurnal photoinhibition in *C. propinqua* after frost in their field experiment. Their investigations showed that cold-induced photoinhibition decreases in the interior of the shrubs as with increasing branch cover the PFDs decrease. My summer study only measured interior leaves of divaricate shrubs and not a gradient of decreasing PFDs towards the inner of the shrubs and the correlated decrease in $F_v/F_m$ ratio. Ögren (1988) found an increase of 10-20% of photoinhibition in sun-exposed willow leaves, when comparing shaded, peripheral and sun exposed positions. He estimated that willow leaves are affected by photoinhibition for up to a third of the days of the growing season and recovery periods can last up to 6 hours after a diurnal decline in $F_v/F_m$. In my study, the evening measurements, which were hypothesised to show a recovery from lower midday $F_v/F_m$ values towards the higher predawn values, were recorded approximately eight hours after the midday measurements. This was assumed to be a sufficient time period, but it is possible that *C. propinqua* leaves require a longer recovery period. In a laboratory study Ögren & Rosenqvist (1991) compared the reaction of several willow species to moderate and high light. They found lower photoinhibition in sun-leaves as well as higher photochemical quenching and faster recovery periods for given stages of photoinhibition. The amount of photoinhibiton was inversely related to the amount of photochemical quenching.

The same species used in the investigations of Howell *et al*. (2002) showed predawn cold-induced photoinhibition, but that study did not investigate the diurnal course of $F_v/F_m$. Judging by the relative decreases seen in $F_v/F_m$, these divaricate plants seem to

be more influenced by frost events in winter than mild drought in summer. It would be worth investigating further by comparing these results to $F_v/F_m$ data of divaricate and non-divaricate plants from higher altitudes as well as coastline or marshland conditions throughout the year. It is possible that divaricate and non-divaricate plants experience photoinhibitory events under different climatic conditions and in different seasons, which could influence their survival and distribution in certain habitats.

Lovelock *et al*. (1994) exposed shade-grown rainforest species to full sunlight and measured abrupt decreases in $F_v/F_m$. In their field study, shade-grown species expressed higher amounts of photoinhibition after exposure to full sunlight than species which inhabit unshaded forest gaps. The originally shade-grown species were found with $F_v/F_m$ values below 0.65 at the midday depression whereas the more sun-tolerating species ranged between 0.64 and 0.75. Plants in shade had $F_v/F_m$ values above 0.76. Recovery to the original midday $F_v/F_m$ values over 0.75 was accomplished by most species after 20 days in the new light environment. Interestingly, well-watered plants did not show declines in their chlorophyll fluorescence after they were moved to the new environment. Lovelock *et al*. (1994) investigated other environmental stress factors to determine the influence of photoinhibition on mortality of seedlings. Species growing in rainforest gaps responded to higher light levels by altering their leaf angles, counterpart species growing in the shade did not show this behaviour. The change of leaf orientation may also have positively influenced the water use efficiency of photosynthesis, and increasing maximum photosynthetic rates. *Alocasia macrorrhiza* plants, transferred from the shade to full sun positions, were able to adapt their photosynthetic capacity to the new environment, but full acclimation was only accomplished after new leaves developed (Sims & Pearcy, 1991). My study found similar $F_v/F_m$ values for the shaded and 'self-shaded' leaves of divaricate shrubs in the field and glasshouse experiments as seen in Lovelock *et al*. (1994). Exposed leaves of divaricate shrubs had lower $F_v/F_m$ values independent of water availability. The differences in $F_v/F_m$ between divaricate and non-divaricate leaves in the glasshouse were not as pronounced as hypothesised and surprisingly, genus had a statistically influence on $F_v/F_m$. As discussed before, it could be related to the young age of these shrubs, the sheltered environment of the glasshouse or the infestation with herbivorous insects, such as scale insects (Section 2.1.3).

Holly *et al.* (1994) investigated cold-induced photoinhibition on *Eucalyptus* seedlings. The seedlings grew under different shelters, which provided either different percentages of shade or a rise above ambient air temperature. All seedlings exhibited a predawn $F_v/F_m$ depression during winter, the extent depending on the shade provided, and recovery in spring. Unsheltered plants expressed the lowest $F_v/F_m$ values around 0.48, those with shade cloth shelters varied between 0.58 and 0.77. Different growth parameter were measured and related to the treatments given. Shade cloth reducing the PFD by 50% gave the highest protection from cold-induced photoinhibition and the greatest stem elongation. The plants in this summer study were either unshaded, exposed or shaded by 25% via shade cloth (Section 2.1.2 and 2.1.3). In the field, the lowest $F_v/F_m$ values of 0.67 were found in exposed leaves of *Corokia* shrubs in the streambed in January 2002/03. Shaded leaves had $F_v/F_m$ values of 0.75 to 0.84 during the midday depression. Shading the plants to 25% might have been not sufficient to see big enough differences compared to unshaded plants, as most plants are over-saturated with sunlight at midday in summer (Horn, 1971). Howell *et al*. (2002) used 32% shading and the $F_v/F_m$ values of those shaded *Corokia* and *Coprosma* leaves were below 0.7. Exposed leaves of these species were lower than 0.6 after frost events in winter 1998.

The $F_v/F_m$ ratio can be reduced either by an increase in $F_0$, which indicates an inactivation of PSII, or a decrease in $F_m$, indicating increased non-photochemical quenching with the xanthophyll cycle involved (Laing *et al*., 1995). Therefore, in this study the two genera probably possess different recovery rates for inactivated PSII or different capacities for non-photochemical quenching. Leaves grown under different light conditions vary in their composition of photoprotective pigments, particularly xanthophyll cycle pigments. As seen in Section 3.1.1, zeaxanthin can increase its heat dissipation under high light conditions and prevent damaging effects of photoinhibitory events. Differences in photoprotective pigments are presented in Section 3.2 and the interaction of non-photochemical quenching and the contents of xanthophylls cycle pigments will be discussed in Chapter 5.

## 3.2 Pigment and a-Tocopherol Composition

Excessive radiation loads and water stress lead to the plant increasing photoprotective pigment and antioxidant levels in leaves. The divaricate growth form was hypothesised to reduce the influence of high light and drought. This part of my study tested the hypothesis that divaricate leaves have lower concentrations of photoprotective pigments and a-tocopherol than their non-divaricate congeners.

### 3.2.1 Introduction

Plants respond to a wide range of biotic and abiotic stress factors. Abiotic stresses like high light loads, heat and drought reduce plant growth and productivity (Lawlor, 2002). $CO_2$ assimilation can be reduced because of reductions in stomatal conductance as well as in concentrations and activity of enzymes of the photosynthetic carbon reduction cycle under adverse conditions (Cornic, 2000; Parry *et al*., 2002). Concentrations of photo-protective pigments and a-tocopherol increase in leaves with increased sun exposure, but also with an increase in other stress factors plants are exposed to, such as heat, drought or frost. Also, combined stress factors like heat, high light and drought can trigger losses of chlorophyll in chloroplasts (Wingler *et al*., 1999; Havaux & Tardy, 1999). This disrupts the absorption of light and thermal dissipation of heat, which normally protects the photosynthetic apparatus (Demming-Adams & Adams, 1996). High light loads stimulate the accumulation of photo-protective pigments, such as antheraxanthin, zeaxanthin or ß-carotene, and antioxidants, such as a-tocopherol in leaves (Foyer *et al.*, 2001). Tausz *et al.* (2001) investigated a multitude of stresses and the complex biochemical responses of 12 variables of *Pinus ponderosa* trees, growing naturally under different levels of drought and altitude as well as human-made pollution. Needles of sun and shade positions from the year of the study were compared with leaves of the previous year. Summarizing the analysis in four different components, they were able to indicate which variables of stress were responsible for high antioxidant defences or low amounts of chlorophyll.

Leaves in Sun and Shade

Schulze *et al*. (2004) described that both within a plant and between plants of different light environments sun and shade leaves are formed. Leaves in sunny conditions have large mesophyll cells with thick cell walls and the number of cell layers is up to 3-fold that of shade leaves. Chloroplasts in sun leaves have a lower number of light harvesting complexes (chlorophyll- protein complexes in the antenna) per electron transport chain, but a high number of acceptors for the electron transport. The amount of plastoquinone is especially high in sun leaves and therefore the electron transport is up to 30-times faster than in shade leaves (Lawlor, 1990). Those sun-exposed leaves show high concentrations of cytochrome *b*/*f*-complexes, ATP-synthase, plastocyanins, ferredoxins and carbon-fixing enzymes (Anderson *et al*., 1995). Shade leaves will show a reduced number of mesophyll layers and lower chlorophyll content per leaf area (Lichtenthaler *et al*., 1981). The efficiency of light absorption under low light conditions is enhanced by an increased size of the antenna system. The amount of chlorophyll a and chlorophyll b per chloroplast volume is four to five times higher than in sun leaves, the number of light harvest complexes (LHC) is higher, whereas the number of photosystem I and II is lower (Lichtenthaler *et al*., 1981).

Changes in light conditions, cold stress and increasing age of leaves drive changes in the contents of pigments and antioxidants (Garcia-Plazaola *et al*., 1999a+b, 2000). Constant shade will change leaf anatomy, morphology and physiology compared to leaves growing under sunny conditions. For example, sun-adapted beech leaves show high amounts of ascorbate, tocopherol, glutathione, ß-carotene and xanthophyll cycle pigments (Garcia-Plazaola & Becerril, 2000). Tausz *et al*. (2003) showed that the biochemical analysis of photoprotective pigments and antioxidants is a successful tool to investigate stress factors that plants are exposed to in their habitats. To cope with a high number of samples at satisfying high accuracy, the analysis via HPLC system was recommended. A higher level of non-photochemical quenching ($q_{NP}$) is achieved by an up to 12-fold greater increase in the de-epoxidation status in sun leaves, which is the ratio between de-epoxidised and epoxidised xanthophyll pigments of the violaxanthin cycle [(antheraxanthin + zeaxanthin) / (violaxanthin + antheraxanthin + zeaxanthin)]. Also the chlorophyll a:b ratio is higher, which indicates a higher ratio of PS I to PSII or a lower ratio of LHC to reaction centres (RC). In contrast, shade leaves have higher photosynthetic efficiencies Garcia-Plazaola & Becerril, 2000).

Oxidative Stress

Chloroplasts become damaged if excessive excitation energy irradiates leaves and the excitation energy is higher than the requirement for the photosynthetic metabolism to produce NADPH + $H^+$ and ATP. If many chlorophyll molecules surrounding the reaction centers are in an excited state, more chlorophyll in a triplet state is produced. The excitation energy can be carried to oxygen and singlet oxygen is produced. This is highly reactive and oxidizes organic molecules. Damage to proteins, lipids and pigments can result, which decreases photosynthetic capacity and causes photo-bleaching. Reactive anions evolve with excessive excitation, if there is not an adequate amount of $NADP^+$ to redirect them (non-cyclic phosphorylation; Arnon *et al.*, 1957). Excessive electrons are transported from photosystem I to oxygen; ATP and $NAPDH^+$ are produced (Richter, 1998). When the non-cyclic electron flow is interrupted, electrons will only be transferred from PSI over cytochrome $b_6$ back to plastoquinone (cyclic phosphorylation; Arnon *et al.*, 1954). This process is combined with ATP synthesis only as plastoquinone transports protons into the thylakoid lumen. Non-cyclic and cyclic phosphorylations probably do not occur independently. The pseudo-cyclic phosphorylation uses both photosystems to transfer electrons to molecular oxygen without redirecting it by $NADP^+$ (Allen, 2002).

Environmental stresses such as high air temperatures, high PFD and low rainfall can result in an increase of the amount of activated oxygen species (AOS) and therefore oxidative stress. Under these conditions antioxidative defences and photo-protection mechanisms are needed in plants (Smirnoff, 1993; Pastori & Foyer, 2002). In phases where water is limited, an increased amount and activity of antioxidants is recognisable due to the stress-induced higher accumulation of active oxygen species (Pastori *et al.*, 2000).

Although AOS are important for intra- and inter-cellular signalling (Foyer & Noctor, 1999), an accumulation can cause damage, for example in the chloroplast membrane (Asada, 1999). Chloroplasts are protected from oxidative damage by the mechanisms of the xanthophyll cycle, photorespiration and changes in metabolic activities (Demmig-Adams, 1996; Osmond *et al.*, 1997), as well as by enzymatic and non-enzymatic antioxidants (Smirnoff, 1995; Foyer *et al.*, 1994; Asada 1999). In particular

tocopherols and carotenoids play an important role in antioxidative protection (Havaux, 1998; Munné-Bosch & Alegre, 2002).

Pigment Composition

Bungard *et al*. (1997) showed higher concentrations of xanthophyll cycle pigments, ß-carotene and lutein on a chlorophyll basis in leaves of *Clematis vitalba*, grown under high light. The $F_v/F_m$ ratio was slightly lower in those sun leaves. Neither the amount of photosynthetically active pigments nor the $F_v/F_m$ ratio was influenced by nitrogen supply. Section 3.1.1 describes the definition and mechanism of Osmond's (1994) so-called dynamic photoinhibition and preventive mechanisms in leaves. Carotenoids and xanthoplyll cycle pigments in particular are able to vary the amount of thermal dissipation and to adapt synergistically the activity of the xanthophyll cycle to existing light conditions. Under high light, less excitation energy is absorbed and excessive excitation energy is dissipated as heat via increased aggregations of LHCs in the antenna (Osmond, 1994). This process is related to changes in the trans-thylakoid pH gradient. A decline in quantum efficiency of PSII during high light events is also related to trans-thylakoid pH changes. The xanthophyll cycle activity is associated with an increased ability to synthesise D1 (polypeptide, integrated in membrane and with chinone acceptor centre $Q_B$) under high light, which is involved in the recycling of inactivated PSII proteins (Osmond *et al*., 1999).

Carotenoids are essential components of all pigment-protein complexes in the photosynthetic apparatus. They are involved in light harvesting and photo-oxidation as well as structuring in antenna and reaction centres (Jahns *et al*., 1998). Carotenoids also counteract against oxidative stress in plastids by reacting with triple oxygen. In the core complexes in photosystem II, ß-carotene quenches singlet oxygen and chlorophylls with excessive excitation energy (Young *et al*., 1997). During the reactions of carotenoids with these reactive oxygen species triplet carotenoids are formed. The excessive excitation energy of the reactive oxygen species and excited chlorophyll are passed to the carotenoids via p-electron system. That is only possible if the excited chlorophyll molecules are very close to the carotenoids. Triplet carotenoid dissipates the excessive energy via heat dissipation and returns to ground energy state. Only molecules with 9 or more double bonds are able to achieve that transformation (Lawlor, 1990). In the xanthophyll cycle, and with a high proton

gradient, plants are able to adapt to high light conditions for short times. Zeaxanthin is synthesised from violaxanthin via the reaction of a de-epoxidase in the lumen and under oxidation of ascorbate. The de-epoxidase enzyme is situated in the thylakoid lumen and activated by light and low pH (Asada, 1999). Violaxanthin is regenerated via zeaxanthin-epoxidase under lower PFDs and in the presence of oxygen and NADPH (Demmig-Adams *et al*., 1996). Therefore, this reaction cycle regulates the amount of NADPH and also the redox state of the chloroplasts via epoxidation (Krinksy, 1978).

Antioxidant Composition

As well as carotenoids, antioxidants prevent membranes and therefore plastids from light-induced damage. Tappel (1962) and Kunert & Ederer (1985) localised the highest concentrations of hydrophilic and lipophilic antioxidants in chloroplasts. There, those components inhibit or reduce damage by reactive oxygen species. In the stroma, hydrophilic antioxidants, e.g. ascorbate and glutathione, react with reactive oxygen species (Polle & Rennenberg, 1994). Tocopherols and carotenoids are lipophilic antioxidants which prevent damage to the thylakoid membrane. Additional functions of tocopherols are maintaining membrane stability and to some extent, participation in intracellular signalling and in the cyclic electron transport around photosystem II (Munné-Bosch & Falk, 2004).

Tocopherols are phenolic substances, which are synthesized in plastids of higher plants. The most common and strongest anti-oxidant tocopherol is a-tocopherol (Chevolleau *et al*., 1993). The highest concentration of a-tocopherol is found in the envelopes of chloroplasts and the osmiophilic plastoglobuli in the stroma of plastids. It is an effective protection against peroxidation *in vivo* and operates synergistically with vitamin C (Kunert & Ederer, 1985).

a-Tocopherol has four major functions, according to Smirnoff (1995): (1) it protects against singlet oxygen, superoxides and hydroxyl radicals, (2) it physically quenches singlet oxygen during energy transfer, (3) it is involved in protection reactions against lipid oxidation via reduction of lipid radicals, and (4) it stabilizes membranes biophysically via the binding of poly-unsaturated fatty acids. a-tocopherol is composed of a chinon-like circlet with a lipophil prenyl chain on one side. The circlet

becomes oxidized by radical oxygen, and the regeneration occurs through a reaction with ascorbate (Asada, 1996). With the prenyl chain on its side it is able to tie itself into lipid membranes and therefore protects thylakoid membranes from oxidation and damage (Polle & Rennenberg, 1994).

But tocopherol seems not only to be involved in antioxidant defence mechanisms, which can affect photosynthesis. Research by Munné-Bosch & Alegre (2002, 2003) and Munné-Bosch (2005) indicates a regulative role for jasmonic acid (growth inhibitor, senescence promoter) in leaves, and therefore in plant development and response. By regulation of lipid peroxidation, a-tocopherol regulates the amounts of lipid hydroperoxides, and therefore the jasmonic acid synthesis which depends on them (Schaller, 2001). Munné-Bosch & Peñuelas (2003) showed a dependency between concentrations of salicylic acid and a-tocopherol in water-limited plants. That implies that regulatory elements and antioxidants adjust the redox state of chloroplasts and therefore the whole cell depending on environmental stress factors (Munné-Bosch & Falk, 2004).

Schupp & Rennenberg (1988) investigated diurnal changes in the glutathione (antioxidant in chloroplasts) contents of spruce needles (*Picea abies* L.). They observed light-dependent responses with the highest concentrations at noon. Increasing concentrations were found with PFDs as low as 100 µmol $m^{-2}$ $s^{-1}$. These diurnal changes were observed on plants growing in maximum day temperatures of either +22 ºC or -4.5 ºC. Similar diurnal light-dependent changes were observed for ascorbate (antioxidant, in redoxreaction with glutathione in chloroplast to reduce reactive oxygen species), confirmed by Wildi & Lütz (1996). Light- and/ or temperature- dependent diurnal rhythms were observed for ascorbic acid, glutathione, photo-protective pigments and, in some cases, tocopherols. Concentrations of ascorbic acid were lowest when temperature were lowest (Schupp & Rennenberg, 1988). For a-tocopherol a day and night rhythm was found, although the authors were not able to confirm whether it was light- or temperature-dependent. That might be due to the rapid turnovers of a-tocopherol in the plants, which are very sensitive to slight environmental changes. Wildi & Lütz (1996) found the total amounts of antioxidants increased as altitude increased and therefore chilling stress, short vegetation period and high irradiation increased too. Polle & Rennenberg (1992) also found altitude-

dependent increases in ascorbate and glutathione as well as seasonal and age-dependent changes in those compounds. Munné-Bosch & Alegre (2003) compared the contents of antioxidative operating carnosic acid in combination to the amounts of a-tocopherol and ascorbate in rosemary, stage and carnosic acid-free lemon balm. Under drought stress, all species had increased amounts of a-tocopherol and ascorbate, with lemon balm chloroplasts reaching the highest values.

Drought Stress

Not only high light loads and high temperatures lead to an increase in active oxygen species and therefore oxidative stress. Drought stress is also very important in this regard. Photoprotective pigments such as carotenoids and antioxidants like tocopherols function against the damaging effects of molecules with excessive energy (Ramachandra Reddy *et al*., 2004). Decreased water supply or increased transpiration can trigger drought stress in plants. As shown by Havaux & Tardy (1999), a loss in chlorophyll is possible, but at the same time changed light absorption and increased thermal dissipation protect the photosynthetic apparatus. Peltzer *et al*. (2002) investigated leaves of water limited plants and recognised changes in chlorophyll photochemistry and observed increased amounts of active oxygen species. Photosynthetic electron transport is disturbed which leads to further formations of superoxide radicals. Ladjal *et al.* (2000) found significant losses of pigments and disorganisation of thylakoid membranes under drought. The accumulation of free radicals can harm DNA and amino acids, and cause protein oxidation and lipid peroxidation (Asada, 1999), which under high levels of stress can cause cell death. Cell detoxification can occur via enzymatic or non-enzymatic reactions. As pointed out by Pastori *et al.* (2000) the amount and degree of activity of antioxidants is highly variable between species and even the same species under different drought stress situations. Foyer *et al.* (1994) reported stress tolerance in plants, which over-produced enzymes like superoxide dismutase. Woody plants have shown less pronounced responses in their antioxidative working enzymes, which was argued as gradual adjustment to the habitat and therefore acclimation to it (Kronfuß *et al*., 1998).

Aim of the Study

Divaricate shrubs posses an extraordinary growth form (Chapter 1), which is supposed to 'self-shade' the interior of the plant. Not only is the exterior of divaricate shrubs marked by the highly interlaced branches, but also the very small leaves are often concentrated in the interior. It is assumed that this growth form offers protection against browsing (Section 1.2.1) or climatic stressful times and influences (Section 1.2.2). Growing under these sheltered conditions, I hypothesised that divaricate leaves would require less protection against high light and/ or mild drought influences than their non-divaricate congeners in summer.

As described above, pigments and antioxidants give good indications of stress in leaves. Therefore, the concentrations of the pigments violaxanthin, antheraxanthin, zeaxanthin (xanthophyll cycle pigments), neoxanthin, lutein, chlorophyll a, chlorophyll b and ?-carotene as well as the antioxidant a-tocopherol were analysed in divaricate leaves of the field trial and divaricate and non-divaricate leaves in the glasshouse experiment via HPLC (Section 3.2.2). The concentrations of photoprotective pigments (xanthophyll cycle pigments, chlorophyll a, chlorophyll b and ?-carotene) and the antioxidant a-tocopherol were thought to be low in divaricate leaves due to their 'self-shading' growth form. Therefore, I investigated whether un-shaded and unprotected non-divaricate leaves express higher levels of xanthophyll cycle pigments, ß-carotene and a-tocopherol then their divaricate congeners. Plants under mild drought are also under stress and therefore I hypothesised that a lesser water availability increases the concentrations of photoprotective pigments and the antioxidant a-tocopherol, as they reduce reactive oxygen species evolved under stress conditions in the chloroplasts.

In the field, *Corokia cotoneaster* and *Coprosma propinqua* grow naturally under different water availabilities, and their light levels were manipulated to provide another factor in the experiment (Section 2.1.2). Pigment and antioxidant concentrations of leaves in all treatments were estimated (Section 3.2.2) to compare the adaptation abilities of both divaricate genera to drought stress and high light loads. Exposing the interior of divaricates to full sunlight could diminish the 'self-shading' effect. These leaves would now experience high light conditions and I wished to

investigate if they had increased concentrations of photo-protective pigments and a-tocopherol.

In a glasshouse experiment, divaricate and non-divaricate plants of similar age and origin were grown under two different light and water treatments (Section 2.1.3). Again, leaf samples of all plants under each treatment were taken and the concentrations of pigments and the antioxidant a-tocopherol were analysed (Section 3.2.2). Here, the different growth forms of divaricate and non-divaricate shrubs were evaluated for their different responses in the concentrations of photoprotective pigments and a-tocopherol. Due to their different growth forms and leaf sizes, I hypothesised that divaricate leaves have lower concentrations of photoprotective pigments and a-tocopherol than their non-divaricate congeners under sun light and well-watered conditions. The shade treatment should lower these concentrations in both growth forms, but I hypothesised to see a bigger decrease in non-divaricate leaves as they have not been shaded or 'self-shaded' in the other light treatment. As argued before, mild drought increases the concentrations of photoprotective pigments and a-tocopherol and therefore an increase should be seen in all plants. As previously described, divaricate shrubs are thought to use water more conservatively and therefore, increases in the concentrations of photoprotective pigments and a-tocopherol were predicted to be less prominent than in non-divaricate leaves.

### 3.2.2 Materials and Methods

Up to 500 mg of fresh leaf material was collected during the summer from plants at the Cass field site (2001/02, 2002/03) and the glasshouse (2002, 2003). The material was weighed and immediately frozen in liquid nitrogen, and stored at -80°C until the analysis was carried out.

Each sample was initially ground in liquid nitrogen, followed by grinding in 100% acetonitrile. The resulting solution was quantitatively transferred to a centrifuge tube and spun at 5000 g for 6 min. Two ml of supernatant were passed through a 0.45 µm syringe filter into HPLC injection vials (Bungard *et al.*, 1997). All samples were analysed within six hours of extraction in a Reverse Phase-HPLC system from Waters (Milford, MA, USA), equipped with a Waters 996 photodiode array detector, a

Waters 474 fluorescence detector and an autosampler. The HPLC system contained a $C_{18}$ radial compression column (Waters Nova-Pak, 4 µm particle size; 8 mm internal diameter x 100 mm length) preceded by a Waters Nova-Pak $C_{18}$ guard column. Both were equilibrated prior to injection with 100% solvent A (acetonitrile-methanol 85:15).

The analysis cycle, with a sample injection volume of 40 µl, consisted of an initial run of 100% solvent A for 18 minutes to elute all xanthophylls. Following this was a 1 minute transition to solvent B (methanol-ethyl acetate 68:32), which was then run for 3.5 minutes to elute chlorophylls a and b. A 3 minute transition to solvent C (ethyl acetate-hexane 50:50) was followed by 0.5 minutes of solvent C to elute ß-carotene. A final concentration change to solvent A was done over 2.5 minutes and solvent A run for another 2 minutes before the next run was automatically initiated. The solvent flow was 1.0 ml $min^{-1}$ for solvent A and B and 2.0 ml $min^{-1}$ for solvent C (Bungard *et al*., 1997).

Pigment retention times were determined by absorption at 445 nm (HPLC software: Millenium® Software version 2.00, Millipore, Milford, Massachusetts, USA). Separated pigments were identified by their retention times and spectra by comparing with spectra of pigment standards as in Bungard *et al*. (1997, 1999). The de-epoxidation status was calculated as shown in Bungard *et al*. (1997) as [(antheraxanthin + zeaxanthin) / (violaxanthin + antheraxanthin + zeaxanthin)*100].

a-Tocopherol was quantified in the same samples extracted for pigment analysis using the same HPLC separation technique. a-Tocopherol was detected after HPLC separation using a fluorometer. The fluorescence detector offers up to 10- fold more sensitive detection of a-tocopherol in the samples than the PDA detector would achieve (Garcia-Plazaola & Becerril, 1999). An excitation wavelength of 295 nm and an emission wavelength of 325 nm produced a retention time of 21 min for a-tocopherol. The recalculation for each sample concentration of a-tocopherol was performed via a calibration curve produced from a serial dilution of a-tocopherol stock (Sigma-Aldrich Chemie GmbH, Steinheim, Germany) [Hansen, 2002 and 2003].

Technical difficulties

The extraction of all samples was performed using acetonitrile rather than acetone as described in the literature (Hansen *et al.*, 2002 and 2003). Using acetonitrile is more work intensive, as acetone helps to break membranes and solubilises pigments. In this case however, difficulties with clear peaks and resolution of initial acetone extracts were experienced. The usage of acetonitrile was able to overcome these difficulties without any further changes in the method necessary. Further technical difficulties were experienced with the quality of de-gasing solutions, automated sample transportation, flow rate adjustment and the consistency of the light source. As the HPLC is set up to shut itself off when problems occur, many samples were lost when the cooling of the sample chamber stopped following internal errors.

### 3.2.3 Results

All results of the pigment and a-tocopherol analysis were divided by total chlorophyll content, leaf area and fresh weight basis. Calculating the pigment and a-tocopherol concentrations on unit leaf area and unit fresh weight depends on the anatomical-morphological differences of the divaricate and non-divaricate leaves, whereas the relation to total chlorophyll is independent of it. These three measures were included to allow comparison to the literature. Due to the high number of ANOVA tables, the statistical results were placed in the Appendix Section A2, but key findings are in the paragraphs below. The statistical results for the pigment and a-tocopherol analysis for the field experiment were shown in Table A2.4 to A2.12 on a total chlorophyll basis, in Table A2.13 to A2.24 on a leaf area basis and in Table A2.25 to A2.36 on a fresh weight basis. The glasshouse results were displayed in Table A2.37 to A2.44 on a total chlorophyll basis, in Table A2.45 to A2.55 on a leaf area basis and in Table A2.56 to A2.66 on a fresh weight basis.

Numerous instrumentation issues (Section 3.2.2) marked the process of analysing the pigment and antioxidant concentrations of samples from the field and the glasshouse. As a result, many samples were lost and the statistical analyses were difficult. The following graphs show the mean results, missing data were marked with ‘NA’ and columns, which resulted from one data point only, were marked with ‘#’.

Cass 2002/03

Samples for the analysis of pigment and antioxidant concentrations of divaricate leaves grown under field conditions were taken in summer 2001/02 and 2002/03 (Section 2.1.2 and 3.2.2). Due to instrumentation issues (Section 3.2.2), the analysis of samples taken from the field in 2001/02 was impossible and therefore data are presented for the 2002/03 season only.

Pigment Concentrations

The de-epoxidation state symbolizes the ratio of epoxidised to de-epoxidised xanthophylls of the violaxanthin cycle (Section 3.2.2). Significant effects for different light levels were demonstrated for antheraxanthin and zeaxanthin on a total chlorophyll basis as well as for pigments in the de-epoxidation state (Table A2.5 to A2.7), confirming my hypothesis of higher concentrations of both photoprotective pigments in leaves grown under high light conditions. The graphs for violaxanthin, antheraxanthin and zeaxanthin per total chlorophyll (Figure 3.2.1) show higher concentrations of those pigments for *Corokia* under exposed and natural light conditions. Violaxanthin per total chlorophyll, leaf area basis and unit fresh weight was significantly higher in plants in the streambed than on the N-facing slope. The graph for the pigments in the de-epoxidation state shows that shaded plants have significantly lower values (Figure 3.2.2) as hypothesised in Section 3.2.1. Calculating the same pigments on a leaf area basis, significantly higher amounts of antheraxanthin and zeaxanthin were found for plants growing exposed to light and under natural light (Table A2.14 and A2.15 and Figure 3.2.3). The amounts of violaxanthin, antheraxanthin and zeaxanthin per unit fresh weight did not show as many significant treatment effects as when calculated on a total chlorophyll content or leaf area basis (Table A2.25 to A2.27and Figure 3.2.4).

Violaxanthin concentrations per unit total chlorophyll were highest in exposed leaves of *Corokia*, grown in a streambed. The lowest amounts were found in leaves of *Corokia* grown under natural light on the N-facing slope (Figure 3.2.1a). Water availability, genus and the light level* water availability interaction demonstrated significant effects on the amounts of violaxanthin per unit total chlorophyll (Table A2.4). The graph 3.2.3a revealed high amounts of violaxanthin per leaf area in

*Corokias*, growing in the streambed and with exposed leaves as well as leaves under natural light. Even in the shade, the concentrations were still high. Genus exhibited significant effects on violaxanthin concentrations per unit leaf area (Table A2.13). Violaxanthin per unit leaf area also displayed significant effects for the interaction of light level* water availability* genus. Significantly higher values of violaxanthin per unit fresh weight (Table A2.25) were found in *Corokia* under shaded conditions in the streambed. Differing water availability results in significant effects on the amount of violaxanthin per unit fresh weight found in the two genera (Figure 3.2.4).

Antheraxanthin per unit total chlorophyll (Figure 3.2.1b) also reached the highest values in *Corokia* with exposed interior leaves, but these were growing on the N-facing slope. Table A2.5 displayed the significant effects of light level on the antheraxanhtin concentration per unit total chlorophyll. On a leaf area basis, antheraxanthin concentrations were higher for all plants with exposed interior leaves and under natural light (Figure 3.2.3b), marking a significant effect of light level (Table A2.14). Comparison on a fresh weight basis did not reveal any significant effects (Table A2.26; Figure 3.2.4b).

The amounts of zeaxanthin per total chlorophyll were generally high (Figure 3.2.1c), except for plants in shaded conditions. Significantly higher concentrations were found for *Coprosma* growing in the streambed. Antheraxanthin, zeaxanthin and the pigments in the de-epoxidation state showed significant effects for the light level when referred on total chlorophyll basis (Table A2.5 to A2.7). *Corokia* and *Coprosma* had high amounts of de-epoxidated xanthophylls, when growing under natural light or when the interior was exposed to full sunlight. Shaded plants expressed lower amounts of de-epoxidised xanthophylls regardless of the water supply for the plants. *Corokia* leaves showed very high concentrations of zeaxanthin, except under shaded conditions (Figure 3.2.1c, 3.2.3c and 3.2.4c). *Coprosma* had only very high concentrations of zeaxanthin for interior leaves when exposed to exterior radiation loads. Light level had a significant effect on zeaxanthin per unit leaf area and per unit fresh weight (Table A2.15 and A2.27). *Coprosma* had the highest zeaxanthin concentration per fresh weight when growing in the streambed and under exposed conditions and natural light (Figure 3.2.4c).

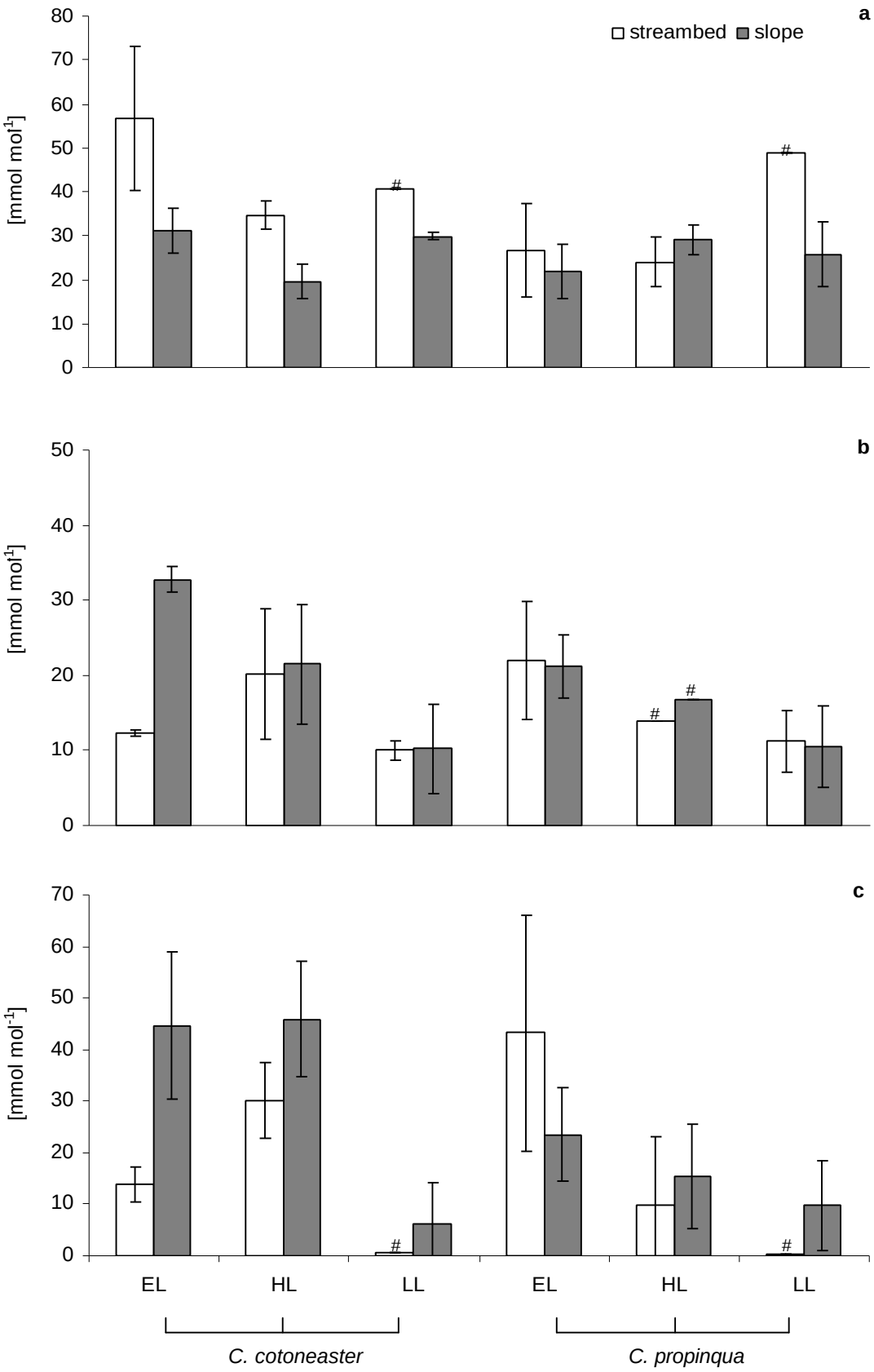



Figure 3.2.1: Concentrations of (a) violaxanthin, (b) antheraxanthin and (c) zeaxanthin per unit total chlorophyll for *Corokia cotoneaster* and *Coprosma propinqua* in 2002/03 at Cass, grown in a streambed and on a N-facing slope and under 3 different light treatments (EL = inner canopy exposed, HL = sun light, LL = shaded) [n = 4].

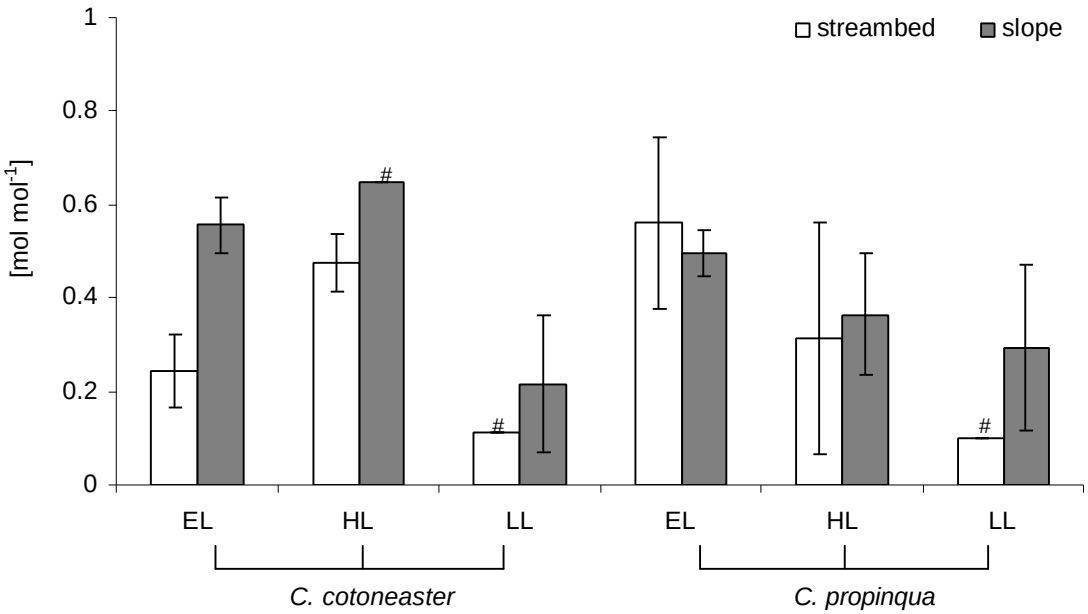



Figure 3.2.2: Concentration of pigments in the de-epoxidation state for *Corokia cotoneaster* and *Coprosma propinqua* in 2002/03 at Cass, grown in a streambed and on an N-facing slope and under 3 different light treatments (EL = inner canopy exposed, HL = sun light, LL = shaded) [n = 4].

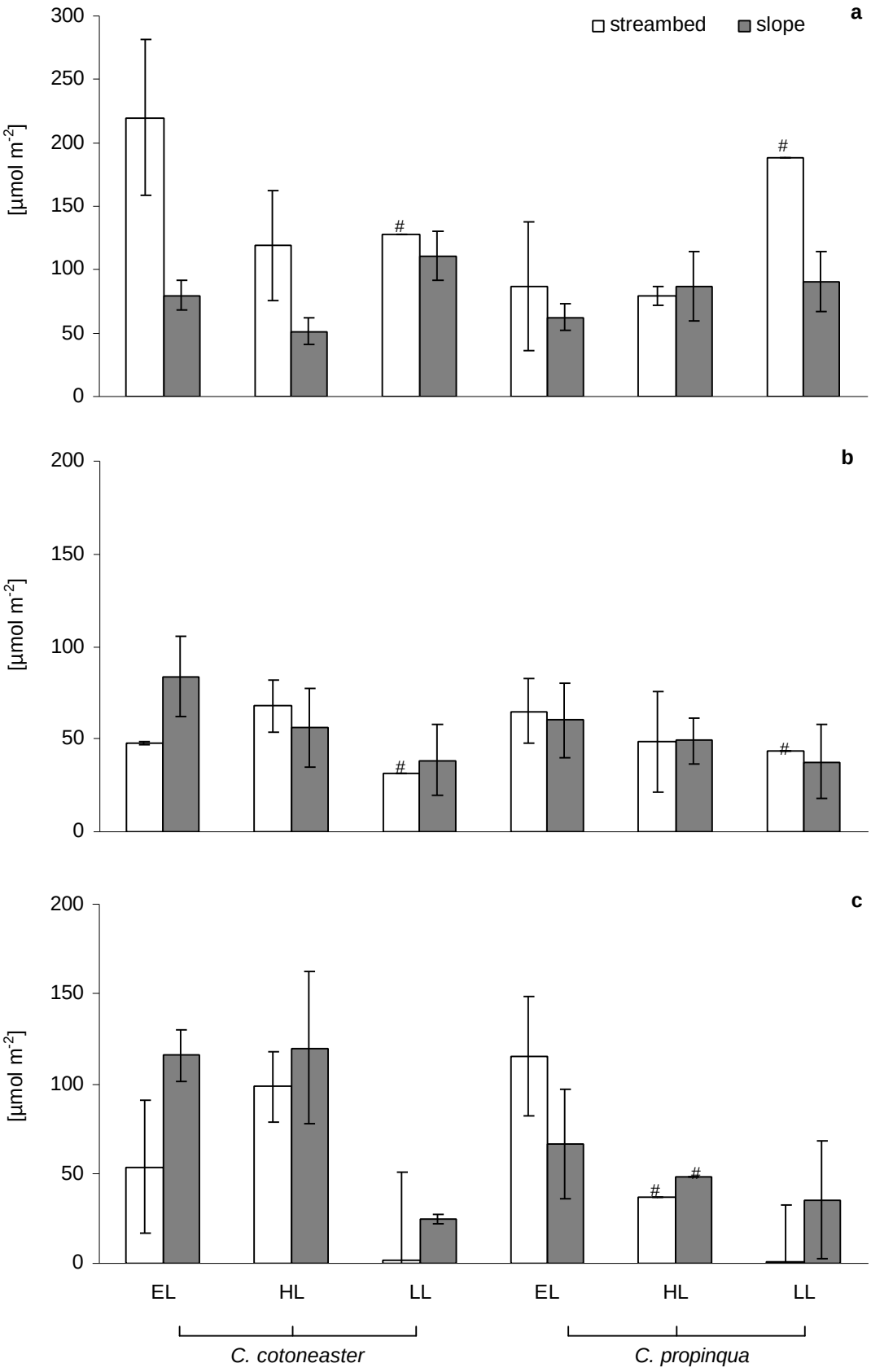



Figure 3.2.3: Concentrations of (a) violaxanthin, (b) antheraxanthin and (c) zeaxanthin per unit leaf area for *Corokia cotoneaster* and *Coprosma propinqua* in 2002/03 at Cass, grown in a streambed and on a N-facing slope and under 3 different light treatments (EL = inner canopy exposed, HL = sun light, LL = shaded) [n = 4].

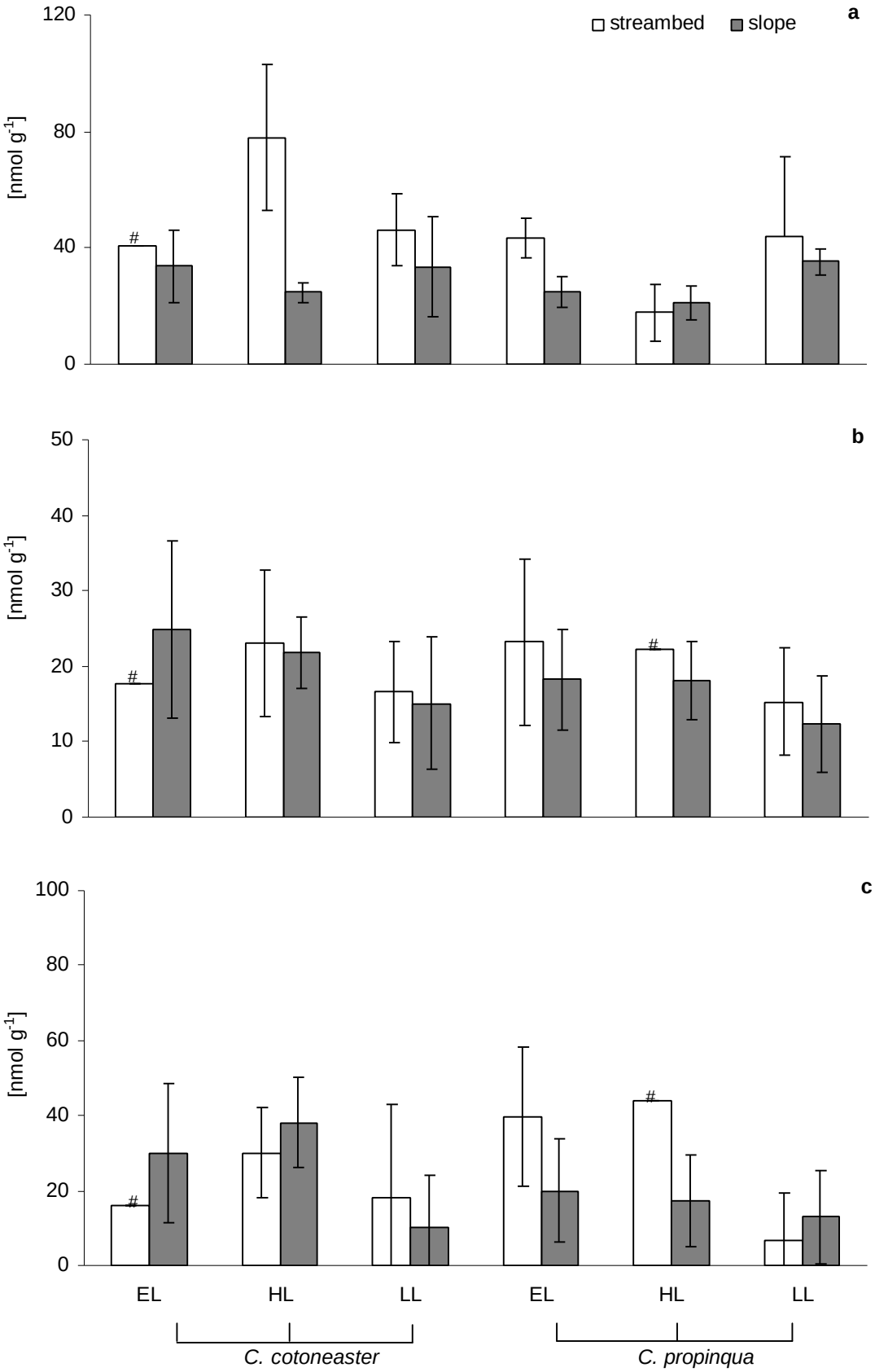



Figure 3.2.4: Concentrations of (a) violaxanthin, (b) antheraxanthin and (c) zeaxanthin per unit fresh weight for *Corokia cotoneaster* and *Coprosma propinqua* in 2002/03 at Cass, grown in a streambed and on a N-facing slope and under 3 different light treatments (EL = inner canopy exposed, HL = sun light, LL = shaded) [n = 4].

Neoxanthin per leaf area was significantly higher in *Coprosma*, with significant effects also found for the interaction of light level* water availability* genus (Table A2.16). The significant effects for genus and for the interaction of light level* water availability* genus was also found for the correlation of neoxanthin per fresh weight (Table A2.28). For this reference a significant difference between the given light treatments was also shown. Lutein per unit total chlorophyll showed significantly higher amounts for unshaded plants (Table A2.9). When compared on a leaf area and fresh weight basis, lutein was significantly different between the two genera (Table A2.17 and A2.29).

The relation of chlorophyll a, chlorophyll b and chlorophyll a+b to the leaf area showed significantly different adaptations between the different light treatments (Table A2.18 to A2.20 and Figure 3.2.5). Chlorophyll a per unit leaf area was also significantly different between the two genera (Table A2.18). Concentrations of chlorophyll a and chlorophyll b per unit leaf area were lower for plants growing on the N-facing slope than for plants in the streambed, in particular for plants under natural light and with exposed interiors (Figure 3.2.5a and 3.2.5b). When compared on a fresh weight basis chlorophyll b and chlorophyll a+b showed significant responses to the three applied light treatments (Table A2.31 and A2.32). For chlorophyll a, chlorophyll b and chlorophyll a+b the interaction of light level* water availability* genus was significant (Table A2.30 to A2.32). The ratio of chlorophyll a to b was significantly different for genus (Table A2.33 and Figure 3.2.6). The graphs for chlorophyll a and chlorophyll b per fresh weight (Figure 3.2.7) show the lowest values for plants grown under shading and drought conditions. Chlorophyll a and chlorophyll b concentration per fresh weight were high for *Corokia* plants growing in the streambed, exposed to exterior light or under natural light as well as for shaded plants on the N-facing slope. *Coprosma* had the highest concentrations of chlorophyll a and chlorophyll b in the shade, when grown in the streambed.

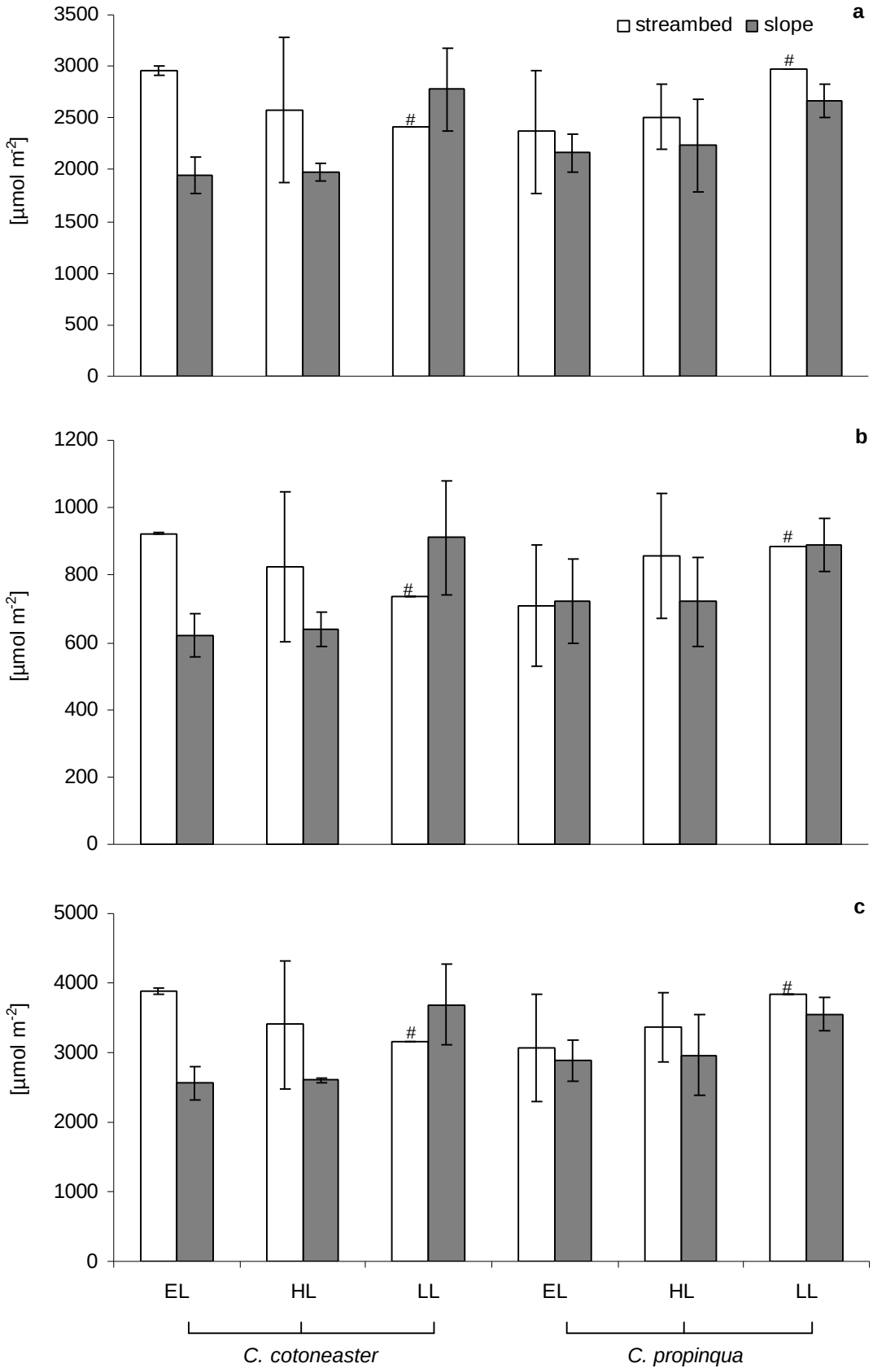



Figure 3.2.5: Concentrations of (a) chlorophyll a, (b) chlorophyll b and (c) chlorophyll a+b per unit leaf area for *Corokia cotoneaster* and *Coprosma propinqua* in 2002/03 at Cass, grown in a streambed and on a N-facing slope and under 3 different light treatments (EL = inner canopy exposed, HL = sun light, LL = shaded) [n = 4].

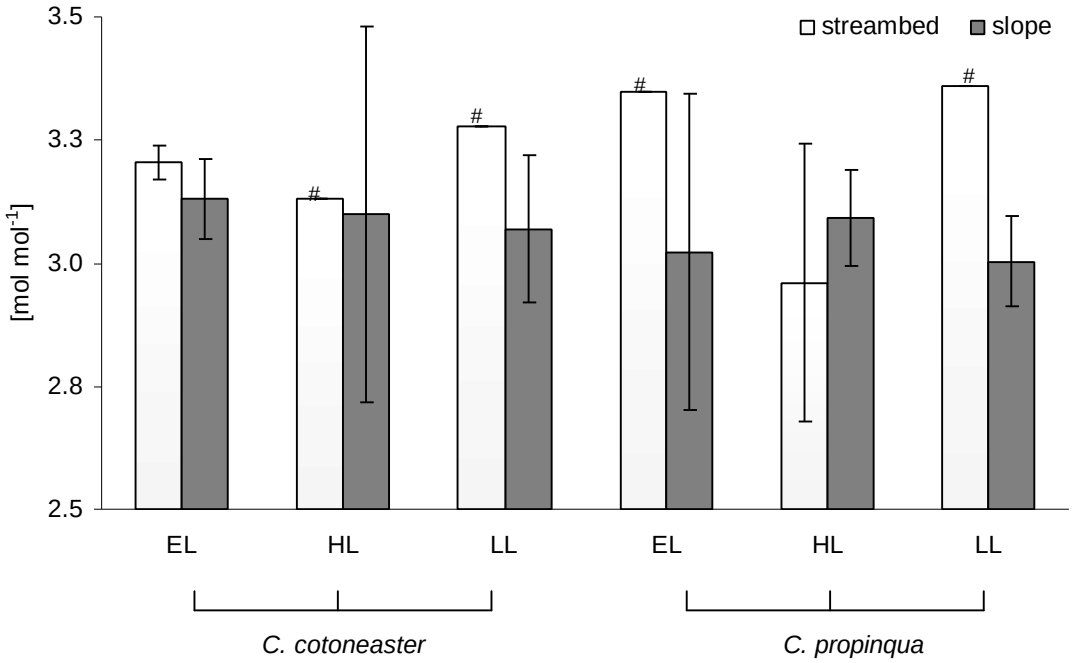



Figure 3.2.6: Chlorophyll a:b ratio for *Corokia cotoneaster* and *Coprosma propinqua* in 2002/03 at Cass, grown in a streambed and on an N-facing slope and under 3 different light treatments (EL = inner canopy exposed, HL = sun light, LL = shaded) [n = 4].

Leaves of the genera *Corokia* and *Coprosma* differed significantly in their ß-carotene concentrations per unit total chlorophyll (Table A2.10). The highest ß-carotene per total chlorophyll concentrations were found in plants with exposed interiors in the streambed (Figure 3.2.8). Exposed *Corokia* leaves had the lowest ß-carotene per total chlorophyll concentration, when grown on the N-facing slope. The amount of light penetrating the leaf and the water availability to the plants significantly influenced the amounts of ß-carotene in the leaves. On a leaf area basis ß-carotene displayed significant responses to light treatment and genus (Table A2.22). Concentrations of ß-carotene per leaf area were also higher for plants growing in the streambed, but especially high for the *Corokia* plants underneath the shade cloth. The amount of ß-carotene per unit fresh weight differed significantly for the two genera (Table A2.34). In contrast to my hypothesis that the concentration of photoprotective pigments would be low in the shade and in divaricate leaves, *Corokia* and *Coprosma* showed high concentrations of ß-carotene in the shade, especially when growing in the streambed. *Corokia* also had high values for plants under natural light and growing in the streambed. In contrast, *Coprosma* leaves displayed the highest concentration in exposed leaves and growing in the streambed.

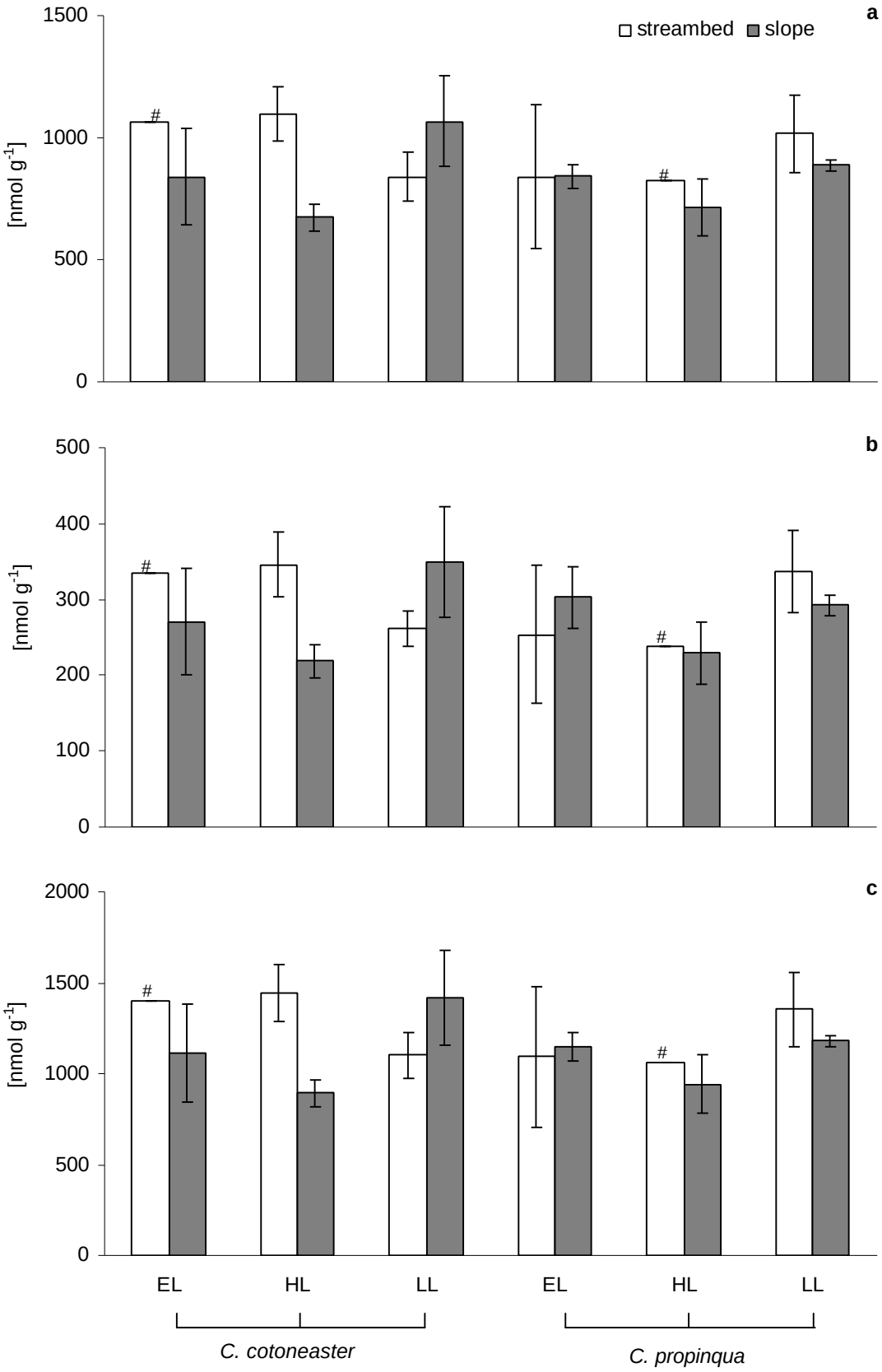



Figure 3.2.7: Concentrations of (a) chlorophyll a, (b) chlorophyll b and (c) chlorophyll a+b per unit fresh weight for *Corokia cotoneaster* and *Coprosma propinqua* in 2002/03 at Cass, grown in a streambed and on a N-facing slope and under 3 different light treatments (EL = inner canopy exposed, HL = sun light, LL = shaded) [n = 4].

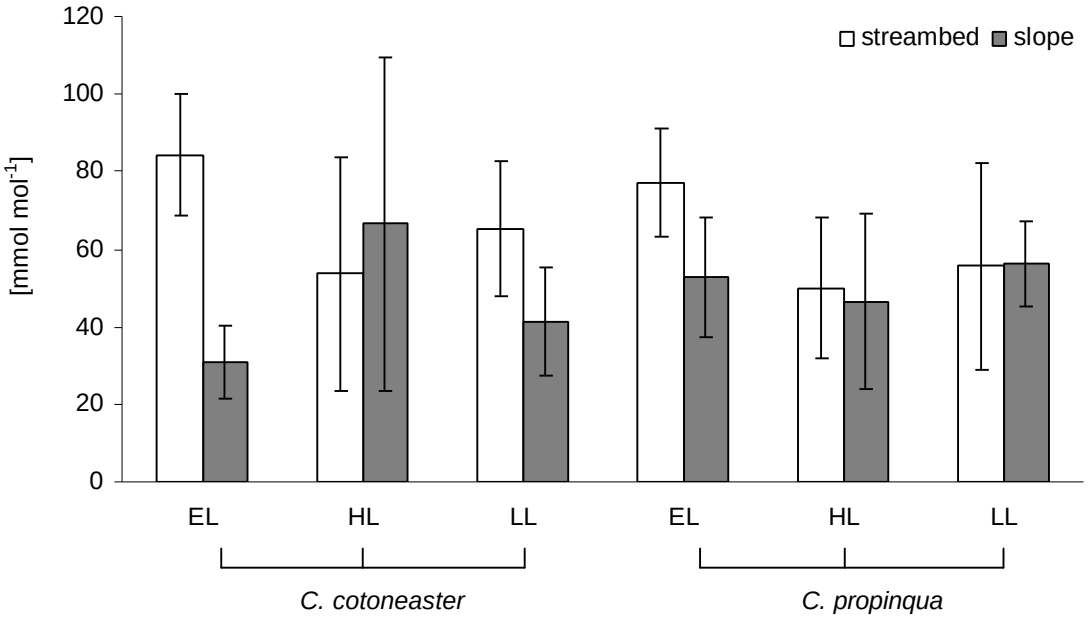



Figure 3.2.8: Concentrations of ß-carotene per unit total chlorophyll for *Corokia cotoneaster* and *Coprosma propinqua* in 2002/03 at Cass, grown in a streambed and on an N-facing slope and under 3 different light treatments (EL = inner canopy exposed, HL = sun light, LL = shaded) [n = 4].

a-Tocopherol Analysis

Significant effects of genus were shown by relating the a-tocopherol samples of 2001/02 and 2002/03 to total chlorophyll as well as leaf area and fresh weight (Table A2.11 and A2.12; A2.23 and A2.24; A2.35 and A2.36). Water availability also had a significant effect on samples taken in 2002/03 for a-tocopherol on a leaf area and a fresh weight basis. Comparing the graphs for a-tocopherol in 2001/02 and 2002/03 (Figure 3.2.9 and 3.2.10), the plants show the same patterns of response in both years when referred on a fresh weight basis (Figure 3.2.9c and 3.2.10c). When compared on total chlorophyll basis (Figure 3.2.9a and 3.2.10a) and on leaf area basis (Figure 3.2.9b and 3.2.10b), the response was reversed in particular for plants with exposed canopies. Here, *Corokia* in the streambed had higher concentrations of a-tocopherol than *Coprosma* in 2001/02. All other data showed the highest a-tocopherol concentrations in *Coprosma*, where it remained high even for leaves under the shade cloth.

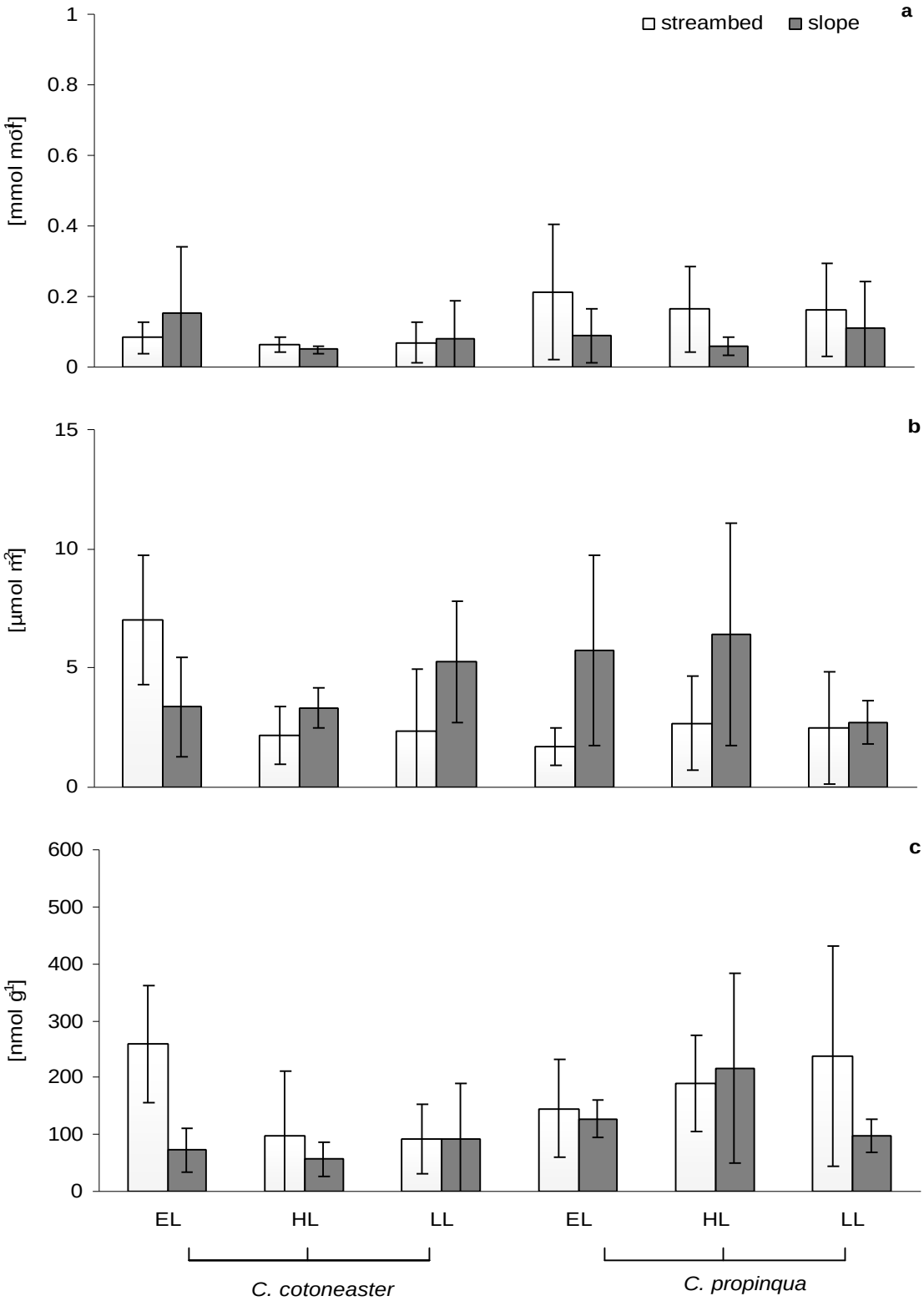



Figure 3.2.9: Concentrations of a-tocopherol (a) per unit total chlorophyll, (b) per unit leaf area and (c) per unit fresh weight for *Corokia cotoneaster* and *Coprosma propinqua* in 2001/02 at Cass, grown in a streambed and on a N-facing slope and under 3 different light treatments (EL = inner canopy exposed, HL = sun light, LL = shaded) [n = 4].

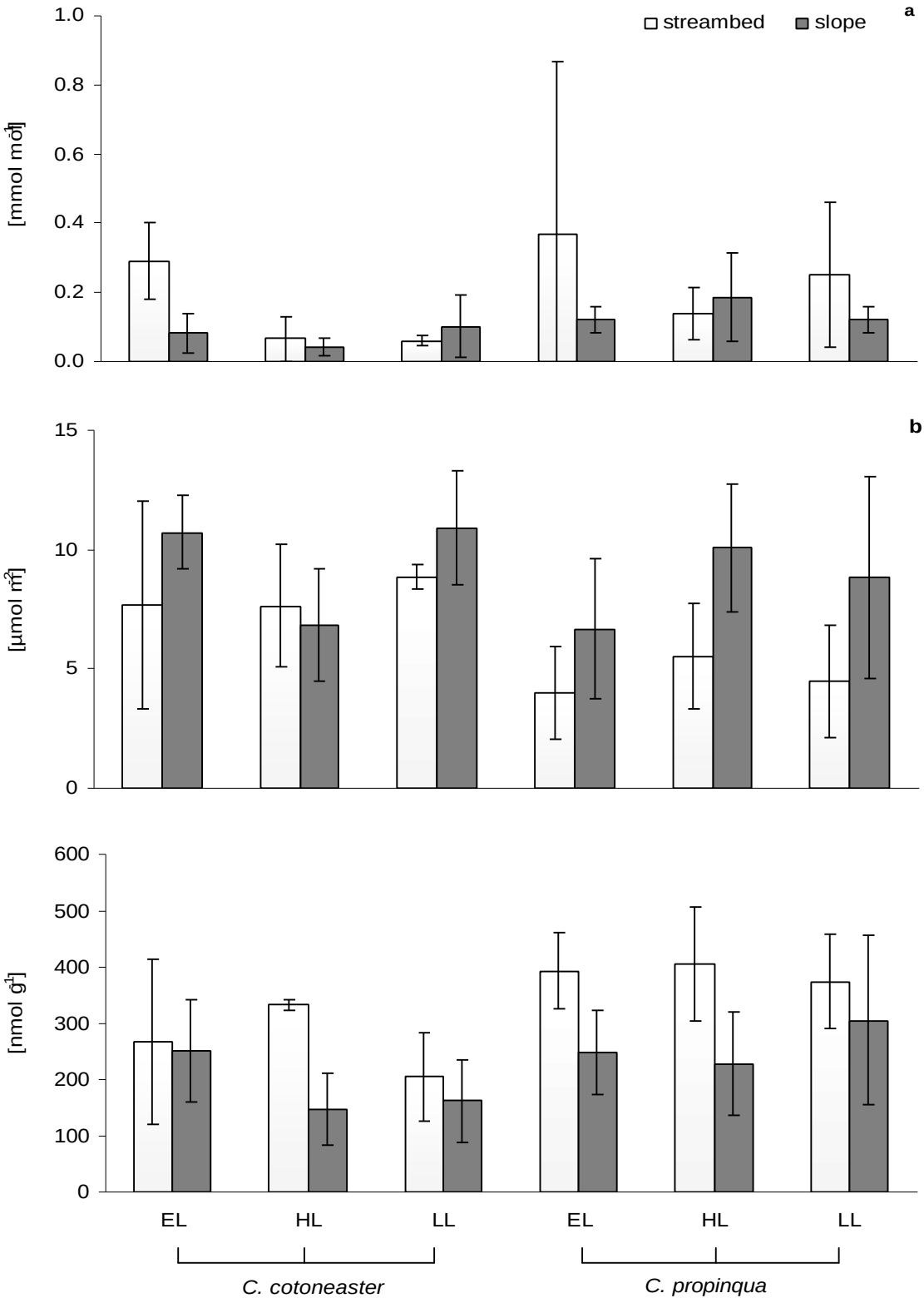



Figure 3.2.10: Concentrations of a-tocopherol (a) per unit total chlorophyll, (b) per unit leaf area and (c) per unit fresh weight for *Corokia cotoneaster* and *Coprosma propinqua* in 2002/03 at Cass, grown in a streambed and on a N-facing slope and under 3 different light treatments (EL = inner canopy exposed, HL = sun light, LL = shaded) [n = 4].

Glasshouse 2002

In 2002 samples for pigment and antioxidant analysis were taken from divaricate and non-divaricate species, grown under different light and water treatments (Section 2.1.3). Due to the difficulties experienced during the HPLC analysis (Section 3.2.2), there were no usable results for the divaricate *Corokia* and *Coprosma* leaves grown under well-watered conditions in the shade. The missing data are marked as 'NA'.

Pigment Compositions

The amounts of the photo-protective pigments of the xanthophyll cycle, expressed on a total chlorophyll basis, were not significantly different in any given treatments or for any species in the glasshouse trial of 2002 (Table A2.37 to A2.39). Also, the pigments in the de-epoxidated state did not differ significantly (Table A2.40). This is in contrast to my hypotheses that divaricate leaves would have lower concentration of de-epoxidised pigments than non-divaricate leaves and that leaves under high light would have higher concentrations of xanthophylls cycle pigments than shaded leaves. Violaxanthin per unit total chlorophyll showed values in a close range of 20 to 40 mmol $mol^{-1}$ for most of the samples (Figure 3.2.11a). The values for antheraxanthin and zeaxanthin per unit total chlorophyll (Figure 3.2.11b and 3.2.11c) were generally very low, only non-divaricate *Coprosma* leaves in high light had highly increased amounts of both pigments.

By examining the violaxanthin concentration per unit leaf area, significant differences for the different water availabilities, genus, habits and the interaction of genus* habit were identified (Table A2.45). In Figure 3.2.14a, the divaricate *Coprosma* leaves reached the highest violaxanthin concentrations, in particular under water-stressed conditions. Here, *C. propinqua* had nearly eight times higher values than *C. cotoneaster*. All *Corokia* plants had similar values, but divaricates had the highest amounts in drought and shaded conditions. The non-divaricate habit appeared to have slightly higher values when the plants were well- watered. The concentrations of antheraxanthin per unit leaf area were significantly different between *Corokia* and *Coprosma* (Table A2.46). *Coprosma* plants reach higher amounts, water-stressed divaricates particularly (Figure 3.2.12b). Zeaxanthin concentrations were not significantly different in this trial (Table A2.47, Figure 3.2.14c).

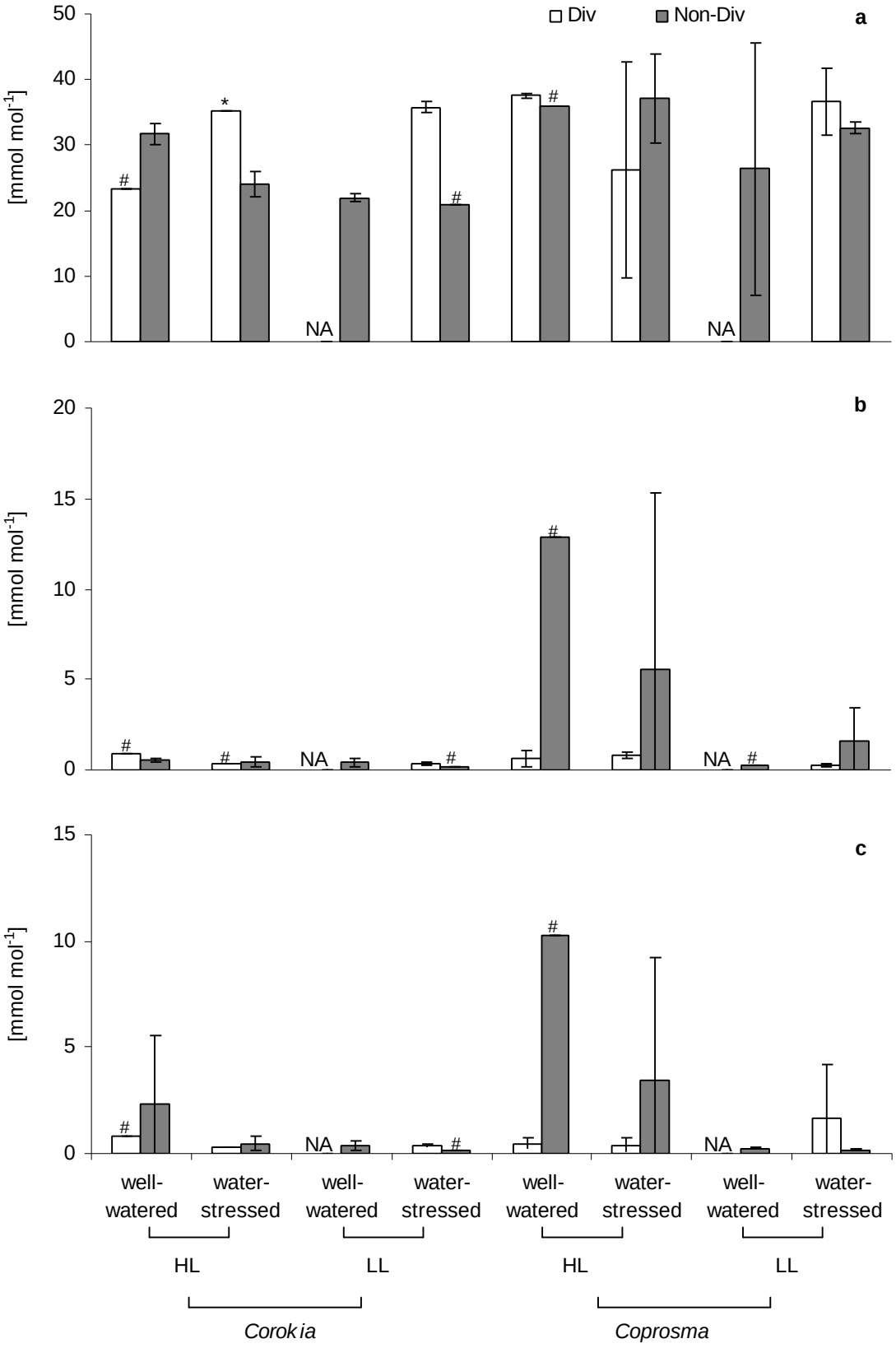



Figure 3.2.11: Concentrations of (a) violaxanthin, (b) antheraxanthin and (c) zeaxanthin per unit total chlorophyll in the Glasshouse in 2002 for divaricate (Div) and non-divaricate leaves (Non-Div) in well-watered and water stressed conditions and two different light treatments (HL = sun light, LL = shaded) [n = 4].

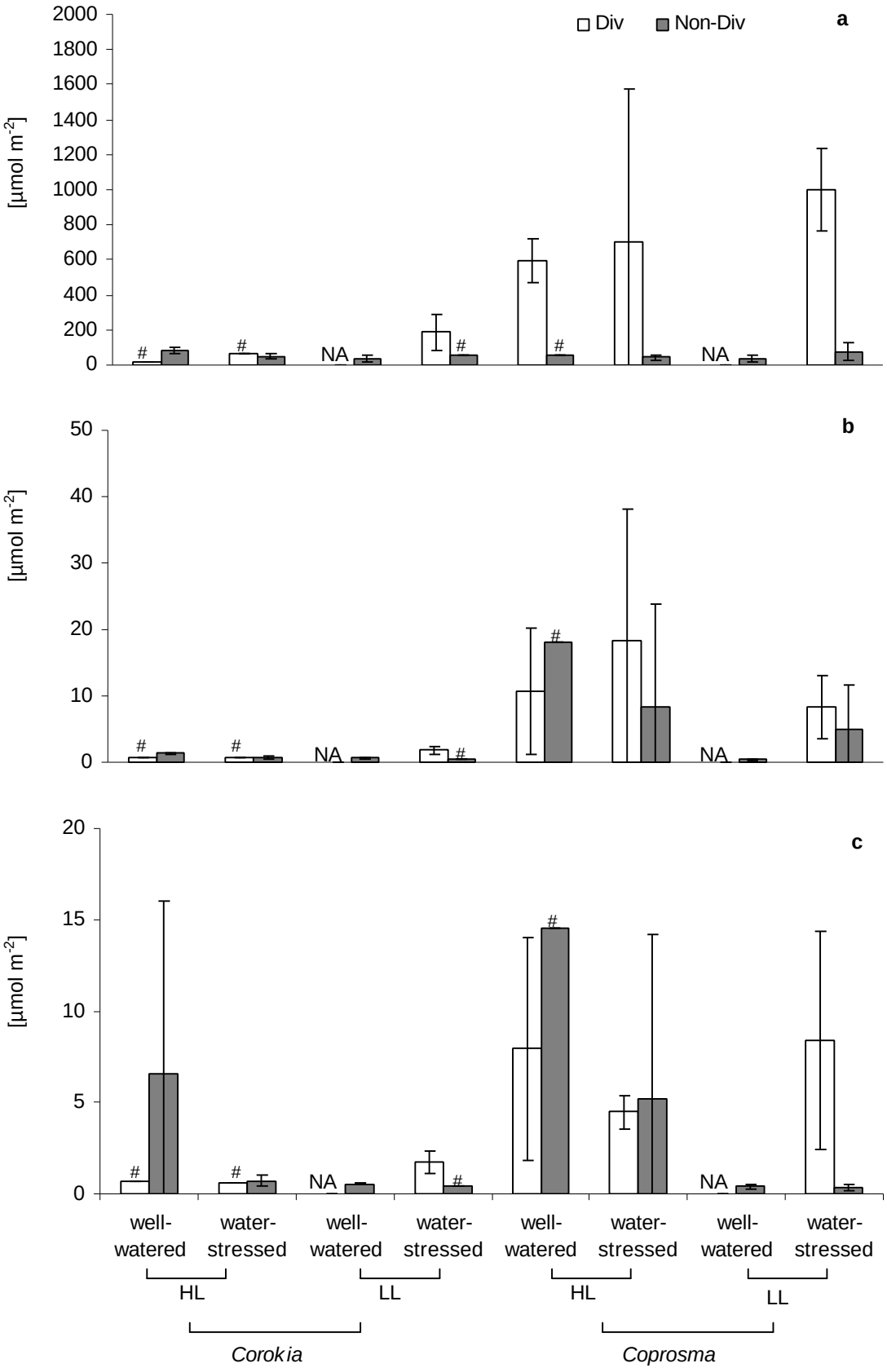



Figure 3.2.12: Concentrations of (a) violaxanthin, (b) antheraxanthin and (c) zeaxanthin per unit leaf area in the Glasshouse in 2002 for divaricate (Div) and non-divaricate leaves (Non-Div) in well-watered and water stressed conditions and two different light treatments (HL = sun light, LL = shaded) [n = 4].

Violaxanthin on a fresh weight basis showed statistically significant effects on the interaction of light level* water availability, light level* habit and genus* habit effects (Table A2.56). The highest values were observed for the non-divaricate *Coprosma* plants under low light and water-stressed conditions (Figure 3.2.13a). Divaricate *Corokias* generally had higher concentrations of violaxanthin per unit fresh weight than the non-divaricate *Corokias*, especially under high light loads. Neither antheraxanthin nor zeaxanthin displayed significant differences on a fresh weight basis (Table A2.57 and A2.58).

Neoxanthin did not differ significantly between treatments when compared on a total chlorophyll, leaf area or fresh weight basis (Table A2.41, A2.48 and A2.59). The only significant effects for pigment concentrations per unit total chlorophyll in the glasshouse trial were found for concentrations of lutein (Table A2.42) for water availability, genus and habit. Lutein per unit leaf area was significantly different for the *Corokia* versus *Coprosma* genus and divaricate versus non-divaricate habit as well as their interaction term (Table A2.49). The divaricate *Coprosma* leaves had higher concentrations of lutein per unit leaf area (not shown). Lutein concentrations expressed on a fresh weight basis showed statistically significant effects for light level* water availability, light level* habit and genus* habit interactions (Table A2.60). In the shade and under drought conditions, *C. robusta* reached the highest concentrations of lutein per unit fresh weight (Figure 3.2.14).

When compared on a leaf area basis, pigment concentrations displayed significant effects for genus, habit and the genus* habit interaction for chlorophyll a, chlorophyll b, chlorophyll a+b (Table A2.50 to A2.52). Significant effects of genus were also found for the chlorophyll a:b ratio (Table A2.53). Graphs for chlorophyll a, chlorophyll b and chlorophyll a+b per unit leaf area showed high amounts of those pigments in leaves of divaricate *Coprosma* plants (Figure 3.2.15). Under shade and drought stressed conditions, divaricate *Corokia* leaves also showed increased amounts of chlorophyll a and chlorophyll b. The chlorophyll a:b ratio was around 2.7 in *Coprosma* and around 2.3 in *Corokia* (Figure 3.2.16). The lowest chlorophyll a:b ratio for divaricates was expressed by shaded *Corokias* in drought conditions. Non-divaricate *Corokias* had the lowest chlorophyll a:b ratio under high and natural light and well-watered conditions. Chlorophyll a, chlorophyll b and chlorophyll a+b

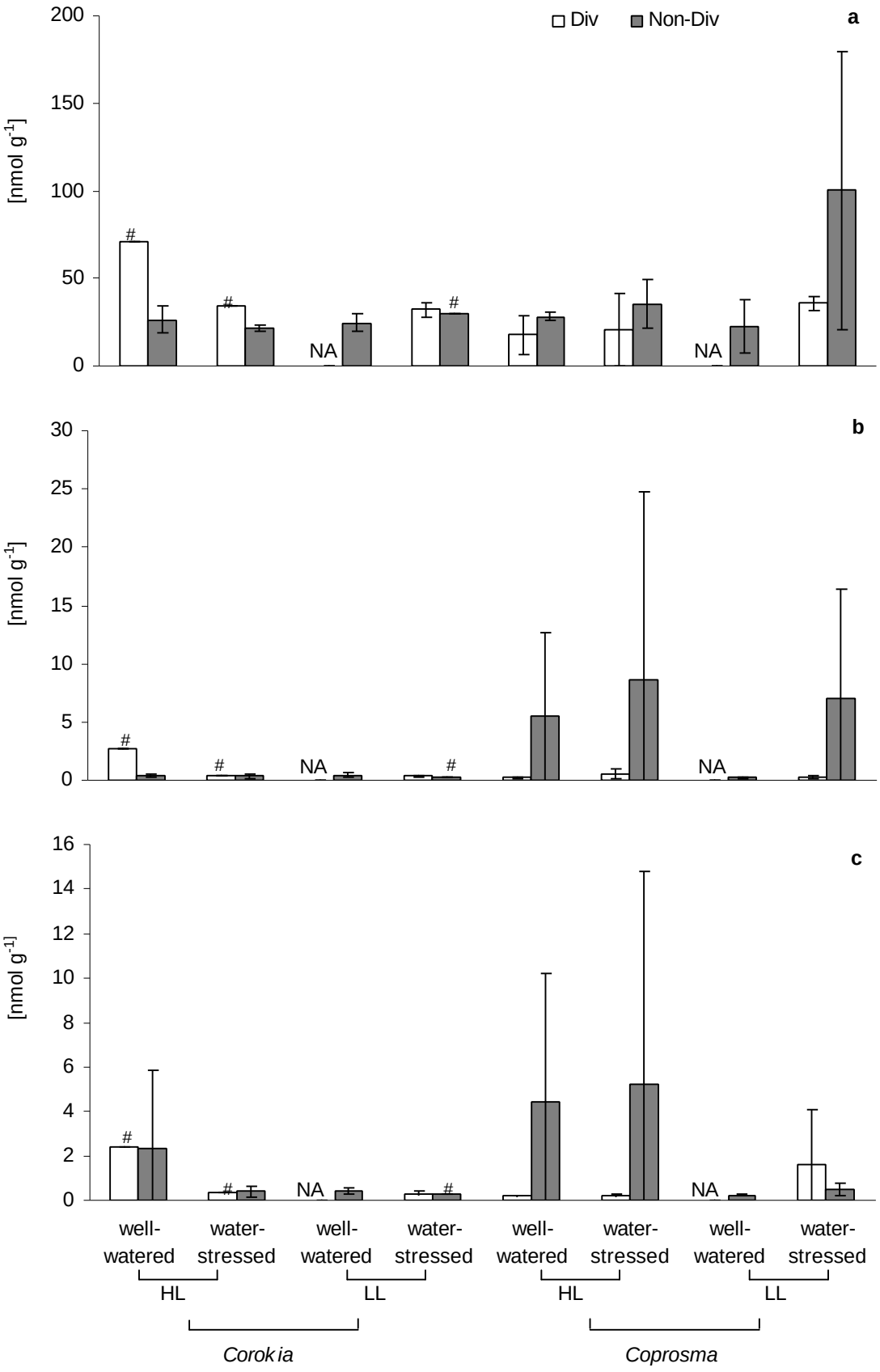



Figure 3.2.13: Concentrations of (a) violaxanthin, (b) antheraxanthin and (c) zeaxanthin per unit fresh weight in the Glasshouse in 2002 for divaricate (Div) and non-divaricate leaves (Non-Div) in well-watered and water stressed conditions and two different light treatments (HL = sun light, LL = shaded) [n = 4].

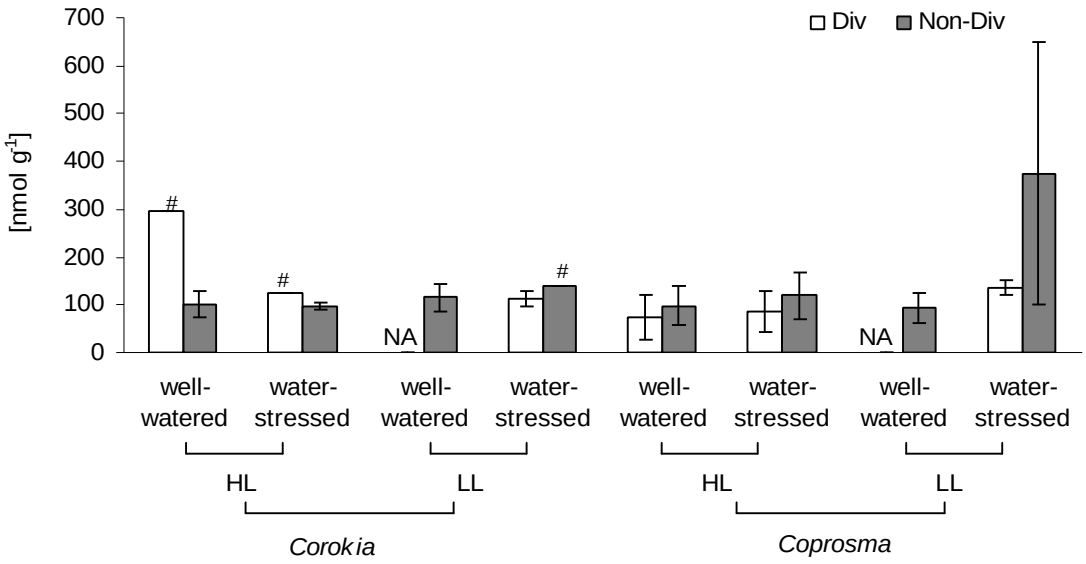



Figure 3.2.14: Concentrations of lutein per unit fresh weight in the Glasshouse in 2002 for divaricate (Div) and non-divaricate (Non-Div) in well-watered and water-stressed conditions and two different light treatments (HL = sun light, LL = shaded) [n = 4].

concentrations on a fresh weight basis showed significant effects for the interactions of light level* habit and genus* habit (Table A2.61 to A2.63). The non-divaricate *Coprosma* had its highest values for chlorophyll a, chlorophyll b and chlorophyll a+b per fresh weight in shaded and drought conditions (Figure 3.2.17).

The concentration of ß-carotene per unit total chlorophyll was almost significant for the different water treatments given (Table A2.43). It was significantly different between the two genera. *Coprosma* had the higher concentrations of ß-carotene per unit total chlorophyll (Figure 3.2.18a). Divaricate *Coprosma* leaves displayed the highest values in shaded and well-watered conditions, whereas the non-divaricate *Coprosma* leaves expressed the highest values under high light and sufficient water supply. Genus, habit and the interaction of genus* habit effects were significant for concentrations of ß-carotene per unit leaf area (Table A2.54). Divaricate *Coprosma* leaves had high amounts of this photo-protective pigment (Figure 3.2.18b). Significant effects on concentrations of ß-carotene per unit fresh weight (Table A2.65) were found for the interactions of light level* water availability, light level* habit and genus* habit. The interaction of water availability* genus* habit also influenced ß-carotene per unit fresh weight. Overall, *C. robusta* had its highest values of ß-carotene per fresh weight under shaded and water stressed conditions

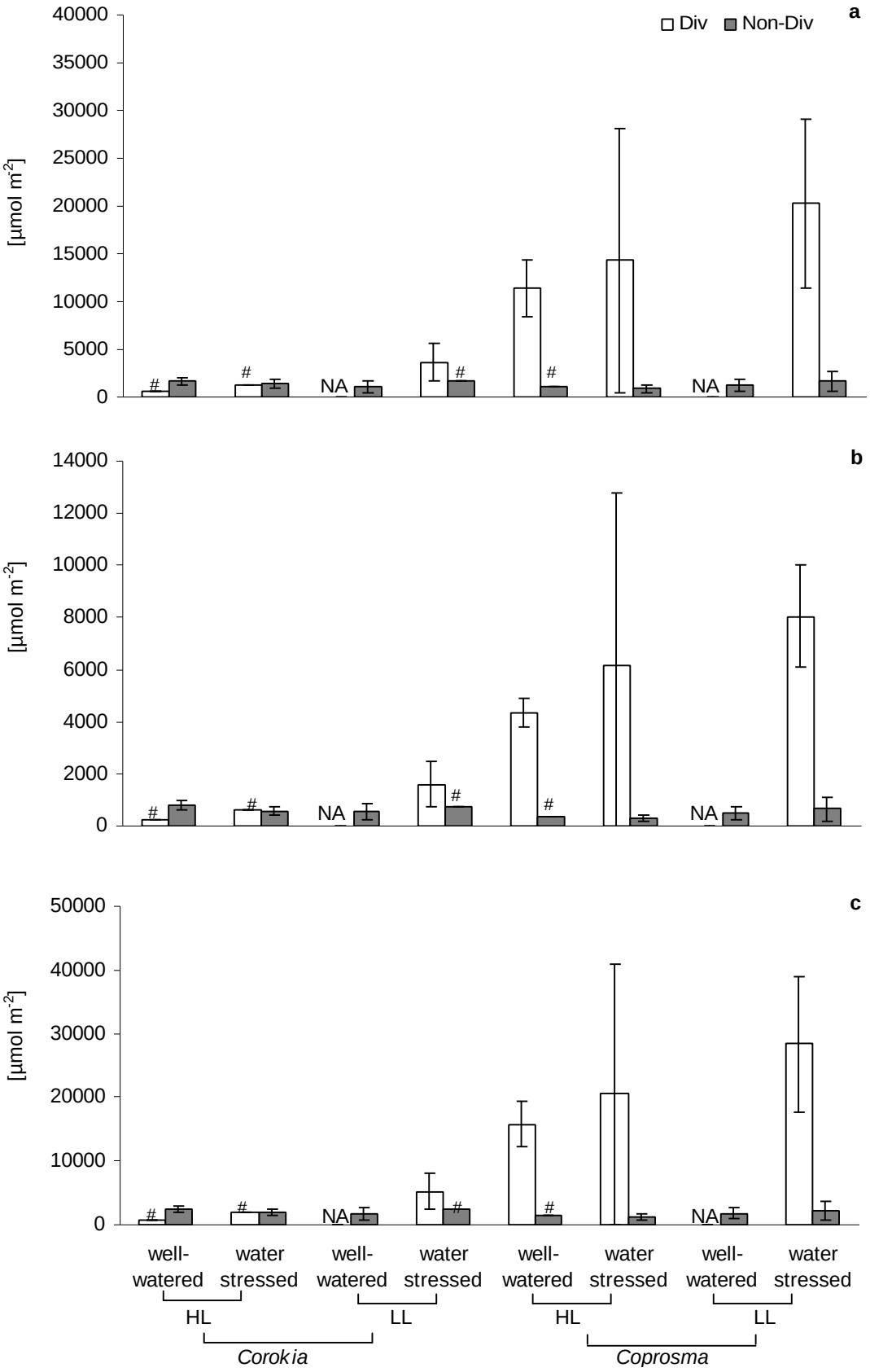



Figure 3.2.15: Concentrations of (a) chlorophyll a, (b) chlorophyll b and (c) chlorophyll a+b per unit leaf area in the Glasshouse in 2002 for divaricate (Div) and non-divaricate leaves (Non-Div) in well-watered and water stressed conditions and two different light treatments (HL = sun light, LL = shaded) [n = 4].

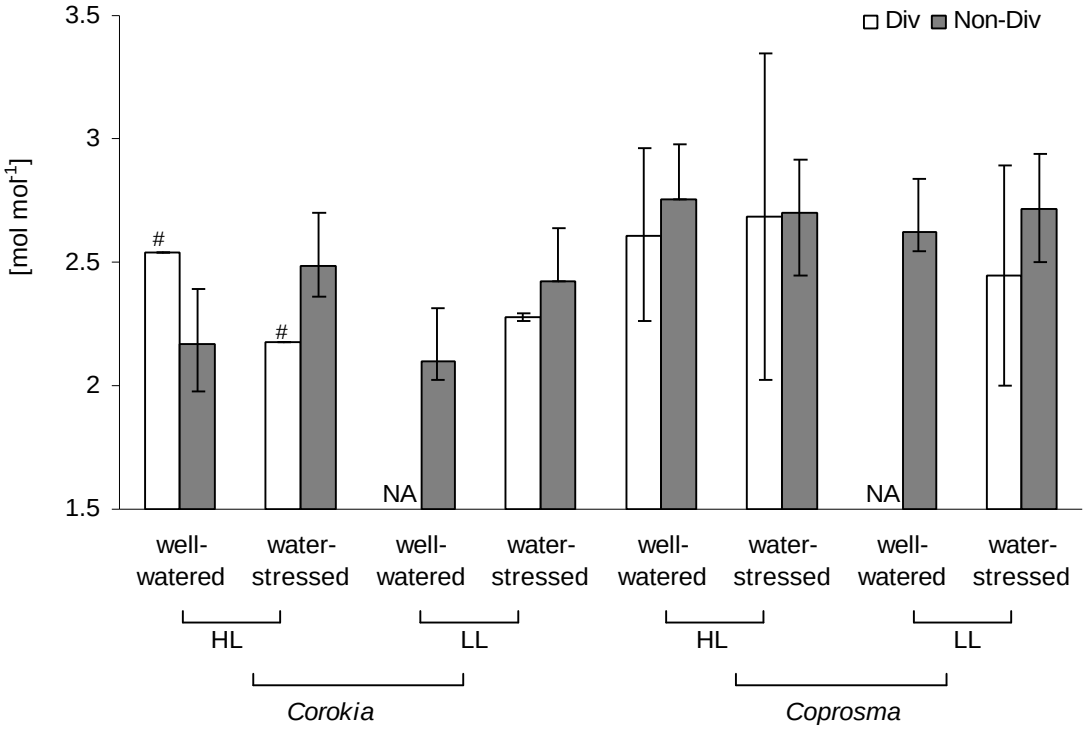



Figure 3.2.16: Ratio of chlorophyll a:b in the Glasshouse in 2002 for divaricate (Div) and non-divaricate (Non-Div) in well-watered and water stressed conditions and two different light treatments (HL = sun light, LL = shaded) [n = 4].

(Figure 3.2.18c). The non-divaricate *C. robusta* showed the highest ß-carotene per fresh weight concentrations in the shade and under drought.

a-Tocopherol Concentrations

Leaf samples used to estimate the a-tocopherol content of all plants grown in the glasshouse were taken in the late afternoons of summer in 2002. Habit showed significant effects on a-tocopherol content per unit total chlorophyll, with light level close to significant (Table A2.44). In all light and watering treatments, divaricates had higher concentrations of a-tocopherol per unit total chlorophyll (Figure 3.2.19a), in contrast to my hypothesis that non-divaricate leaves would have higher a-tocopherol concentrations than divaricate leaves. On a leaf area basis, a-tocopherol was significantly affected by light level, water availability, genus and habit (Table A2.55). The difference between the amounts of a-tocopherol per unit leaf area for divaricates versus non-divaricates was particularly prominent (Figure 3.2.19). *Coprosma propinqua* had the highest values overall. a-tocopherol per unit fresh weight was significantly affected

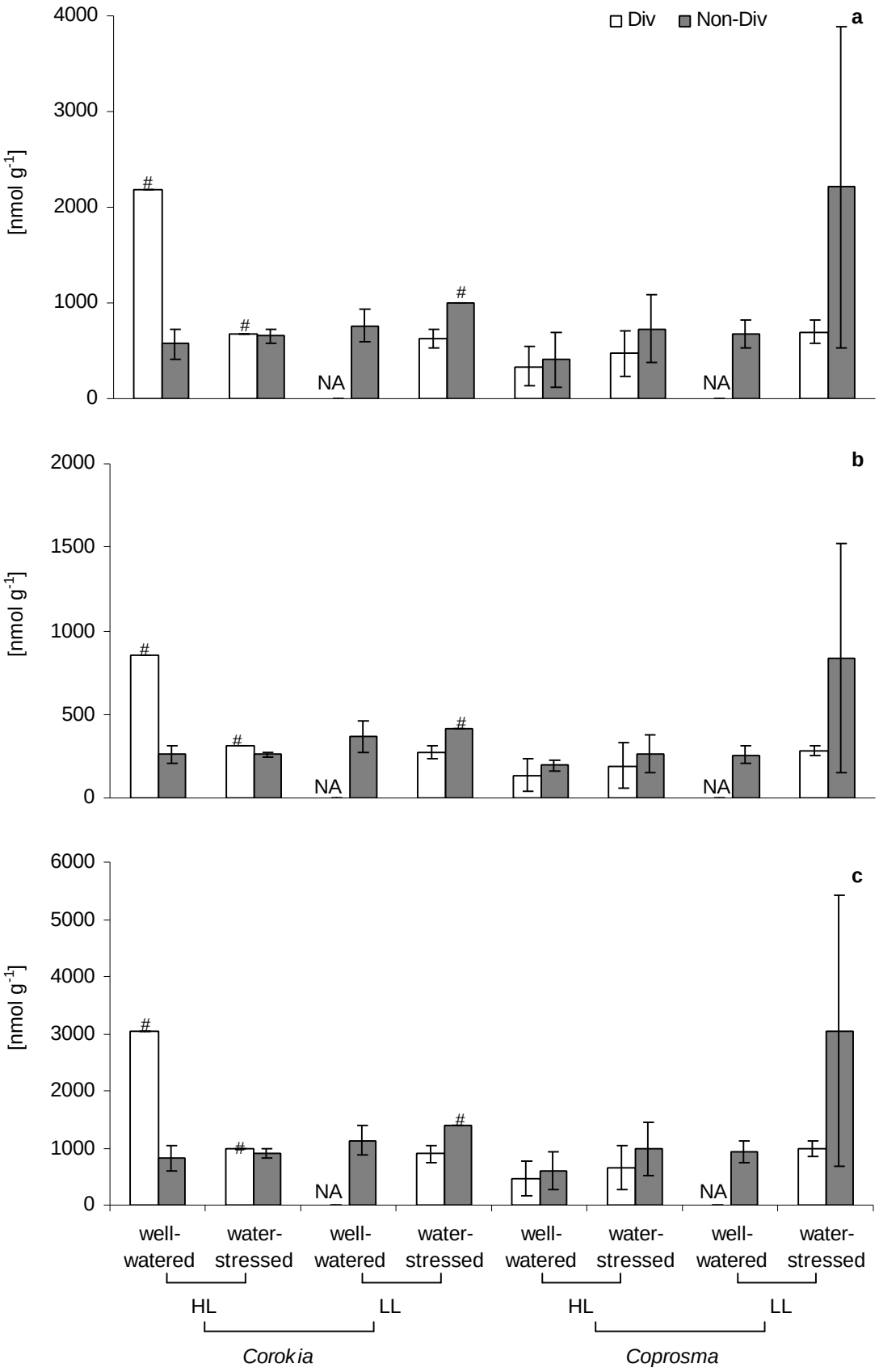



Figure 3.2.17: Concentrations of (a) chlorophyll a, (b) chlorophyll b and (c) chlorophyll a+b per unit fresh weight in the Glasshouse in 2002 for divaricate (Div) and non-divaricate leaves (Non-Div) in well-watered and water stressed conditions and two different light treatments (HL = sun light, LL = shaded) [n = 4].

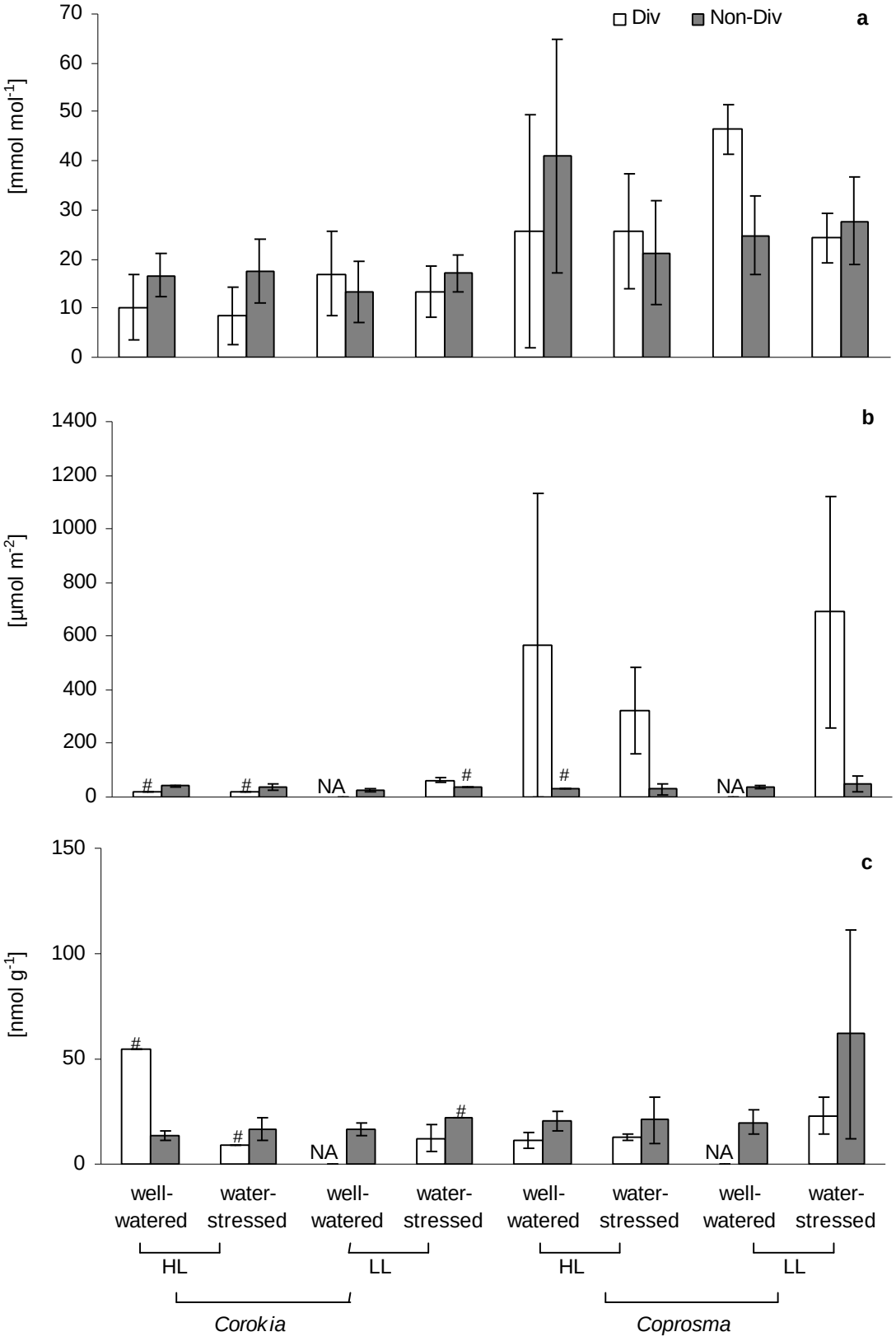



Figure 3.2.18: Concentration of ß-carotene per (a) total chlorophyll, (b) leaf area and (c) fresh weight in the Glasshouse in 2002 for divaricate (Div) and non-divaricate leaves (Non-Div) in well-watered and water stressed conditions and two different light treatments (HL = sun light, LL = shaded) [n = 4].

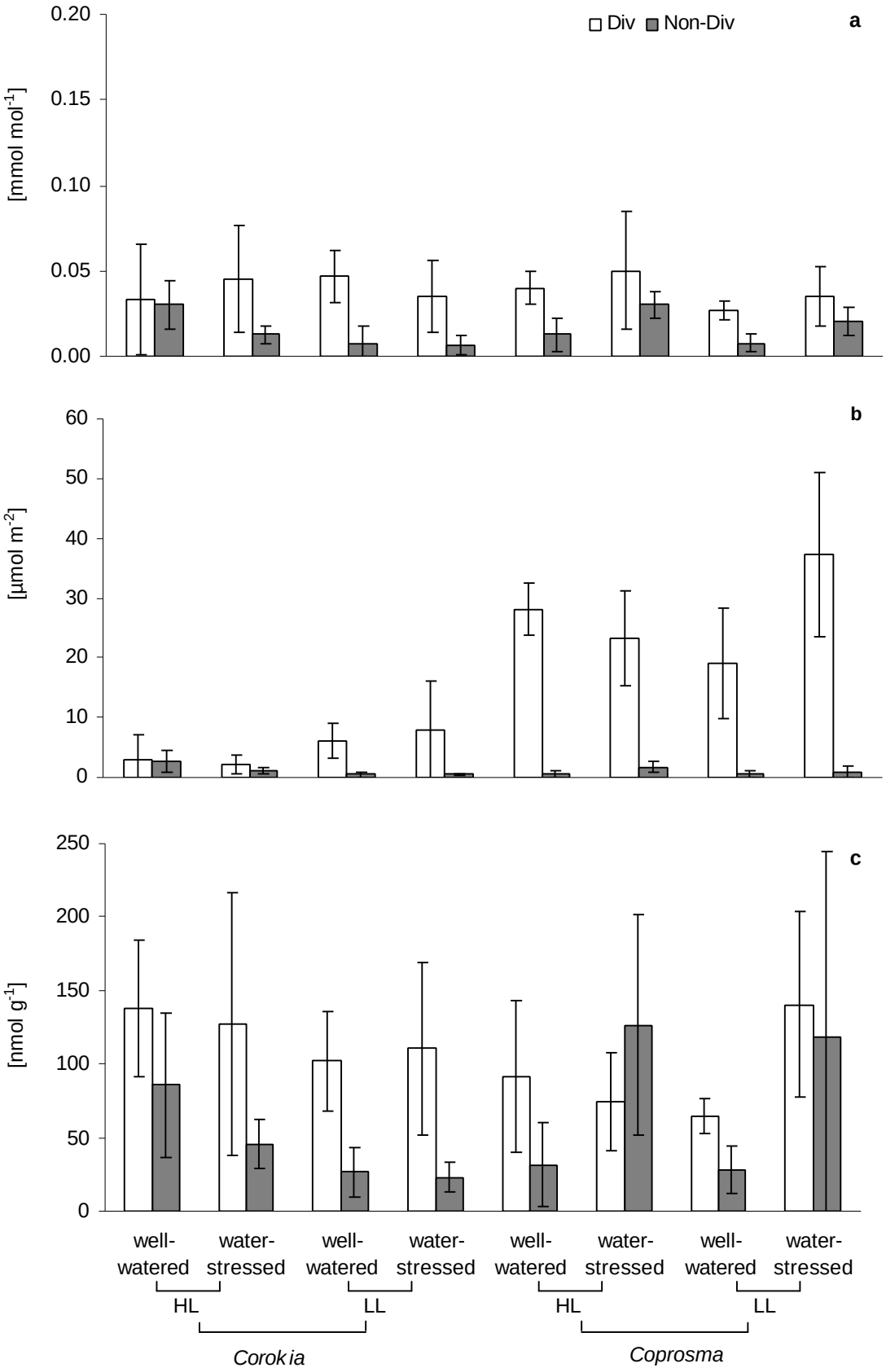



Figure 3.2.19: Concentrations of a-tocopherol per unit (a) total chlorophyll, (b) leaf area and (c) fresh weight in the Glasshouse in 2002 for divaricate (Div) and non-divaricate leaves (Non-Div) in well-watered and water stressed conditions and two different light treatments (HL = sun light, LL = shaded) [n = 4].

by water availability (Table A2.66). Divaricates and non-divaricates possessed higher a-tocopherol concentrations per unit fresh weight under well-watered conditions than under drought-stressed ones (Figure 3.2.19c). Only *Coprosma robusta* plants expressed rather high amount of a-tocopherol per fresh weight when grown in high light and under water-stress.

Summary of Results

Summarising the field trial and experimental set up, the experimental plants possessed higher concentrations of photoprotective pigments under high light conditions than in the shade. Also, drier habitats increased the concentrations of photoprotective pigments. In contrast to my hypothesis, divaricate leaves had at least similar concentrations of photoprotective pigments and a-tocopherol as non-divaricate leaves. Only antheraxanthin and zeaxanthin concentrations were significantly higher in non-divaricate leaves. The pigment concentrations found in the field were higher than the concentrations found in the glasshouse, in particular for de-epoxidised xanthophylls.

In the field experiment, pigment concentrations of *Corokia* and *Coprosma* were compared to sites of good and poor water availabilities and different light levels imposed in them (Section 2.1.2). As hypothesised, the installed light treatment had significant effects on the concentration of antheraxanthin, zeaxanthin, pigments in the de-epoxidation state as well as on chlorophyll a and chlorophyll b. All pigments of the xanthophyll cycle reached their highest concentrations in leaves grown in natural light or when interior leaves were exposed to exterior radiation loads. The naturally existing difference in water availability had significant effects only on the concentrations of violaxanthin and a-tocopherol, contrary to my hypothesis (Section 3.2.1). A significant effect of genus was found on nearly all pigments. The ratio of chlorophyll a:b was significantly different between *Corokia* and *Coprosma*. Concentrations of ß-carotene were increased in exposed leaves, but also in plants of the streambed. a-tocopherol was also higher in plants growing in the streambed compared to plants grown on the N-facing slope when calculated on unit total chlorophyll or unit fresh weight. When displayed on leaf area basis, the leaves of the N-facing slope expressed higher a-tocopherol values than the leaves from plants of

the streambed. A significant effect of genus was found for a-tocopherol by reference on total chlorophyll, leaf area and fresh weight basis.

Experimental plants were also grown under different water and light treatments in the glasshouse (Section 2.1.3). Significant effects of light level were only determined for the content of a-tocopherol. The water availability showed significant effects on the concentrations of ß-carotene per unit total chlorophyll and a-tocopherol per unit leaf area and fresh weight only. Most pigment concentrations and the content of a-tocopherol (calculated on unit leaf area) were significantly affected by genus and habit as well as the interaction of genus* habit. The interaction of light level* water availability significantly influenced the concentrations of violaxanthin, lutein and ß-carotene when calculated on unit fresh weight. The light level* habit interaction had significant effects on the concentrations of violaxanthin, lutein, chlorophyll a, chlorophyll b (calculated on unit fresh weight) and a-tocopherol (per unit leaf area). Antheraxanthin displayed its highest concentrations in non-divaricate leaves in high light. Divaricate leaves had higher concentrations of lutein, shaded *Coprosma* leaves showed the highest values. The concentrations of chlorophyll a and chlorophyll b were highest in divaricate leaves of *Coprosma*. The ratio of chlorophyll a:b expressed the lowest values in shaded *Corokia* leaves under water-stressed conditions. Per unit fresh weight, *Corokia cotoneaster* possessed the highest concentrations of ß-carotene in high light and under drought, whereas *Coprosma robusta* had its highest concentrations in shaded and well-watered conditions. a-tocopherol concentrations were greatest in divaricate leaves, but also showed increases in high light and in well-watered plants.

### 3.2.4 Discussion

During the summer months, divaricate and non-divaricate plants were exposed to a wide variety of stress factors. This research investigated the combination of high light loads and drought stress and the biochemical response of leaves of two genera which both express different growth forms in closely related species. Response patterns of pigment and a-tocopherol concentrations towards different light and water availabilities were examined for two divaricate species in an alpine field experiment (Section 2.1.2), and for divaricate and non-divaricate leaves under experimental conditions (Section 2.1.3). On the basis of their unique growth forms, divaricate leaves are seen as 'self-shaded', whereas their non-divaricate congeners receive high radiation loads in 'unprotected' leaves. In Section 3.2.1, I hypothesised that divaricate leaves would display lower concentrations of photoprotective pigments and a-tocopherol than their non-divaricate congeners due to their unique growth form. The divaricate habit was hypothesised to provide shade to interior leaves and therefore lower the stress of high light and drought to these leaves.

#### Result Evaluation

During the summer months leaf samples from the field trial and the glasshouse experiment were taken from all divaricates and non-divaricates in the late afternoon. In the field experiment, the light treatment had significant effects on antheraxanthin, zeaxanthin, pigments in the de-epoxidation state, chlorophyll a and chlorophyll b. Interestingly, the light treatment applied to the glasshouse plants had significant effects only on the content of a-tocopherol, but not on the photoprotective pigments. That result contrasts my hypothesis and the results found in the literature (Section 3.2.1). Concentrations of these pigments differ significantly between shade and sun leaves (Lichtenthaler *et al.*, 1981). Photoprotective pigments should have appeared in higher concentrations in high light situations such as in the sunny positions in the glasshouse as well as in sun light positions and in exposed leaves in the field. Additional drought stress was thought to increase the concentrations of the xanthophyll cycle pigments and a-tocopherol in particular, because of the hypothesised increase in contents of reactive oxygen species in the chloroplasts of these stressed plants. As discussed before (Section 2.2.4 and 3.1.4), it is possible that the shading in the glasshouse was not sufficient enough to embrace big differences in

the physiological response of divaricate or non-divaricate leaves. The light level* habit interaction was significantly affecting violaxanthin, chlorophyll a, chlorophyll b and a-tocopherol concentrations in plants in the glasshouse.

The different water availabilities of the streambed and N-facing slope in the field experiment affected the concentrations of violaxanthin and a-tocopherol significantly. In the glasshouse experiment, the concentrations of ß-carotene and a-tocopherol were significantly affected by the water treatments. Garcia-Plazaola & Becerril (2000) compared beech seedlings (*Fagus sylvatica* L.) from different regions and their ability to adapt to water limited habitats. Initially, the differences in pigment and antioxidant concentrations were significant, but diminished with the age of the leaves. Cotyledons expressed the highest concentrations of antioxidants and de-epoxidised xanthophylls, implying increased requirements for protection against photo-oxidative damage. Short-term experiments by Tausz *et al*. (2001) showed decreases in maximal stomatal conductance in *Pinus canarienis* seedlings in mild drought conditions, when compared with irrigated plants. Higher concentrations of de-epoxidised xanthophylls were found in unshaded needles of seedlings under drought conditions. ß-carotene and a-tocopherol concentrations did not differ with drought exposure. In this present study species displayed a response of ß-carotene as photoprotective pigment and a-tocopherol as antioxidant towards increasing light and drought stress. In particular, drier habitats (streambed, see Section 2.2.4) increase the amount of photoprotective pigments such as xanthophyll cycle pigments and ß-carotene in leaves in the field experiment. Peñuelas *et al.* (2004) have also pointed to the significance of photoprotective mechanisms for plants growing under drought. They determined amounts of photoprotective and antioxidative substances in irrigated and non-irrigated plants. There was no photo- or oxidative damage found (Peñuelas *et al.*, 2004), even with a decrease of the net photosynthetic rate and stomatal conductance by 90%. Amounts of zeaxanthin and a-tocopherol increased threefold, but ß-carotene decreased to 50%. There was no significant effect between irrigated and non-irrigated plants in respect to maximum efficiency of photosystem II. Peñuelas *et al.* (2004) summarised their findings as photoprotective mechanisms avoiding photoinhibition in mediterranean plants under summer drought.

A significant effect of genus was found for most pigments and the a-tocopherol concentrations in the field and glasshouse trial. Also, in the glasshouse experiment a significant effect of habit and genus* habit on pigments and a-tocopherol concentrations was found. I hypothesised to find a difference in pigment concentrations and a-tocopherol between different habits, because the divaricate and non-divaricate growth forms were assumed to adapt differently to high light and drought conditions found in summer months. The divaricate growth form was hypothesised to provide an advantage by sheltering and 'self-shading' its leaves (Section 3.2.1). In contrast to my hypothesis, ß-carotene and a-tocopherol concentrations per leaf area basis were significantly higher in divaricate than non-divaricate leaves. Also in contrast to my hypothesis, the difference in pigment and a-tocopherol concentrations in the response to the light and water treatments was greater for each species than for the growth forms.

Technical Difficulties

Unfortunately, large numbers of samples were lost during the HPLC analysis due to technical difficulties (Section 3.2.2). Choosing the right samples for the analysis of pigments and antioxidants was difficult for divaricate plants. The largest apparently functional leaves are situated in the middle of the shrub with a dense branch structure around. Difficulties arose from the hard to determine leaf age, because divaricate plants are semi-deciduous. To harvest 0.5g of leaf material for the analysis via the HPLC system (Section 3.2.2), a substantial number of leaves had to be picked from each sample site. Therefore, it was not possible to mark new leaves before the experiment or to distinguish between leaves of different ages in any other practical way. Altogether, it made it not only challenging to reach leaf samples in sufficient numbers in a given sample area, but also to judge age and amount of senescence or photobleaching per sample taken. It is possible that in some samples, and in particular for samples from the field trial, aged leaves or leaves with unusually high amounts of stress signs were included. This may partly explain the high variability in the pigment and antioxidant concentrations.

Öquist *et al*. (1978) analysed seasonal changes in chlorophyll concentrations in *Pinus sylvestris*. They found that chlorophyll was synthesized in spring and reached its maximum concentrations in summer. During the synthesis of chlorophyll, the

chlorophyll a:b ratio also increases up to a maximum in summer. In autumn the chlorophyll decreases and yellowing of the leaves results from relative increases in carotenoids in the needles. Seasonal changes in pigments of the xanthophyll cycle were described for *Pinus sylvestris* by Ottander *et al*. (1995). Total xanthophyll concentrations and amounts of de-epoxidised xanthophylls increased from April through September, but decreased in the winter months. Even fully developed leaves are influenced by radiation; post-expansional acclimatisation can be induced by internal and external factors affecting leaves. Considering the seasonal changes in the pigment compositions it is possible that response of divaricate leaves to light and drought are blurred due to the collection of leaves of different age.

Tegischer *et al.* (2002) compared needles of different ages of Norway spruce in respect to the amounts of photoprotective pigments and antioxidants and their biomasses. Second year needles contained higher concentrations of chlorophyll, de-epoxidised xanthophyll cycle pigments and a-tocopherol per biomass as well as per leaf area than leaves of the recent year. With increasing tree age, the amounts of chlorophyll and a-tocopherol per leaf area declined, but increased or did not change when compared on a mass basis. Due to the difficulties to determine the age or stage of senescence of divaricate leaves, inaccuracies in the determination of pigment and a-tocopherol concentrations could have occurred and influenced the statistical analyses in my study. Tausz *et al.* (1996) characterized losses in chlorophylls, xanthophylls, and carotenes with increased needle ages. Electron-microscopically determined reductions in the thylakoid membranes were associated with decreased a-carotene/ ß-carotene ratios. Higher amounts of neoxanthin were found in needles with lightened plastoglobuli. As argued above, it was not possible to determine the exact age of divaricate leaves. In Section 3.2.3, high neoxanthin concentrations per leaf area basis were shown for divaricate *Coprosma* leaves in the field and glasshouse trial. A relationship between decreased chlorophyll, xanthophyll or ß-carotene concentrations and increased neoxanthin concentrations could not be established due to the experimental layout of my study. The accurate determination of the time when divaricate leaves shed would be of advantage for further predictions.

Response to Light

In summer, along with reduced water availability, higher PFDs and temperatures are also stress factors for the plants. An increase in photoprotective pigment concentrations, such as xanthophyll cycle pigments and a decrease in pigments in the de-epoxidated state show a reaction to excessive PFD. Additionally, an increase in the concentrations of antioxidants, such as ?-tocopherol, indicates a protective reaction against high PFDs. Divaricate shrubs, with self-shaded leaves were hypothesised to not have high concentrations of photoprotective pigments or antioxidants compared to concentrations found in non-divaricate leaves.

In high light conditions, antheraxanthin and zeaxanthin, developed via de-epoxidase reaction from violaxanthin, dissipate excessive excitation energy (Lawlor, 1990). During times of low light availabilities, violaxanthin is recycled (Section 3.2.1). Field-grown divaricate *Corokia* and *Coprosma* leaves displayed an increase in the concentrations of xanthophyll cycle pigments in response to natural light or high light exposure. Significant effects of light level were found on antheraxanthin, zeaxanthin and the pigments in the de-epoxidation state, whereas violaxanthin was significantly affected by the water availability. Therefore, both divaricate genera expressed forms of photoprotection via the xanthophyll cycle in response to increasing light, even with good water supply. The water availability provoked different responses in the concentrations of the xanthophyll cycle pigments for *Corokia* versus *Coprosma*. In the glasshouse, concentrations of the xanthophyll cycle pigments violaxanthin and antheraxanthin varied between genera. Violaxanthin concentrations differed in response to water availability and habit. High light and good water supply decreased the amounts of violaxanthin found in divaricate and non-divaricate plants. The highest antheraxanthin concentrations were detected in non-divaricate leaves under high light. Obviously, in all plants, mechanisms of photoprotection increased under high light conditions. However, the increase of xanthophyll cycle pigments was higher in non-divaricate leaves than divaricate leaves under similar growing conditions. This is consistent with the hypothesis that divaricate leaves have a better protection against high light loads due to their ‘self-shading’ growth form. In particular when the pigment concentrations were calculated on a fresh weight basis, non-divaricate leaves had high concentrations of antheraxanthin and zeaxanthin. By comparison to the

concentrations found in divaricate leaves in the field, the glasshouse grown non-divaricate leaves had only very small concentrations of xanthophyll cycle pigments. Analysis of pigment concentrations of divaricate and non-divaricate plants grown under natural conditions would provide a better test of my hypothesis.

Normally, concentrations of neoxanthin and lutein do not differ with light exposure, because both pigments have structural functions (Logan *et al*., 1998). Yamamoto & Bassi (1996) argued that lutein is to be found in the minor and bulk LHCs of photosystem II. In my study some differences (light level, genera) in the concentrations of neoxanthin and lutein between treatments and divaricate and non-divaricate leaves were found. So far, it is not clear why the concentrations of neoxanthin and lutein changed with different light treatments and between the species. Outer antennae have more neoxanthin in photosystem II and lutein in photosystem I and II compared to the core complexes (Yamamoto & Bassi, 1996). In *Coprosma* leaves, not only high amounts of neoxanthin and lutein but also a higher chlorophyll a:b ratio were found. This correlation implies increased concentrations of LHC-bound chlorophyll compared to core complex chlorophyll in *Coprosma*. Typically, leaves under shaded conditions have a bigger antennae size, which enhances the efficiency of photon absorption. In self-shaded leaves it was predicted that more shade characteristics in the pigment concentrations would be found (Section 3.2.2). An increase in the antennae size could be interpreted in that respect. In contrast, a higher chlorophyll a:b ratio was found in sun leaves, whereas shade leaves have lower ratios. The amount of chlorophyll a and chlorophyll b per chloroplast volume was eminently higher in shade leaves than in sun leaves. A large amount of the total chlorophyll is located in the chlorophyll b rich light harvesting complexes (Section 3.2.1). Therefore, the ratio of chlorophyll a:b should be lower in self-shading leaves than in sun leaves.

Pigment characteristics also indicate particular structural features of the photosynthetic apparatus. Garcia-Plazaola *et al*. (2000) showed acclimation patterns for *Fagus sylvatica* L. in a vertical light gradient, where the highest chlorophyll a:b ratios were reached in sun leaves. In shade leaves, the amount of chlorophyll in the antennae compared to the reaction centers is higher. Lichtenthaler *et al.* (1981) investigated the adaptation of *Fagus sylvatica* L. leaves in the sun and shade. In their

study, the ratios for chlorophyll a:b were much higher that those of Garcia-Plazaola *et al*. (2000) and in this present study. Neither group described experimental light conditions and this makes it hard to relate their findings to each other or my study. In the field, chlorophyll a and chlorophyll b concentrations decreased in water-limited habitats as well as under natural light conditions and exposed leaves compared to shaded leaves. Significant effects were displayed on light level and genus. The glasshouse set up showed concentrations of chlorophyll a and chlorophyll b were highest in divaricate leaves of *Coprosma*, significant effects were found on water availability, genus, habit and genus* habit. Anderson *et al*. (1988) argued that the photosynthetic apparatus and therefore the light-harvesting antennae of the photosystems, components of the electron transport chain and the ATP synthase change in respond to varying light quality and quantity. These changes affect thylakoid membrane organisation as well as photosynthetic capacity. Further, morphological differences between sun- and shade-adapted leaves also occur. Shaded plants have larger chloroplasts with greater areas of thylakoid membranes and more thylakoids per granum. Qualitatively, the amount of total chlorophyll associated with LHC II increases, whereas the number of photosystem I LHC complexes decreases. With increased sun light, the amount of P680 can increase by up to 70% (Chow & Anderson, 1987). Chlorophyll b is located in the outer antennae of both photosystems and is enriched in photosystem II versus photosystem I (Yamamoto & Bassi, 1996). In my study, higher concentrations of chlorophyll b could be attributed to higher concentrations of LHC bound chlorophyll to the total chlorophyll in *Coprosma*s.

The ratio of chlorophyll a:b was significantly affected by genus in the field experiment. In the glasshouse trial, chlorophyll a:b ratio was also significantly affected by genus with water-limited and shaded *Corokia* leaves expressing the lowest ratios of 2.3. The higher ratios of 2.7 were expressed by *C. propinqua*. Greater ratios of chlorophyll a:b are normally expressed in leaves acclimated to high PFDs compared to leaves grown in shaded positions. Therefore, the hypothesis that ‘self-shaded’ divaricate leaves would have lower chlorophyll a:b ratios was not supported. Johnson *et al*. (1993) could not reveal a close relationship between the chlorophyll a:b ratio and growth PFD. A higher ratio of photosystem II to photosystem I could explain the high chlorophyll a:b ratios. As seen above, increased antennae sizes indicate shade leaf responses, but the increased chlorophyll a:b contradicts these

responses. Kelly & Ogle (1990) measured a variety of microclimatic factors in different areas in divaricate shrubs and compared them to the outside climate. Although most of the measured parameters showed only small differences, some were significant. Therefore, it is possible that the self-shading of divaricate shrubs gives these leaves some protection but is not efficient enough to formulate true 'shade leaf' characteristics. The pigment composition of *Coprosma* leaves is similar to that found in shade- adapted leaves. Higher LHC chlorophyll to core complex chlorophyll concentrations could explain most of the pigment characteristics of *Coprosma* versus *Corokia*. That suggests a higher contribution of outer antennae chlorophyll to total chlorophyll in the photosynthetic apparatus of *Coprosma* leaves, indicating shade characteristics. Genetically determined differences in the response to light by chloroplasts can be as important as specific structural features in *Coprosma* shrubs as chloroplasts can be exposed to different internal radiation environments in the mesophyll.

Carotenoids are essential components of pigment-protein-complexes. They have functions in the light harvesting complexes, are structurally required in the antenna and reaction centers and protect against photo-oxidation. ß-carotene quenches singulet oxygen in the core complexes of photosystem II and dissipates excessive excitation energy (Section 3.2.1). In the field experiment, significant effects of genus and the interaction of light level* genus on the ß-carotene concentration were found. Concentrations of ß-carotene were highest in the interior leaves of divaricate plants, exposed to outside radiation loads, supporting my hypothesis that increased ß-carotene concentrations should be found in plants under high light stress. Oddly, higher quantities of ß-carotene were also found in plants grown in the streambed. In contrast to my hypothesis, the streambed was the site with lower water availability (Section 2.2.4). In the glasshouse, concentrations of ß-carotene were significantly affected by water availability and genus. *Corokia cotoneaster* possessed the highest concentrations of ß-carotene per unit fresh weight in high light and under drought, whereas *Coprosma robusta* expressed the highest concentrations in shaded and well-watered conditions. Carotenoids have photoprotective functions in the chloroplasts. Their contents are generally correlated to the amount of excessive excitation energy that has to be thermally dissipated (Osmond, 1994). High growth PFD results in higher demands of xanthophyll cycle pigments and ß-carotene to meet the greater

necessity of photoprotection. In the field and glasshouse trial, divaricate and non-divaricate leaves displayed an increase in ß-carotene with increasing light exposure as well as water-limiting conditions. In contrast to my hypothesis (Section 3.2.2) that the self-shading growth form of divaricate leaves reduces high light loads and therefore divaricate leaves would have low concentrations of photoprotective pigments, significantly higher concentrations of ß-carotene per leaf area basis were found in divaricate leaves than non-divaricate leaves. But, the highest concentrations were found in exposed divaricate leaves, which lost their self-shading branch layer due to the experimental set up. Drought stress also increased the amounts of ß-carotene and therefore photoprotective response in leaves of plants in the glasshouse trial. That finding is in accord with results of Smirnoff (1993), Pastori *et al*. (2000) and Pastori & Foyer (2002), who found high amounts of photoprotective pigments and antioxidants in plants under water-limited conditions, due to stress-induced higher accumulations of active oxygen species.

Demmig-Adams (1994) investigated pigment compositions of sun-loving and shade-tolerant species under their natural light environment. The sun-exposed species had higher concentrations of carotenoids and therefore thermal dissipation. By reversing the light conditions, shade-acclimated plants adapted to the new PFD conditions with increased concentrations of xanthophyll cycle pigments, in particular antheraxanthin and zeaxanthin. Xanthophyll cycle pigments and ß-carotene concentrations increase with increasing light exposure (Grace & Logan, 1996). The present study supports their photoprotective role in *Corokia* and *Coprosma* species under different light treatments. The relation of de-epoxidated xanthophylls to the sum of the xanthophyll cycle pigments, the de-epoxidation state, shows higher values in sun-exposed leaves than in shaded leaves. A high epoxidation state shows that the photon absorption is higher than energy conversion in the dark reactions (Lawlor, 1990).

Response of Antioxidants to Light

a-Tocopherol is not only stabilizing membranes biophysically by binding poly-unsaturated fatty acids in the membranes, but also protects membranes against singlet oxygen and lipid peroxidation (Section 3.2.1). Expression of antioxidant systems generally increases with the light consumption (Logan *et al*., 1998). The highest concentrations of hydrophilic and lipophilic antioxidants are found in chloroplasts,

where they prevent damage to structural components and membranes by active oxygen species (Tappel, 1962 and Kunert & Ederer, 1985). This correlates with the function of dissipating excessive excitation energy. For plants under similar radiation loads, the amount of antioxidants also depends on other external stress factors (Garcia-Plazaola *et al*., 1999). Mehlhorn *et al.* (1986) showed increased antioxidant concentrations for conifers under elevated ozone and sulphur dioxide treatments/ concentrations. The concentration of a-tocopherol per total chlorophyll increases with increased light exposure, which reflected an increased load of active oxygen species (Garcia-Plazaola *et al.*, 2000). In my study, the two genera responded to given treatments in the field but displayed different a-tocopherol concentrations. A clear difference was seen between plants growing in the streambed and plants grown on the N-facing slope. In contrast to my hypothesis that water- limited plants on the N-facing slope would show increased antioxidant concentrations, the two genera had high amounts of a-tocopherol in the streambed, where the water supply was hypothesised not to be limited, but where more negative shoot water potentials were found. This effect of low water potentials was even more pronounced in the lower rainfall year (2002/03). The plants grown in the glasshouse expressed high concentrations of a-tocopherol in high light, but also under good water supply. The highest concentrations were found in divaricate leaves. Significant effects of light level, water availability, genus and habit in particular were found in the glasshouse trial when related on leaf area basis. Overall, divaricate and non-divaricate leaves both showed increased antioxidant concentrations under stress conditions. The difference between the genera was more pronounced than between divaricate and non-divaricate habits.

As argued in other studies (Hansen *et al*., 2003), a-tocopherol is not only an antioxidant, but also accumulates in the cell. Kunert & Ederer (1985) showed increased a-tocopherol concentrations in *Fagus sylvatica* and *Abies alba* with increasing age of needles and leaves. a-Tocopherol is stored in plastoglobuli of the chloroplast stroma. The large storage pools can sometimes over rite seasonal changes. In the case of the semi-deciduous divaricate leaves, the higher concentrations could reflect an accumulation rather than an active antioxidant defence mechanism. Lichtenthaler *et al*. (1981) showed that a-tocopherol accumulation could result from storing excessive prenylquinones, mainly a-tocopherol and reduced plastochinones, in plastoglobuli in aged leaves. Ontogenetically, old leaves sometimes exhibit relatively

high amounts of a-tocopherol. Therefore, a photo-protective reaction or acclimatisation to stress factors via a-tocopherol concentration can be masked by these stored amounts in the leaves. Only small concentrations of a-tocopherol could be localised in thylakoids. Whether or not the stored a-tocopherol is involved in photoprotective reactions against reactive oxygen species in leaf cells is not always clear. More research is necessary to identify the reasons for a-tocopherol accumulation in mature leaves. I did not investigate the component of the a-tocopherol occurrence, but used the whole leaf to determine a-tocopherol concentrations. An extraction method which could divide the plastoglobuli storage pool from the rest would be desirable.

Ellenberg (1986) found a connection between increased a-tocopherol concentrations and leaves growing under water shortages. It is usually explained with an increased shortage of water in sun versus shade leaves, but also with a higher possibility of understorey trees to face water restrictions relative to mature trees. I found significant effects of water availability on the a-tocopherol concentration in the field and in the glasshouse experiment. Unlike Ellenberg, I investigated only interior leaves of divaricate shrubs, which were supposed to be self-shaded. The non-divaricate shrubs of the glasshouse trial were equally exposed to sun or shade treatments, they were too young to develop a crown structure, which could have affected the a-tocopherol concentration as argued by Ellenberg. As seen in Section 3.2.1, divaricate shrubs are argued to be developed from understorey trees. I used mature shrubs in the field, which were not shaded by other vegetation. Therefore, it is not possible to refute or verify Ellenberg's argumentation with my study.

Conclusions

An adjustment in the pigment compositions in response to light and water regime was expressed for all species. The original assumption that the 'self-shading' growth form of divaricates would reduce the amount of required photoprotection, expressed by an increase of photoprotective operating pigments, was not completely verified. As seen for the xanthophyll cycle pigments divaricate and non-divaricate leaves showed a response to increasing light, and in the glasshouse non-divaricate leaves had the highest concentrations of de-epoxidised pigments. But compared to the concentrations of de-epoxidised pigments found in divaricate leaves in the field, all concentrations in

the glasshouse experiment were substantially lower. The increased concentrations of neoxanthin and lutein indicate increased antennae sizes in divaricate leaves, which were normally found in shade leaves. The chlorophyll a:b ratio was highest in divaricate *Coprosma* leaves and the concentrations of ß-carotene were increased in divaricate leaves, indicating sun leaf characteristics. The concentrations of the antioxidant a-tocopherol were high in divaricate leaves, in contrast to their hypothesised 'self-shading' and therefore protecting characteristics. As discussed above, the 'self-shading' of divaricate leaves might not be sufficient to characterise these leaves as shade leaves and additional photoprotective adaptations to high light loads might be found. The glasshouse trial also had the disadvantage that all plants grew in pots. That limits water supply and root growth and could have had influences on the growth of those shrubs stems and leaves, even when the pot size was regularly up-sized. Niinemets *et al*. (2003) and Garcia-Plazaola *et al.* (2004) found slower responses in antioxidant and xanthophyll cycle compositions in plants grown under natural conditions than of plants in the growth chambers. As growth chambers and glasshouses give the opportunity to optimise experimental set ups, plants are also removed from a multitude of environmental factors, such as wind, rain or browsing animals. Therefore, results from experimental set up measurements have to be taken in careful consideration and might not always reflect the natural response of plants to the treatment applied.

In my study, all plants under reduced sun light were covered by shade cloth, which reduced the sun light by 25%. The leaf samples for divaricate shrubs were taken from the interior of these plants. As argued by McGlone & Webb (1981), the divaricate growth form provides already kind of 'self-shading' for interior leaves. In this context it is possible that the shading applied in the field and experimental set ups was not sufficient to evoke drastic changes in the pigment and antioxidant compositions of divaricate leaves. The exposure of interior leaves of divaricate shrubs in the field experiment showed clearly that these exposed leaves had increased concentrations of photoprotective pigments such as the xanthophyll cycle pigments, and here the pigments in the de-epoxidised state in particular as well as concentrations of ß-carotene. The a-tocopherol concentrations also increased in these exposed leaves compared to leaves under natural light or shade cloth.

The growth form of divaricate shrubs has been hypothesised to be a 'self-shading' mechanism for the leaves in the interior of these plants (McGlone & Webb, 1981). Therefore, it was hypothesised in my study that these leaves would receive less excessive radiation loads from the summer sun. In contrast, McGlone & Webb (1981) argued that the evolution of divarivate shrubs started in understorey plants exposed to shaded conditions. This could be argued in two directions [(1) and (2)].

(1) The divaricate leaves are genetically determined as understorey leaves, which are already adapted to shade conditions and therefore do not show dramatic changes to further shading. The concentrations of photoprotection would be low in such shaded leaves. Anderson *et al*. (1988) argued that on the forest floor PFD is the sum of two different components. About 40% of the total PFD is diffuse radiation, primarily of far red light. Up to 60% of the total PFD is recorded in sun flecks, with high PFDs over a short time period. Therefore, the photosynthetic apparatus has to adapt to use the PFD most effectively but also has to resist damaging effects as well as short- and long-term changes in light quality and quantity (Anderson & Osmond, 1987). Under low PFDs, light- harvesting is maximised by having the larger PS II photosynthetic units, less chlorophyll a-proteins and fewer $P_{680}$ units.

2) The divaricate shrub could be thought of as an already stressed plant, because the shading canopy trees were removed as a consequence of human arrival in New Zealand. Consequently, these plants have experienced higher light loads for a long time. Therefore we would expect them to show low or only minor increases in the amount of photoprotective pigments or antioxidants after the interior of the field plant was exposed to the exterior light conditions. Under high light loads the damaging effects of photoinhibition have to be avoided. Photosystem II antennae are smaller, more photosystem II reaction centres are accumulated and the photosystem II photosynthetic units are smaller but more frequent (Anderson & Osmond, 1987).

My field study did find low concentrations of photoprotective pigments and a-tocopherol in shaded divaricate plants and increased concentrations in exposed divaricate leaves, whereas the plants under natural light had a high content of photoprotective pigments and a-tocopherol. The low concentrations of photoprotective pigments and a-tocopherol in the shaded leaves would support the

assumption of McGlone & Webb (1981) and classify divaricate shrubs as understorey plants. The high concentrations of chlorophyll a and chlorophyll b as well as chlorophyll a:b ratio in all divaricate leaves indicate characteristics opposite of sun leaves. Also, the rather high concentration of photoprotective pigments and a-tocopherol in unchanged plants disapproved the understorey origin of divaricate shrubs. It could be argued that those former shade or understorey plants are in the process of adapting to human-made high light conditions, but the transition is far from completion yet due to the short elapsed time in evolutionary terms.

# 4. PHOTOSYNTHESIS AND RESPIRATION

Divaricate shrubs were hypothesised to avoid photoinhibition and the costs of photoprotection via their 'self-shading' growth form. Therefore it was hypothesised that divaricate plants have a higher carbon gain than their non-divaricate congeners, which lack this adaptation. This part of my study observed the influence of summer drought and high light loads on the daytime respiration, quantum efficiency and maximum photosynthetic rate of divaricate and non-divaricate leaves to test this hypothesis.

## 4.1 Introduction

The leaves of higher plants have mechanisms to prevent damage from high radiation loads, such as adaptable amounts of photoprotective pigments and antioxidants as well as thermal dissipation of excessive excitation energy. High light loads do not only cause photoinhibition and therefore damage to photosystem II but also reduce quantum efficiency of leaf photosynthesis (Osmond, 1994). Powles & Björkmann (1982) showed that the light-saturated rate of photosynthesis is also affected by photoinhibition. Björkman & Powles (1984) found quenched variable fluorescence ($F_v$) as well as reduced electron transport activity in isolated chloroplast membranes in water stressed *Nerium oleander* leaves. They showed that inhibited photon yield and inhibited $CO_2$ uptake were effects of photoinhibition and effects of water stress, which they found to be at least partly dependent on each other. Ludlow & Powles (1988) found that water stress induces photoinhibition and therefore reduces growth and grain yield.

Biomass production of terrestrial plants is a compromise between photosynthesis and transpiration, often limited by water availability. Photosynthesis is measured by $CO_2$ uptake, production of organic material and oxygen release. $CO_2$ uptake defines the rate of the Calvin cycle, whereas oxygen release reflects the non-cyclic electron transport. The respiratory metabolism of a plant, characterized by $O_2$ uptake and $CO_2$ release, interacts with the photosynthetic metabolism. It is dependent on light and temperature as well as the $CO_2$ concentration of the ambient air. This present study investigated the gas exchange parameters, such as daytime respiration and

photosynthetic rates of leaves with contrasting leaf sizes and plant growth forms grown under different light and water availabilities. Different leaf sizes and therefore boundary layers should cause different stomatal behaviour, which would be reflected by differences in the internal $CO_2$ concentrations and finally in different $CO_2$ uptake and carbon gain under water limitations or high PFDs.

Stomatal Behaviour

The response of photosynthetically active tissue to micro- and macroclimate is often measured by the response of the stomata to environmental changes. $CO_2$ and water share the same pathway for their diffusion into and out of the plant tissue via stomata (Geber & Dawson, 1997). Jarvis (1980) showed a direct relationship between low leaf water potential and stomatal closure, which also reduces carbon gain (Farquhar & Sharkey, 1982; Schulze, 1986). A negative feedback response among stomatal conductance, internal $CO_2$ concentration as well as PFD was found by Raschke *et al.* (1978). Raschke (1975) and Wong *et al.* (1979) hypothesised a constant internal $CO_2$ concentration, maintained by stomatal control and reaction, involving a response to carbon fixation rate and the metabolites of carbon fixation. Lange *et al.* (1971) found changes in stomatal conductance are directly related to changes in atmospheric humidity, whereas Barrs (1973) could not show a relationship between the two. In a turgid plant, the size of the stomatal aperture and hence stomatal conductance regulate the rate of photosynthesis. Water vapour diffusion is linearly related to stomatal conductance and therefore transpiration is restricted by low stomatal conductance. The balance between $CO_2$ assimilation and respiratory water loss is defined as water-use efficiency (Mohr & Schopfer, 1995). Stomatal limitations result in a lower actual photosynthetic rate. The maximum photosynthetic rate occurs when the stomata are fully open. Changes in temperature, $CO_2$ concentration and atmospheric water are sensitively registered and jointly determine stomatal behaviour (Farquhar & Wong, 1984; Ball & Farquhar, 1984; Ball *et al.*, 1987).

Other environmental factors such as light, vapour pressure deficit, molar fraction of $CO_2$ and air temperature influence stomatal conductance. Lowered water loss under conditions of low soil moisture and high vapour pressure deficits via stomatal control is crucial for plant tissue (Mielke *et al.*, 2000). Also, changes in wind speed determine the boundary layer conditions, energy balance and evapotranspiration of the canopy

and soil surface. Cheeseman (1991) argued that stomatal patchiness, meaning number of stomata, their distribution and relative conductance, is one of the most vital response factors in plants. As shown by Geber & Dawson (1997), genetic lines with larger leaves showed both higher specific leaf weight due to greater leaf thickness, and increases in mesophyll resistance. That limits maximum photosynthetic rate by limiting the $CO_2$ influx to the site of carboxylation, even when those leaves have high stomatal densities (Nobel, 1991).

The internal water balance or degree of water saturation of plant tissue is determined by the relative rates of water uptake and transpiration and is more important to plant growth than the absolute rate of water uptake or transpiration (Kramer, 1937). Changes in evaporation also alter the leaf temperature through the energy balance of the leaf and stomatal conductance and vice versa (Farquhar *et al*., 1980a).

Biochemistry

Photosynthesis of $C_3$ plants is driven by two main regulation mechanisms, electron transport capacity (Kirschbaum & Farquhar, 1984) and Calvin cycle biochemistry, in particular the concentration and activity of ribulose-1,5-bisphosphate carboxylase (Rubisco) and the regeneration of ribulose-1,5-bisphosphate (Farquhar & Caemmerer, 1982). The photosynthetic biochemistry is directly affected by temperature, in particular in the kinetic properties of the carboxylation reaction and ribulose-1,5-bisphosphate regeneration, which are affected by the solubility of $CO_2$ and $O_2$ and Rubisco affinity for $CO_2$ and $O_2$ (Farquhar & Caemmerer, 1982; Long, 1991). The demand for assimilates influences the rate of photosynthesis as well as the concentration of accumulated assimilates, such as starch and/ or sucrose; so called source-sink regulation similar to the end-product inhibition of biochemical reactions (Neales, 1968). Azcon-Bieto (1983) observed a decrease in photosynthesis at high carbohydrate concentrations resulting from impaired functioning of the Calvin Cycle. Edwards & Walker (1983) also found reduced quantum yields with declined concentrations of NADPH and ATP, which depended on the concentrations of inorganic phosphate in the chloroplasts. The movement of inorganic phosphate, released out of sucrose synthesis, influences the oppositional movement of the triose phosphate, which is produced in the Calvin Cycle (Fitter & Hay, 1987). Triose phosphate is synthesized to starch in the chloroplasts, or after transport to the cytosol,

to sucrose. Farrar (1993) modelled the feedback inhibition between starch and sucrose production and their inverse relationship via inorganic phosphate and the concentrations of ATP. Lower ATP concentrations also lowers the rate of ribulose-1,5-bisphosphate regeneration and therefore the carbon fixation rate.

Internal $CO_2$ concentration limits Rubisco activity and therefore is one of the major limitations to carbon fixation. At high internal $CO_2$ concentrations, photosynthesis can be limited by the regeneration rate of ribulose-1,5-bisphosphate. As Farquhar & Sharkey (1982) argue, $C_3$ plants should operate at an internal $CO_2$ concentration which co-limits the consumption and regeneration of ribulose-1,5-bisphosphate. Geber & Dawson (1997) showed a strong correlation between stomatal conductance and biochemistry in genetic lines of *Polygonum arenastrum*. High transpiration rates were positively related to high biochemical activities (high activities of Rubisco and a high electron transport capacity).

Environmental Influences

Considering the whole plant, the rates of carbon fixation have to be in balance with assimilate production and utilisation. Powles (1984) and Ludlow (1987) found that plants have the ability to avoid or at least tolerate water stress as they are able to avoid or tolerate photoinhibition. Björkman & Powles (1984) showed that water stress affects photosynthesis and growth long before photoinhibitory effects take place. Björkman *et al*. (1980) found correlations between high light and water stress affecting the photosynthesis of sclerophyllous *Nerium oleander*, but water stress on its own reduced the photosynthetic rate and electron transport similarly. Water-stressed leaves of sclerophyllous shrubs showed midday depressions in carbon assimilation in the study of Demming-Adams *et al*. (1989), and Mooney *et al*. (1977) showed decreased photon yield and light-saturated photosynthetic rates in the desert shrub *Larrea divaricate* under water stress. The internal $CO_2$ concentration did not affect the measurements of Mooney *et al*. (1977), as measurements under limited and saturated $CO_2$ concentrations obtained the same results. Boyer (1971) found the photosynthesis in sunflower leaves under severe water stress insensitive to changes in the leaf temperature and external $CO_2$ concentration, in particular under high light. Lowering water potentials led to stomatal closure, which decreased the photosynthetic rate.

Plant tissue shows two types of respiration. Dark respiration involves different ways of substrate oxidation, such as glycolysis or the tricarboxylic acid cycle. It includes the oxidation of NADH and $FADH_2$. Photorespiration produces $CO_2$ in the photorespiratory carbon oxidation cycle, in which ribulose-1,5-bisphosphate is oxygenated (Ögren, 1984). McCree (1970) related respiration linearly to total photosynthesis, reporting up to 25% of the total photosynthetic production in plant dry weight to be respired in the canopy.

Assimilates produced in plants are allocated to different structural components, such as leaves, stems, roots and seeds. Also, allocation varies with changing demands during plant development. Larcher (1995) divided plants into two groups, depending on their photosynthetic production and growth. Annual plants are characterized by a high photosynthetic capacity and a high proportion of photosynthetically active tissue and plant mass. They are also characterized by small leaf sizes, high stomatal conductance and low water use efficiency (Geber & Dawson, 1997). Biennial and perennial plants adopt a more conservative strategy, with a lower net rate of photosynthesis, and therefore growth. In contrast to the annual plants, which invest most of their carbon to produce photosynthetically active leaves, perennial plants can accumulate large storage pools to survive periods of unfavourable conditions, such as drier or colder periods. Wilson (1988) showed a growth and allocation response in plants under limiting resources. It was shown that increasing the relative mass of the plant component that reduced the stress factor could offset the negative effects. Increased root: shoot ratios were observed by Rufty (1984) in plants on nutrient-poor soils or under water stress, whereas decreased root: shoot ratios were observed in plants under low light.

Interspecific variation in taxa with shorter life spans are found, often with higher gas exchange rates and higher organic nitrogen per leaf area (Evans, 1989) as well as increased biochemical capacities to regulate photosynthesis than in slow-growing plants (Wullschleger, 1993). So far, genetic differences in photosynthetic rates of the same species have been assigned to changes in the concentration and activity of Rubisco (Caemmerer & Farquhar, 1981). Geber & Dawson (1997) showed in genetic lines with high gas exchange rates, plants with small leaves and early flowering also had high biochemical capacity with respect to Rubisco activity and electron transport.

They also correlated limitations on photosynthesis by stomatal closure to low photosynthetic rates and low intercellular $CO_2$ concentrations as well as late flowering and larger leaf sizes. Geber & Dawson (1997) also found evidence that slower plant development and maturation is related to longer life span and a lower gas exchange rate to a relative tolerance to drought stress. Givinish (1979) and Nobel (1991) argued that smaller leaves should be functionally favoured in areas of water restriction, as the lesser leaf area also reduces the transpiration surface and keeps a lower average temperature. Turnbull *et al*. (2002) showed a positive relationship between higher leaf photosynthesis and habitats with naturally greater water supply in *Acer*, whereas *Quercus* was able to maintain low transpiration rates and a high photosynthetic rate in drier habitats. In contrast, Grossnickle *et al*. (2004) could not find a relationship between gas exchange and long-term water use efficiency as a response to environmental conditions. Geber & Dawson (1997) argued that the transition from stress avoidance towards stress tolerance is linked not only with the transition from short-lived towards long-lived species, but also with gas exchange rates. Fast metabolisms are correlated with fast adaptations in biochemical activity rates and therefore stress avoidance.

Schwinning *et al.* (2002) investigated the gas exchange rates of shrubs grown in arid zones and their seasonal use of large and rare rain events. Interestingly, the three species investigated used the additional water differently, which was partly due to different zones of water uptake via roots, but all showed increased gas exchange rates after rain events. An increase in the rate of photosynthesis up to 4-fold was seen in *Hilaria jamesii*, accompanied by a 2.7-fold increase in water use efficiency after a summer rain. Following a rain event in spring, gas exchange rates in this species increased about four fold.

Kemp *et al.* (1997) showed different capacities of water uptake, which also varied in their capacity to extract water from certain soil layers. They also showed that the rate of extraction was independent of the soil layer from which the water was extracted. In the study of Schwinning *et al*. (2002), shrubs used a third of the transpirational water from the rainwater of lower soil layers, additional to the water uptake from deeper soil layers, whereas grasses of the same vegetation zone used only deeper soil layer water. Similarities can also be found between low night temperatures and photosynthesis

under water stress, which was slowly applied to grapevines (Flexas *et al.*, 1999). Water stress induced by chilling and drought can enhance $O_2$ uptake and rates of electron flow, whereas the $CO_2$ exchange rate decreases towards zero in stressed leaves.

Aim of the Study

Overall, my study investigated the influence of the combination of physiological drought, high PFDs and high temperatures on divaricate and non-divaricate plants during summer in a field trial and glasshouse experiment.

High PFD's above the optimum of a plant species negatively affect photosynthesis and induce photoinhibition (Chapter 3). Photoinhibition is usually reversible and plants are capable of light acclimatisation by the expression of resistance mechanisms. Water stress affects leaf water potential as well as net photosynthesis and leaf conductance because of stomatal closure during drought. Internal $CO_2$ concentration is lowered during drought. Predawn water potential and leaf gas exchange are variable with rainfall and water content of the soil and therefore vary seasonally. Kramer & Boyer (1995) showed that the water equilibrium between plant and soil is well indicated by the predawn leaf water potential. As seen above, terrestrial plants can be limited by stomatal behaviour as well as leaf biochemical capacity. Geber & Dawson (1997) indicate in their study on *Polygonum arenastrum* that changes in biochemistry are related to changes in stomatal characteristics and vice versa.

This part of the study determined the photosynthetic reactions of divaricate and non-divaricate leaves to different light and water availabilities. Previous studies have investigated the single factors of drought or high light loads, but the combination of them and their relation to different growth forms of related species has not been previously studied. The species investigated are not only closely related, but can also form natural hybrids. The most interesting feature of them though, is their contrasting growth forms (Chapter 1). The divaricate species are often found under more extreme climatic conditions than their non-divaricate congeners. The differences in water and radiation loads between the divaricate and non-divaricate habitats make these species an ideal model system to test whether the divaricate growth form is an adaptation which provides benefits in conditions of drought and high irradiation. Additionally,

the combination of field experimentation and glasshouse trials (Section 2.1) gave my study added breadth. This made it possible to observe and study divaricate and non-divaricate plants, grown from known sources and habitats, under customized and well-defined conditions.

The experiments described in this chapter were guided by a series of hypotheses. The small leaf area of divaricate shrubs was hypothesised to assist these plants to maintain tissue turgor pressure even with increasing water vapour deficit and therefore increased evaporation rates in summer. The daytime respiration rate in divaricate leaves should not increase dramatically under high temperatures or PFD, because of the much smaller leaf surface and self-shaded growth form, which was hypothesised to reduce temperature increases in the leaves. Avoiding stomatal closure, when temperatures are high and air humidity and/ or soil moisture low, should keep the internal $CO_2$ concentration more constant and therefore the photosynthetic rate should be buffered, hence high maximum photosynthetic rates should be found. Therefore, divaricate leaves were hypothesised to have higher quantum efficiencies, as shaded have higher quantum efficiencies than sun leaves (Tognetti *et al*., 1997) and reductions in maximum photosynthetic rates should also be seen in reductions in the quantum efficiency (Ball *et al*., 1994). Water stress should decrease the quantum efficiency, in particular in non-divaricate leaves. A higher maximum photosynthetic rate and therefore more positive carbon balance even during summer droughts could be expected from divaricate leaves than from non-divaricate leaves.

## 4.2 Materials and Methods

Leaves of divaricate shrubs growing at the sub-alpine field station at Cass (Section 2.1.2) were used for gas exchange measurements and these were compared to gas exchange measurements on leaves of divaricate and non-divaricate shrubs grown in the glasshouse (Section 2.1.3).

Gas exchange parameters were determined using a portable Infrared Gas Analyser (IRGA; LI-6400, LI-COR, Lincoln, Nebraska, USA). Light response curves for divaricate and non-divaricate leaves were recorded between 10:00 am and 8:00 pm in summer. PFD was started with 2000 µmol photons $m^{-2}$ $s^{-1}$ and then reduced in steps down to 0 µmol photons $m^{-2}$ $s^{-1}$ (2000, 1500, 1250, 1000, 500, 200, 100, 75, 50, 25, 0 µmol photons $m^{-2}$ $s^{-1}$) using a red/ blue light source. The last measurement was used to estimate the daytime dark respiration rate after the shoot was equilibrated in the dark for at least 10 minutes. Constant air flow rates of 500 ml $min^{-1}$ and a chamber temperature of 20 °C were programmed. A constant $CO_2$ concentration in the chamber was adjusted to 360 ppm in the leaf chamber.

Non-divaricate leaves were large enough to fill the 3 x 2 cm cuvette, whereas divaricate leaves are much smaller, having leaf areas between 1.8 and 3.2 $cm^2$ (data not shown). To calculate the light response curves for leaves of divaricate species, a section of a branch with a satisfactory amount of leaves for one measurement was used. The leaves were removed afterwards to determine the actual leaf area, and to allow the determination of the rate of gas exchange of the stem alone. Stem light response curves were recorded 20 minutes after the leaves were removed, and these values were used to calculate the actual divaricate leaf gas exchange rate.

From the light response curves following parameters were calculated: daytime respiration (Rd) as described above; quantum efficiency (QE) and maximum photosynthetic rate ($A_{max}$). These were calculated using the 'Photosynthesis Assistant' software (Dundee Scientific, Dundee, UK). $A_{max}$ were determined by single readings of each leaf's light response curve in the 'Photosynthesis Assistant' software, QE by linear regression of the slope of light response curve.

## 4.3 Results

Cass 2003

At the University field station light response curves were estimated for leaves of divaricate *Corokia cotoneaster* and *Coprosma propinqua* plants in the summer of 2002/03 (Section 4.2). The light response curves for *C. cotoneaster* and *C. propinqua* were very similarly shaped. Rd of both species was around 0.7 µmol $CO_2$ $m^{-2}s^{-1}$ and $A_{max}$ reached up to 8 to 9 µmol $CO_2$ $m^{-2}s^{-1}$, the parameters extracted from the light response curves are shown in Figures 4.1.

The divaricate species show no major differences in their respiration rates between the streambed and the N-facing slope habitats (Figure 4.1.a). Originally, the streambed was assumed to provide a better water availability to the plants then the N-facing slope, but lower water potentials were found in plants in the streambed than on the N-facing slope (Section 2.2.4). Only shaded plants of both species respired more on the N-facing slope. *Corokia* leaves under natural light had higher respiration rates than leaves which were exposed to the exterior climate. The ANOVA analysis (Table 4.1) showed only one close to significant effect for the light levels to which the leaves were exposed.

QEs (Figure 4.1.b) of both species reached average values between 0.03 and 0.04. Leaves of *Corokia* had higher QEs under natural light and under shaded conditions than the exposed leaves, supporting the hypothesis that shaded or 'self-shaded' leaves have higher QEs. Shaded *Corokia* leaves had the highest values on the N-facing slope, whereas in the streambed shaded *Corokia* leaves showed the lowest QE. In contrast to my hypothesis (Section 4.1), leaves of *Coprosma* plants did not show big differences in QEs, with only the shaded plants on the slope reaching QEs over 0.04. The different water availabilities at the streambed and the N-facing slope had significant effects on the QEs of the two species and the light level* water availability combination (Table 4.2). In contrast to my original hypothesis, QE was low in shaded divaricates in the streambed. Here, in contrast to my original hypothesis, plants

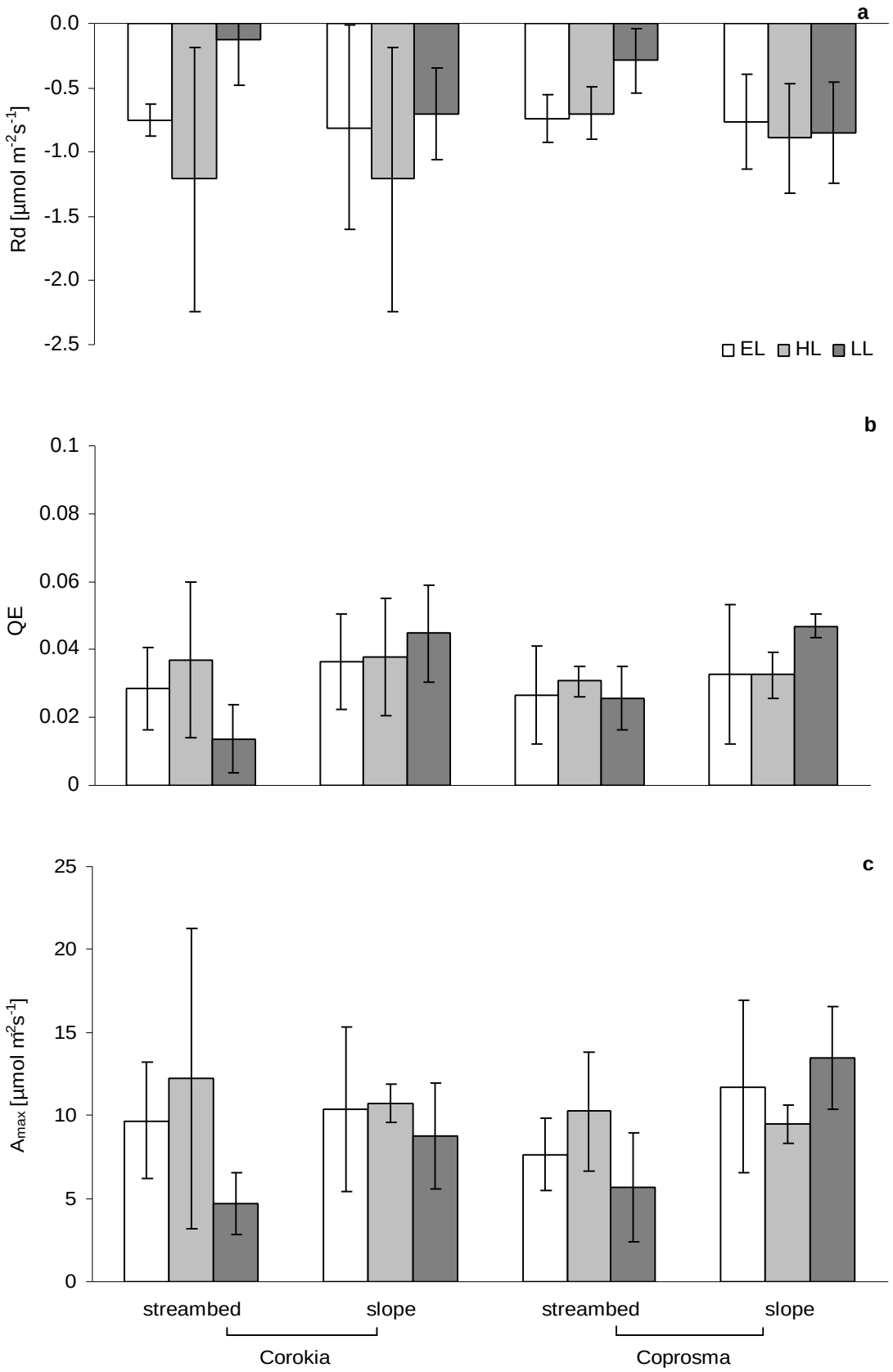



Figure 4.1 Gas exchange measurements in season 2002/03 at Cass for (a) daytime respiration ($R_d$), (b) quantum efficiency (QE) and (c) for maximum photosynthetic rate ($A_{max}$) for *Corokia cotoneaster* and *Coprosma propinqua*, grown in a stream bed and on a slope and under 3 different light treatments (EL= inner canopy exposed, HL= sun light, LL= shaded).

Table 4.1 Analysis of variance table for respiration measurements at the Cass field site, taken in March 2003. Light level (natural daylight, shaded and exposed plants), water availability (streambed and slope) and genus (*Corokia cotoneaster* and *Coprosma propinqua*) as treatments [significant results in bold,].

| | Df | Sum of Sq | Mean Sq | F Value | Pr(F) |
|---|---|---|---|---|---|
| Light level | 2 | 1.33924 | 0.6696181 | 3.0406 | 0.056 |
| Water availability | 1 | 0.46449 | 0.4644858 | 2.1091 | 0.152 |
| Genus | 1 | 0.03315 | 0.0331496 | 0.1505 | 0.700 |
| Light level*Water availability | 2 | 1.13240 | 0.5662012 | 2.5710 | 0.086 |
| Light level*Genus | 2 | 0.40611 | 0.2030559 | 0.9220 | 0.404 |
| Water availability*Genus | 1 | 0.12990 | 0.1299033 | 0.5899 | 0.446 |
| Light level*Water availability*Genus | 2 | 0.28158 | 0.1407902 | 0.6393 | 0.532 |
| Residuals | 54 | 11.89209 | 0.2202239 | | |

Table 4.2 Analysis of variance table for measurements of QE at the Cass field site, taken in March 2003. Light level (natural daylight, shaded and exposed plants), water availability (streambed and slope) and genus (*Corokia cotoneaster* and *Coprosma propinqua*) as treatments [significant results in bold].

| | Df | Sum of Sq | Mean Sq | F Value | Pr(F) |
|---|---|---|---|---|---|
| Light level | 2 | 0.00022675 | 0.000113377 | 0.5749 | 0.566 |
| **Water availability** | 1 | 0.00182653 | 0.001826526 | 9.2620 | **0.004** |
| Genus | 1 | 0.00002972 | 0.000029721 | 0.1507 | 0.699 |
| **Light level*Water availability** | 2 | 0.00173368 | 0.000866841 | 4.3956 | **0.017** |
| Light level*Genus | 2 | 0.00040110 | 0.000200548 | 1.0169 | 0.368 |
| Water availability*Genus | 1 | 0.00004488 | 0.000044883 | 0.2276 | 0.635 |
| Light level*Water availability*Genus | 2 | 0.00008450 | 0.000042249 | 0.2142 | 0.808 |
| Residuals | 55 | 0.01084633 | 0.000197206 | | |

Table 4.3 Analysis of variance table for measurements of Amax at the Cass field site, taken in March 2003. Light level (natural daylight, shaded and exposed plants), water availability (streambed and slope) and genus (*Corokia cotoneaster* and *Coprosma propinqua*) as treatments [significant results in bold].

| | Df | Sum of Sq | Mean Sq | F Value | Pr(F) |
|---|---|---|---|---|---|
| Light level | 2 | 39.2222 | 19.61109 | 1.1944 | 0.311 |
| **Water availability** | 1 | 80.3199 | 80.31986 | 4.8917 | **0.031** |
| Genus | 1 | 2.8104 | 2.81044 | 0.1712 | 0.681 |
| **Light level*Water availability** | 2 | 129.7309 | 64.86545 | 3.9505 | **0.025** |
| Light level*Genus | 2 | 66.2777 | 33.13885 | 2.0182 | 0.143 |
| Water availability*Genus | 1 | 25.0897 | 25.08970 | 1.5280 | 0.222 |
| Light level*Water availability*Genus | 2 | 7.4455 | 3.72277 | 0.2267 | 0.798 |
| Residuals | 55 | 903.0819 | 16.41967 | | |

showed lower water potentials, which also influenced the gas exchange parameters of these plants.

The highest $A_{max}$ (Figure 4.1c) of 13 µmol $CO_2$ $m^{-2}$ $s^{-1}$ was reached by shaded *Coprosma* leaves on the N-facing slope, and 12 µmol $CO_2$ $m^{-2}$ $s^{-1}$ by *Corokia* leaves under natural light in the streambed. Shaded streambed plants of the two genera had the lowest values for $A_{max}$, between 5 and 6 µmol $CO_2$ $m^{-2}$ $s^{-1}$. Water availability and light level* water availability had significant effects on photosynthetic capacity (Table 4.3). In contrast to my hypothesis that the small leaf area should support constant photosynthetic rates even under stress, low water potentials and differing light levels led to lower $A_{max}$ than in plants under sun light and with less negative water potentials.

Glasshouse 2002

In a glasshouse environment, light response curves of divaricate and non-divaricate leaves were recorded in summer 2001/02 (Section 4.2). Comparing the light response curves, both divaricate species showed a decreased $CO_2$ uptake when exposed to over 500 µmol photons $m^{-2}$ $s^{-1}$ (data not shown). Higher PFDs decreased their photosynthetic rates further. In general, the leaves of *Coprosma* plants had lower photosynthetic rates than *Corokia* leaves. Divaricate *Coprosma* leaves showed the biggest reduction in the photosynthetic rate under high PFDs. This is in contrast to my hypothesis that the small leaves and 'self-shading' growth form of divaricates would enable these leaves to a constantly high photosynthetic rate even under stress conditions (Section 4.1).

From the light response curves parameters such as Rd, QE and $A_{max}$ were calculated (Figure 4.2). Rd (Figure 4.2a) of divaricate leaves was higher than Rd of non-divaricate leaves in all treatments and in the two genera, which was in contrast to my hypothesis that the small divaricate leaves would display lower respiration rates than the large non-divaricate leaves. The highest respiration rates were found under high light conditions. The ANOVA analysis (Table 4.4) showed significant effects for the different light levels and habits.

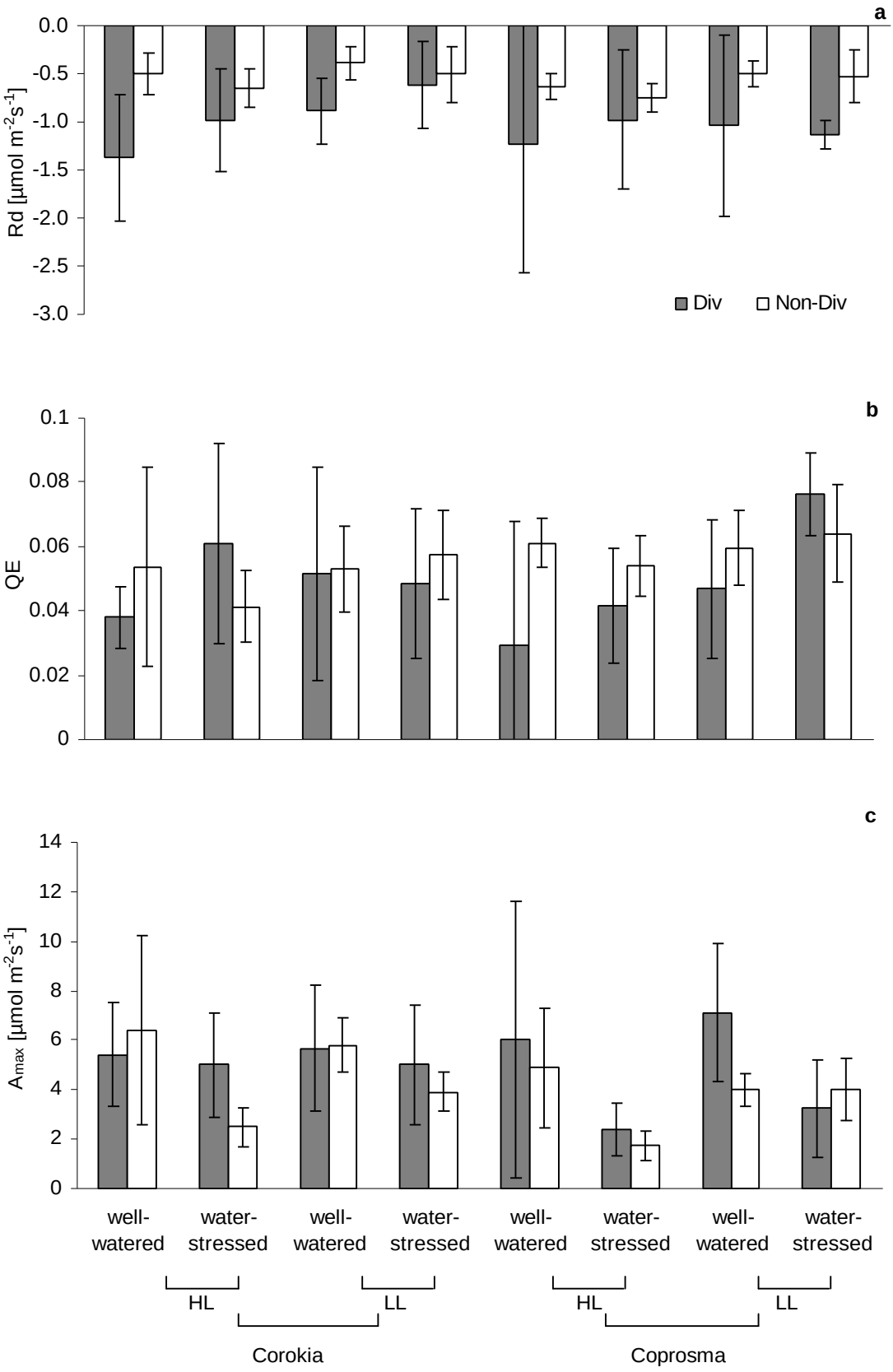



Figure 4.2 Gas exchange measurements for (a) daytime respiration ($R_d$), (b)quantum efficiency (QE) and (c) maximum photosynthetic rate ($A_{max}$) in well-watered and water stressed conditions and two different light treatments (HL= sun light, LL= shaded) in the Glasshouse, summer 2001/02. *Corokia cotoneaster* (Div) and *Corokia buddleioides* (Non-Div) were investigated as well as *Coprosma propinqua* (Div) and *Coprosma robusta* (Non-Div).

Table 4.4 Analysis of variance table for respiration measurements in the glasshouse, taken in June 2002. Light level (100% vs 75% of natural daylight), water availability (200 mls every 2 days and 50mls every 3 days), genus and habit (*Corokia cotoneaster*, *Coprosma propinqua*, *Corokia buddleioide* and *Coprosma robusta*) as treatments [significant results in bold].

| | Df | Sum of Sq | Mean Sq | F Value | Pr(F) |
|---|---|---|---|---|---|
| **Light level** | 1 | 2.16872 | 2.168724 | 7.2033 | **0.009** |
| Water availability | 1 | 0.05481 | 0.054811 | 0.1821 | 0.671 |
| Genus | 1 | 0.00414 | 0.004144 | 0.0138 | 0.907 |
| **Habit** | 1 | 4.92211 | 4.922114 | 16.349 | **0.0001** |
| Light level*Water availability | 1 | 0.09635 | 0.096345 | 0.3200 | 0.573 |
| Light level*Genus | 1 | 0.01629 | 0.016287 | 0.0541 | 0.817 |
| Water availability*Genus | 1 | 0.16033 | 0.160333 | 0.5325 | 0.467 |
| Light level*Habit | 1 | 0.31921 | 0.319205 | 1.0602 | 0.306 |
| Water availability*Habit | 1 | 0.45048 | 0.450482 | 1.4963 | 0.224 |
| Genus*Habit | 1 | 0.06913 | 0.069133 | 0.2296 | 0.633 |
| Light level*Water availability*Genus | 1 | 0.02496 | 0.024957 | 0.0829 | 0.774 |
| Light level*Water availability*Habit | 1 | 0.31062 | 0.310622 | 1.0317 | 0.312 |
| Light level*Genus*Habit | 1 | 0.09679 | 0.096789 | 0.3215 | 0.572 |
| Water availability*Genus*Habit | 1 | 0.41981 | 0.419815 | 1.3944 | 0.241 |
| Light level*Water availability*Genus*Habit | 1 | 0.21018 | 0.210177 | 0.6981 | 0.406 |
| Residuals | 95 | 28.60177 | 0.301071 | | |

Table 4.5 Analysis of variance table for measurements of quantum efficiency (QE) in the glasshouse, taken in June 2002. Light level (100% vs 75% of natural daylight), water availability (200 mls every 2 days and 50mls every 3 days), genus and habit (*Corokia cotoneaster*, *Coprosma propinqua*, *Corokia buddleioide* and *Coprosma robusta*) as treatments [significant results in bold].

| | Df | Sum of Sq | Mean Sq | F Value | Pr(F) |
|---|---|---|---|---|---|
| Light level | 1 | 0.00163891 | 0.001638911 | 3.5543 | 0.062 |
| Water availability | 1 | 0.00066691 | 0.000666914 | 1.4464 | 0.232 |
| Genus | 1 | 0.00012714 | 0.000127143 | 0.2757 | 0.601 |
| Habit | 1 | 0.00168585 | 0.001685848 | 3.6561 | 0.059 |
| Light level*Water availability | 1 | 0.00002849 | 0.000028485 | 0.0618 | 0.804 |
| Light level*Genus | 1 | 0.00052043 | 0.000520432 | 1.1287 | 0.291 |
| Water availability*Genus | 1 | 0.00020847 | 0.000208471 | 0.4521 | 0.503 |
| Light level*Habit | 1 | 0.00013561 | 0.000135607 | 0.2941 | 0.589 |
| **Water availability*Habit** | 1 | 0.00182636 | 0.001826359 | 3.9609 | **0.049** |
| Genus*Habit | 1 | 0.00088956 | 0.000889562 | 1.9292 | 0.168 |
| Light level*Water availability*Genus | 1 | 0.00038242 | 0.000382425 | 0.8294 | 0.365 |
| Light level*Water availability*Habit | 1 | 0.00093139 | 0.000931386 | 2.0199 | 0.158 |
| Light level*Genus*Habit | 1 | 0.00120492 | 0.001204925 | 2.6132 | 0.109 |
| Water availability*Genus*Habit | 1 | 0.00013483 | 0.000134827 | 0.2924 | 0.590 |
| Light level*Water availability*Genus*Habit | 1 | 0.00089608 | 0.000896083 | 1.9434 | 0.167 |
| Residuals | 98 | 0.04518793 | 0.000461101 | | |

Table 4.6 Analysis of variance table for measurements of maximum photosynthetic rate (Amax) in the glasshouse, taken in June 2002. Light level (100% vs 75% of natural daylight), water availability (200 mls every 2 days and 50mls every 3 days), genus and habit (*Corokia cotoneaster*, *Coprosma propinqua*, *Corokia buddleioide* and *Coprosma robusta*) as treatments [significant results in bold].

| | Df | Sum of Sq | Mean Sq | F Value | Pr(F) |
|---|---|---|---|---|---|
| Light level | 1 | 6.8545 | 6.8545 | 1.1902 | 0.278 |
| **Water availability** | 1 | 122.9067 | 122.9067 | 21.341 | **0.000** |
| Genus | 1 | 18.8979 | 18.8979 | 3.2813 | 0.073 |
| Habit | 1 | 22.4302 | 22.4302 | 3.8946 | 0.051 |
| Light level*Water availability | 1 | 13.8637 | 13.8637 | 2.4072 | 0.124 |
| Light level*Genus | 1 | 3.8123 | 3.8123 | 0.6619 | 0.418 |
| Water availability*Genus | 1 | 2.5667 | 2.5667 | 0.4457 | 0.506 |
| Light level*Habit | 1 | 0.0316 | 0.0316 | 0.0055 | 0.941 |
| Water availability*Habit | 1 | 0.4229 | 0.4229 | 0.0734 | 0.787 |
| Genus*Habit | 1 | 2.5312 | 2.5312 | 0.4395 | 0.509 |
| Light level*Water availability*Genus | 1 | 1.6169 | 1.6169 | 0.2807 | 0.597 |
| Light level*Water availability*Habit | 1 | 9.2273 | 9.2273 | 1.6022 | 0.209 |
| Light level*Genus*Habit | 1 | 1.5244 | 1.5244 | 0.2647 | 0.608 |
| **Water availability*Genus*Habit** | 1 | 30.0958 | 30.0958 | 5.2256 | **0.024** |
| Light level*Water availability*Genus*Habit | 1 | 0.6732 | 0.6732 | 0.1169 | 0.733 |
| Residuals | 98 | 564.4141 | 5.7593 | | |

QE (Figure 4.2b) was lowest in leaves of *Coprosma propinqua*, except for the plants under shade cloth and water-stressed conditions, where QE reached its highest value of 0.08. In general, water stressed leaves of divaricate species had lower quantum efficiencies than water stressed non-divaricate congeners, in contrast to my hypothesis (Section 4.1). This difference was most pronounced in the *Coprosma* species. Divaricate *Coprosma* leaves under high light and well-watered conditions showed the lowest quantum efficiency, approaching 0.03. The water availability* habit interaction had significant effects on the quantum efficiencies (Table 4.5).

As shown in Figure 4.2c, $A_{max}$ of divaricate and non-divaricate leaves were reached at different PFDs. In Figure 4.2c it becomes obvious that water stressed *Coprosma* leaves under sun light had the lowest $A_{max}$ of 2.4 $CO_2$ $m^{-2}$ $s^{-1}$ for divaricate and 1.7 $CO_2$ $m^{-2}$ $s^{-1}$ for non-divaricate leaves, respectively. Divaricate *Coprosma* leaves had higher $A_{max}$ than non-divaricate leaves, except for the shaded, water-stressed divaricate leaves of *Coprosma*. Similarly, in *Corokia*, divaricate leaves exhibited higher $A_{max}$ than their non-divaricate congeners. This supports my hypothesis that divaricate leaves maintain higher photosynthetic rates than their non-divaricate congeners under stress conditions. Water-stressed *C. cotoneaster* leaves reached photosynthetic rates of about 5 µmol $CO_2$ $m^{-2}$ $s^{-1}$, whereas in the same environment *C.*

*buddleioides* only reached 2.5 µmol $CO_2$ $m^{-2}$ $s^{-1}$. Well-watered divaricate *Coprosma* leaves had the highest $A_{max}$ of 7 µmol $CO_2$ $m^{-2}$ $s^{-1}$. Significant effects for the $A_{max}$ were found for water availability and water availability* genus* habit (Table 4.6) This supports my hypotheses that water stress would influence the photosynthetic rate and that divaricate leaves would maintain higher photosynthetic rates under water stressed conditions than their non-divaricate congeners (Section 4.1).

Further Results

All plants in the field experiment at Cass displayed a significant effect of water availability on the transpiration rates (E) measured at 0, 100 and 2000 µmol photons $m^{-2}$ $s^{-1}$. The plants on the N-facing slope had higher E's than the plants growing in the streambed. A significant effect of the interaction of water availability* genus at 500 and 2000 µmol photons $m^{-2}$ $s^{-1}$ was also founed. *Coprosma* plants on the N-facing slope had the highest E, followed by *Corokia* plants on the N-facing slope. The lowest E were found in *Coprosma* plants in the streambed. Stomatal conductance ($g_s$) was significantly influenced by water availability, the higher values for $g_s$ were displayed by plants grown on the N-facing slope. The interaction of light level* water availability, light level* genus, water availability *genus and light level* water availability *genus had also a significant effect on $g_s$. As seen for the transpiration data, *Coprosma* plants grown on the N-facing slope had the highest values, whereas *Coprosma* plants in the streambed showed the lowest values of $g_s$.

The plants grown in the glasshouse showed significant effects of light level, water availability, habit, genus* habit and water availability* genus* habit on the transpiration rate of all investigated plants. Plants with good water availability had higher transpiration rates than plants under water stress. As seen in the field experiment, *Coprosma* plants had the highest values for transpiration rate, especially the divaricate *Coprosma* leaves in particular. $g_s$ was significantly influenced by light level, water availability, habit, light level* water availability and water availability* genus* habit. The highest values for $g_s$ were found in divaricate *Coprosma* leaves grown under good water availability, the lowest values for $g_s$ were found in leaves of non-divaricate *Coprosma* leaves under water stress.

Summary of the Results

In contrast to my hypothesis that divaricate leaves would show lower Rd rates than non-divaricate leaves, high Rd rates were found in divaricate leaves in the field and glasshouse experiment. The quantum efficiencies of divaricate leaves in the field were lower than the quantum efficiencies of divaricate and non-divaricate leaves in the glasshouse. As hypothesised, high $A_{max}$ were found in divaricate leaves in the field and in the glasshouse experiment, but both growth forms had higher photosynthetic rates under well-watered conditions than under water stress.

The field experiment showed significant effects of the different water availabilities and a significant interaction between light level and water availability for QE and $A_{max}$. This is in contrast to my hypothesis that divaricate leaves would maintain high photosynthetic rates in particular under stressful conditions. Both species of divaricate shrubs expressed acclimatisation patterns to the given light treatments as well as to the different water availabilities they grew in. Respiration rates were high in *Corokia* leaves under high light, but these leaves also expressed high quantum efficiencies and $A_{max}$. Shaded plants on the N-facing slope in particular, had high quantum efficiencies. Leaves of divaricate *Coprosma* plants had the highest quantum efficiencies and the highest $A_{max}$ under shade and when growing on the N-facing slope.

The glasshouse experiment showed significant effects of light level and habit for Rd. As mentioned above, the highest respiration rates were found in divaricate leaves, in contrast to my hypothesis (Section 4.1). Divaricate leaves showed higher respiration rates than non-divaricate leaves in all treatments, but in sun light in particular. QE was significantly influenced by the water availability* habit interaction. Non-divaricate leaves of the two genera generally showed higher quantum efficiencies than divaricate leaves, in particular under well-watered conditions. $A_{max}$ were significantly influenced by water availability as well as the interaction of water availability* genus* habit. $A_{max}$ were mostly higher in divaricate leaves of the two genera, but they reached $A_{max}$ at PFDs of 500 µmol photons $m^{-2}$ $s^{-1}$ and below.

## 4.4 Discussion

Divaricate shrubs are characterized by a unique growth form and nowhere else in the world do they reach such a high percentage of the flora as in New Zealand. As described in Chapter 1, divaricate shrubs have been hypothesised to use their growth form as a 'self-shading' mechanism to protect their leaves in the interior from high radiation loads and transpirational water loss (McGlone & Webb, 1981). It was also hypothesised that internal $CO_2$ concentration should remain relatively constant over short periods of dry soil and air. Partly shaded or 'self-shaded' leaves still receive a satisfactory photon flux density (Kelly, 1990) to maintain a high photosynthetic capacity and a positive carbon gain, but the investments in photo-protective mechanisms should be less. Therefore photosynthetic rates were thought to stay constantly high in leaves of divaricates over the summer period, which was observed by taking light response curves with an infrared gas analyser (Section 4.2).

Stem Photosynthesis

Howell *et al*. (2002) discussed the possibility that the stems of divaricate shrubs might contribute to carbon gain via photosynthetic reactions. They found positive rates of photosynthesis on stems of *Aristotelia* even when most leaves were lost. As shown in previous studies, such as Bossard & Rejmanek (1992), branches can contribute to the photosynthetic carbon gain, but the major contribution is still made by leaves (Nilsen 1992). In my study, the light response curves of divaricate leaves were first taken from branchlets with leaves and then compared with light response curves of the same branchlet without leaves (Section 4.2). The evaluation of the light response curves of branchlets without leaves showed that almost all branchlets showed net release of $CO_2$ at all PFDs (data not shown). There was presumably some photosynthesis offsetting respiration in the stems, but the amount of this must be small. Therefore, the suggestion of Howell *et al.* (2002) that there would be some gain in photosynthesis via stems of *Corokia* and *Coprosma* was not supported by my findings. Stems of the divaricate shrubs of *Corokia* and *Coprosma* in my study did not contribute to the net carbon gain via photosynthesis. The finding of Howell *et al*. (2002) that the stems of *Aristotelia fruticosa* are photosynthetic was not further investigated here. The role of stems of other divaricate shrubs in photosynthetic carbon gain is still unclear. It seems

possible that in semi-descidious divaricate shrubs, like *Discaria toumatou* and *Plagianthus divaricatus*, stems could have a more pronounced role in photosynthesis.

Daytime Respiration

Similar or slightly lower values were found for Rd in *C. cotoneaster* and *C. propinqua* in my field study in comparison to those found by Howell *et al*. (2002) at the same field site. Respiration was high on all plants on the N-facing slope, and in that habitat was highest in leaves under natural light. That result is in contrast to the finding in Section 2.2.4, where it was shown that the plants on the N-facing slope maintained higher water potentials than the plants growing in the putatively wetter streambed. The values for Rd in the glasshouse trial were comparable with the values found in the field, reaching maximal values of 1.2 µmol $CO_2$ $m^{-2}$ $s^{-1}$ to 1.4 µmol $CO_2$ $m^{-2}$ $s^{-1}$. Leaves of non-divaricate plants in the glasshouse trial showed Rd rates between 0.38 µmol $CO_2$ $m^{-2}$ $s^{-1}$ and 0.75 µmol $CO_2$ $m^{-2}$ $s^{-1}$. As seen in the study of Howell *et al*. (2002), the Rd values vary considerably. Even though my study used a longer adaptation period to estimate the Rd rate after recording the light response curve (Section 4.2), this method seems not ideal. The statistical analyses showed in particular for the glasshouse trial significant effects of light and habit on the Rd rate. Raven (1989) compared the co-occurrence of photoinhibition and its avoidance and repair mechanisms in differently light adapted *Oxalis* leaves and an increase in respiration rates in those leaves at the same time. Depending on the frequency and intensity of sunflecks, avoidance or repair mechanisms were favourable.

Quantum Efficiency

The values of QE were similar to Howell e*t al*. (2002). Generally, QE's were higher in the glasshouse trial than in the field experiment: Divaricate leaves had values between 0.029 and 0.076, non-divaricates between 0.040 and 0.061. The field experiment of Howell *et al*. (2002) showed values between 0.012 and 0.029 for divaricate *Corokia* and *Coprosma* leaves. *Corokia* leaves had slightly higher values in my study and varied under different water availabilities. Under shaded conditions in particular, QE of *C. cotoneaster* in the streambed was lower than the published values of Howell *et al*. (2002), whereas the values from plants on the N-facing slope were much higher. Comparing divaricate and non-divaricate leaves, higher QE's were found in non-divaricate leaves in my study. That was in contrast to my hypothesis

(Section 4.1). Tognetti *et al*. (1997) reported that shade leaves normally have higher QEs than sun leaves, which was not seen as clearly in my study in the 'self-shaded' leaves. In the field, only the shaded leaves on the N-facing slope showed higher QE's than the unshaded leaves. Howell *et al*. (2002) found reduced $A_{max}$ in plants under high light, but no correlation between the light treatment and respiration or QE in *C. cotoneaster*. In the glasshouse trial QE was strongly influenced by water availability* habit. Divaricate leaves under water-stressed conditions had higher QE's than well-watered ones, in particular in the shade. I hypothesised that water stress would decrease QE, whereas shade would increase QE. Non-divaricate leaves of the two genera and divaricate *Coprosma* leaves had the same or higher QE's in the shade when water-stressed. Also there was no correlation between low QE's and reduced $A_{max}$ in the glasshouse study, in contrast to findings of Ball *et al*. (1994). The lowest $A_{max}$ were found in water-stressed plants in the glasshouse, but these plants did not necessarily express the lowest QE's. Only shaded field plants in the streambed showed a correlation between low QE's and reduced $A_{max}$, indicating photoinhibitory effects under water stress in divaricate plants.

Photosynthetic Rate

Interestingly, the same divarivate species reached considerably higher values of $A_{max}$ in the field than in the glasshouse in all treatments. The values seen in the glasshouse trial seem rather low and there are several possible reasons for that. By comparison, the plants used in the glasshouse trial were considerably younger and had limited root development due to the pot size. The characteristic divaricate growth form was just developing in the glasshouse plants and no complete self-shading of the interior leaves was established. Incomplete shading of the interior leaves could increase stress in these young leaves and therefore reduce the proposed benefits of the divaricate habit and induce photoinhibitory effects. This could have had considerable influence on the actual effect of the light treatments and therefore the gas exchange measurements. In particular divaricate *Coprosma* leaves had higher $A_{max}$ in the shade treatment, indicating that these young plants have higher carbon gain when they are less exposed. The higher carbon gain in shaded positions could have vital influences on the growth of these shrubs. Interestingly, interior leaves which were exposed to the exterior climate in the field experiment, showed respiration rates as well as quantum efficiencies and $A_{max}$ which were at levels intermediate between leaves under sun

light and shaded leaves. As seen in both divaricate species in the glasshouse trial, Rd was higher and QE lower under high light. That could indicate that divaricate leaves experience photoinhibitory effects under high light (which is exacerbated under additional mild drought), which could have negative consequences on carbon gain and growth on young plants in particular. Also, regular insecticide usage was necessary in the glasshouse, due to repeated infestations by scale insects and aphids (Section 2.1.3). The glasshouse plants were also sheltered from the influence of wind, which affects gas exchange via $CO_2$ diffusion rates and leaf temperature because of boundary-layer effects. These factors can influence the respiration rate and photosynthetic rate of leaves (Lawlor, 1990).

As mentioned in Section 4.3, divaricate leaves in the glasshouse showed reduced photosynthetic rates when exposed to PFDs above 500 µmol photons $m^{-2}$ $s^{-1}$. That could indicate photoinhibitory effects in divaricate leaves, which were exposed to PFDs above 500 µmol photons $m^{-2}$ $s^{-1}$. Water stress additionally decreased the photosynthetic rates of divaricate leaves, in contrast to my hypothesis (Section 4.1). As in divaricate leaves, water stress decreased the photosynthetic rates in non-divaricate leaves. Howell *et al*. (2002) showed lower $A_{max}$ in leaves exposed to exterior radiation loads than in shaded interior leaves. They argued that the exposed leaves experienced photoinhibitory effects, higher photosynthetic rates were found in shaded leaves. In my study, photosynthetic rates of shaded divaricate leaves dropped considerably when the light intensity exceeded 500 µmol photons $m^{-2}$ $s^{-1}$, indicating an over-saturation with light. That finding is consistent with Horn (1971), who showed that leaves of a variety of higher plants are light-saturated, even when exposed to intensities as low as at 20% of full sun light. Howell *et al*. (2002) also calculated a positive net carbon balance for foliage exposed to PFDs greater than 500 ?mol photons $m^{-2}$ $s^{-1}$. Higher PFDs induce photoinhibitory effects, which can reduce photosynthesis (Powles, 1984). In my field experiment, no reduced photosynthetic rates for PFDs over 500 µmol photons $m^{-2}$ $s^{-1}$ were found. That could suggest that only young divaricate plants (as used in the glasshouse experiment) experience photoinhibitory effects when PFDs rise above 500 µmol photons $m^{-2}$ $s^{-1}$ and that in older plants the leaves become protected from high PFDs as well as from photoinhibitory effects as soon as their ‘self-shading’ growth form is fully developed.

Findings supporting this hypothesis were that the $A_{max}$ of shaded leaves of divaricate shrubs grown on the N-facing slope (less negative water potentials) in the field were higher than the unshaded leaves.

$A_{max}$ estimated in my study were considerably higher for *C. cotoneaster* and *C. propinqua* than found by Howell *et al.* (2002). In my study the values of $A_{max}$ varied between 4.7 µmol $CO_2$ $m^{-2}$ $s^{-1}$ and 13.4 µmol $CO_2$ $m^{-2}$ $s^{-1}$ for divaricate leaves in the field. With one exception (Figure 4.2), the exposed leaves had higher $A_{max}$ than shaded leaves of the same species in the same water treatment. The values of $A_{max}$ estimated in the field were comparable with published values, such as Prider & Facelli (2004) observing chenopod shrubs in arid habitats (*Atriplex vesicaria, Enchylaena tomentosa, Rhagodia spinescens*). In the glasshouse, the values for $A_{max}$ of divaricate *Corokia* and *Coprosma* leaves were between 2.4 µmol $CO_2$ $m^{-2}$ $s^{-1}$ and 7.1 µmol $CO_2$ $m^{-2}$ $s^{-1}$, respectively. Non-divaricate leaves showed values between 1.7 µmol $CO_2$ $m^{-2}$ $s^{-1}$ and 6.4 µmol $CO_2$ $m^{-2}$ $s^{-1}$. Howell *et al*. (2002) found values between 1.9 µmol $CO_2$ $m^{-2}$ $s^{-1}$ and 3.9 µmol $CO_2$ $m^{-2}$ $s^{-1}$. $A_{max}$ in my study were not influenced by light level alone: neither the shading nor the exposing of leaves showed significant effects on the photosynthetic rates of those leaves, but the light level* water availability interaction had significant effects on $A_{max}$. Plants on the N-facing slope and under natural light or shading expressed higher photosynthetic rates than plants in the streambed. This is in contrast to the findings of Howell *et al*. (2002), who found reductions in $A_{max}$ for leaves of *A. fruticosa* and *C. cotoneaster* under high light and cold temperatures. In the same study, no reductions of $A_{max}$ were found for exposed *C. propinqua*. In the glasshouse the water availability and the water availability* genus* habit interaction influenced the response in $A_{max}$, supporting my hypothesis of high $A_{max}$ in divaricate leaves even under stressful conditions. Interestingly, low photosynthetic rates did not linearly correlate with high Rd rates or quantum efficiencies in divaricate and non-divaricate leaves.

Although the divaricate leaves in the field study showed reasonable high values in their $A_{max}$, it is hard to argue that divaricate leaves have higher photosynthetic rates than non-divaricate leaves. $A_{max}$ was close to significant for habit. Unfortunately, in my study no non-divaricate leaves were available in the field experiment, where divaricate leaves not only showed high photosynthetic rates but also a lesser decrease

in their photosynthetic rate at high PFDs. This is, of course, because the non-divaricate congeners do not naturally occur in the Cass area (Chapter 1). Due to the difficulties in the glasshouse trial, the absence of non-divaricate plants in the field trial, and the different age of the plants in the glasshouse and field experiment, it is difficult to synthesise all findings in a satisfactory manner. Further investigations in a habitat with a natural occurrence of divaricate and non-divaricate plants together would be favourable.

Impacts of Drought Stress

Grossnickle *et al*. (2004) investigated variations in the gas exchange rate of different red cedar (*Thuja plicata*) populations under summer conditions. Although a variety of responses to a regional precipitation gradient were detected, there was a better relationship to foliar conductance than net photosynthesis of the population. Inconsistency was found in gas exchange measurements as well as in foliage carbon isotope measurements. This present study found significant effects of water availability on QE, $A_{max}$, E and $g_s$ in the field, supporting the hypothesis (Section 4.1) that divaricate leaves can maintain constantly high photosynthetic rates when transpiration rates increase. In the glasshouse experiment, water availability had a significant effect on $A_{max}$, E and $g_s$. Water stress reduced $A_{max}$, E and $g_s$ of divaricate and non-divaricate leaves, which was hypothesised for non-divaricate leaves only. In divaricate leaves E and $g_s$ were higher than in non-divaricate leaves, which is in contrast to the hypotheses (Section 4.1). I hypothesised a decrease in $A_{max}$ and an increase in E and $g_s$ in non-divaricate leaves under water stressed conditions, but not in divaricate leaves. Different responses to mild drought conditions were found in divaricate and non-divaricate plants, but plants of the two genera also varied in their response to given water treatments.

Summary of the Discussion

As discussed before (Chapter 1), most leaves of divaricate shrubs are situated in the interior of the shrub and are therefore at least partly shaded (Greenwood & Atkinson, 1977; McGlone & Webb, 1981). Therefore, the balance of carbon gain from interior and exterior leaves in divaricates is more heavily weighted toward interior leaves. At the whole-plant level, carbon gain may also be enhanced by the fact that the costs of photoinhibition and photoprotection would be reduced by the reduced number of

leaves on the outside of the canopy. My study was undertaken to investigate two related hypotheses:

(1). That divaricate leaves would have a reduced transpiration surface and therefore could avoid stomatal closure during summer drought periods. High internal $CO_2$ concentrations would allow the divaricate leaves more constant photosynthetic rates and a more positive carbon balance under these adverse conditions. The results actually show a less uniform result than predicted. Not only were the values of Rd, E and $g_s$ of divaricate leaves high in the field, but also higher than the non-divaricate leaves in the glasshouse trial. Therefore this hypothesis was not supported.

(2). That the divaricate growth form mitigates the potentially photoinhibitory impacts of high light loads in combination with drought. In contrast to this hypothesis, divaricate leaves had higher Rd and E rates as well as $g_s$ and lower quantum efficiencies than non-divaricate leaves. Divaricate leaves showed a decrease in their photosynthetic rate when the light intensity exceeded 500 µmol photons $m^{-2}$ $s^{-1}$ in the glasshouse but not in the field trial. $A_{max}$ were higher in the field than in the glasshouse experiment. In the glasshouse, $A_{max}$ of divaricate leaves were higher than in non-divaricate leaves, supporting the hypothesis above.

The difference in the carbon balance of divaricate and non-divaricate leaves under summer drought conditions was not calculated in my study. However, observing the gas exchange parameters only, the hypothesised advantages of the divaricate growth form under summer drought and high light loads could only be shown for $A_{max}$. The interaction of all measured parameters with the divaricate and non-divaricate growth forms will be discussed in Chapter 5.

Divaricate shrubs have been shown to compensate for the costs of self-shading by avoiding cold-induced photoinhibition and by higher carbon fixation rates per day in those ‘self-shaded’ leaves (Howell *et al*., 2002). Unfortunately, the picture in relation to water stress is not as clear. The balance between costs of photoinhibition and photoprotection versus costs of ‘self-shading’ are still not fully understood nor calculated. As seen in my study and the study of Howell *et al*. (2002), light does reach deeply into the interior of divaricate shrubs and is substantially used for positive

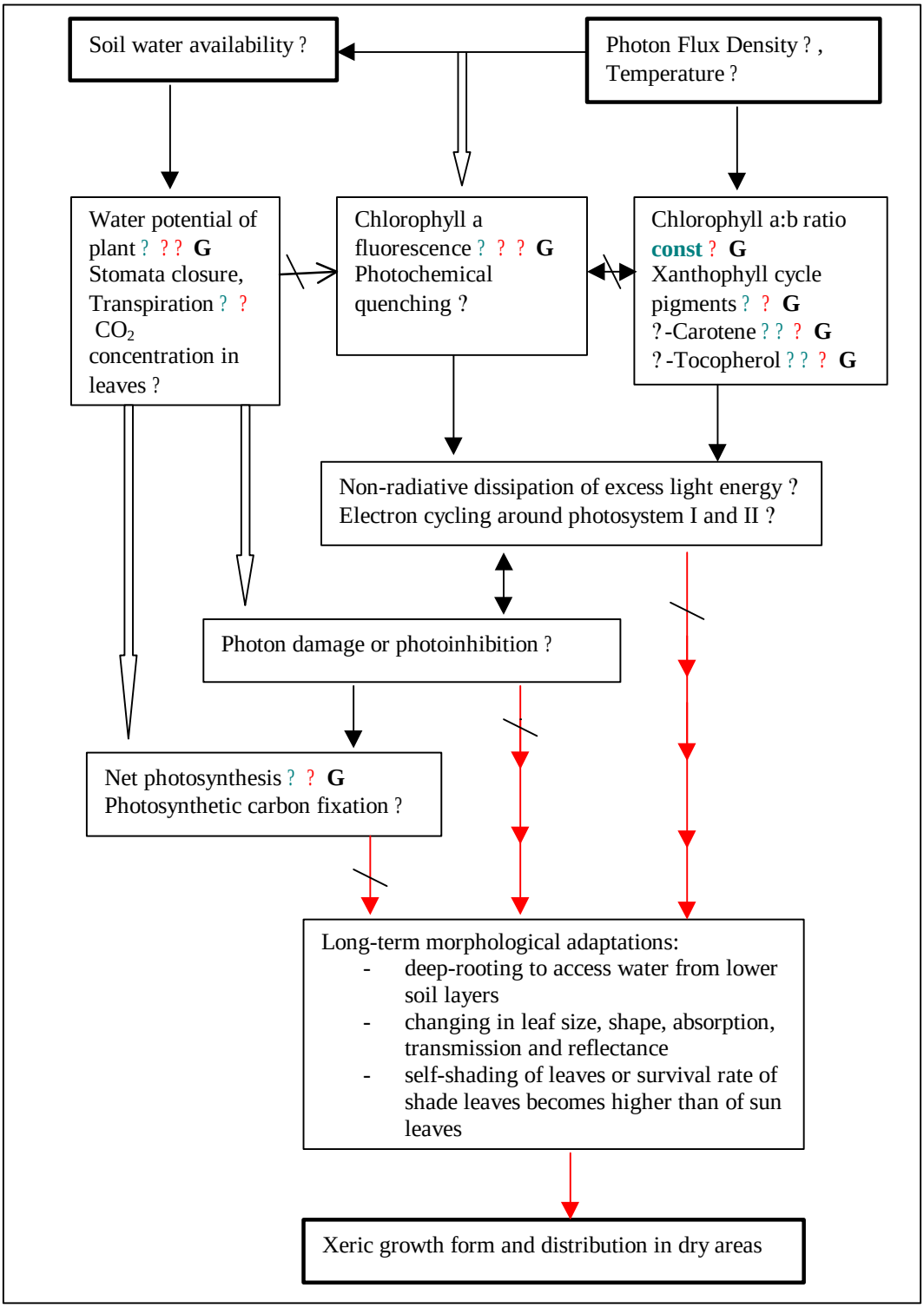
carbon gain; the photosynthetic rates found in the glasshouse experiment are at least comparable with those of their non-divaricate congeners.

In a post-doctoral project R. Christian (unpublished data) estimated biomass allocation of divaricate and non-divaricate species in a detailed growth analysis. On average, the leaf area index of non-divaricate species was found to be twice as high as in divaricate shrubs. Also, over 50% of the biomass of divaricate shrubs was allocated in stems, whereas non-divaricates only invested around 30% of their carbon gain in stems. Preliminary carbon allocation calculations by Christian (unpublished data) showed that divaricate leaves would need a two-fold higher net photosynthesis per unit leaf area to accomplish the same carbon gain for those structural components. My study did not show such high photosynthetic rates in divaricate leaves in the glasshouse or in the field study. So far, it is still arguable as to the extent to which cost of photoprotection and photoinhibition are outweighed by the structural costs of 'self-shading'.

# 5. GENERAL DISCUSSION

The main hypothesis of my study was that the morphological structures and physiological mechanisms of divaricate shrubs (Figure 1.2) allow them to maximize their photosynthetic production by minimizing the damaging effects of high light loads and mild drought. This idea was tested with a field trial (Section 2.1.2) and a glasshouse experiment (Section 2.1.3). Due to the results in this study, the conceptual model was adapted accordingly (Figure 5.1). As found in the literature, summer climates with high PFDs and high temperature reduce the water availability in the soil (soil humidity sensor readings, Section 2.1.4). The water potential in plants in the field and glasshouse was more reduced in non-divaricate plants, than in divaricate plants, but a strong genus effect was also found. In contrast to the hypothesis of this study (Section 1.3), transpiration was lower in non-divaricate than in divaricate plants. Reduced water potential and increased transpiration had a negative influence on the net photosynthesis of divaricate and non-divaricate plants (Figure 5.1). In Figure 1.2 only a negative result for non-divaricate plants was hypothesised. As hypothesised, high PFDs increased the amount of photoprotective pigements and ?-tocopherol, but in contrast to the hypothesis of this study, divaricate plants also showed high concentrations. Additionally, photoinhibitory events were found in divaricate and non-divaricate plants under high PFDs and different water availabilities, but the genera difference was greater than the difference between the contrasting growth forms, which is in contrast to the conceptual model (Figure 1.2). Additionally, the hypothesised connections between low water potentials, high fluorescence and high amounts of xanthophyll cycle pigments could not been shown in either growth form, probably due to the strong genus effect. These results do not support the conclusion, found in Howell et al. (2002), that the ‘self-shading’ growth form reduces photoinhibition or protects interior divaricate leaves from photodamage. Hypotheses that the divaricate growth form evolved as an adaptation to xeric climates (Figure 1.2) were not explicitly supported in this study (Figure 5.1) due to the greater difference between the two genera than divaricate and non-divaricate growth forms.

Figure 5.1: Model of plant responses to summer conditions.




Symbols: **colour**… results found for divaricate plants, **colour**… results found for non-divaricate plants, ? … low amount or descrease, ? … high amount or increase, **const**… no change or constant, ? … inconsistent, **G**… strong Genus effect found, → … as found in the literature, ⇒ … hypothesised in this study, ⇨ … found in this study, \ …not found in this study

## 5.1 Environmental Factors Influencing Photoinhibiton

The degree to which leaves experience photoinhibition is influenced by environmental factors, such as light loads, temperature, water availability, soil fertility, phenotype (sun or shade leaves, angle of leaves), and physiological factors (such as carbon metabolism). When plants already experience high light intensities, any additional stress or stresses will increase the amount of photoinhibition (Krause, 1988; Long *et al*., 1994). Plant defence mechanisms against photoinhibition can be on different levels, such as morphological (e.g. leaf angle, thick cuticle), metabolic (e.g. pH gradient, thermal dissipation), biophysical (e.g. fluorescence or heat emission) and biochemical (e.g. accumulation of formate). There are several different definitions of photoinhibition in the literature (Section 3.1). Powles (1984) described photoinhibition as damage to the photosynthetic apparatus, with photodestruction making photosynthesising pigments non-functional. Photo-oxidation is visible as a bleaching of the leaves and it can cause cell death or death of the entire leaf. Photo-oxidation first becomes visible as a decrease in photosynthetic activity, but does not change the content of pigments in the leaves (Powles, 1984; Long *et al*., 1994). Long *et al*. (1994) included reductions in photosynthetic efficiency, which are slow and reversible, in the definition of photoinhibition. These reductions depend on radiation loads received. The production of dry matter and therefore growth will be reduced due to a partial loss in the capacity to use radiant energy for photosynthesis. In my study the definition of Long *et al*. (1994) was used.

Osmond (1987, 1994) showed reduced photosynthetic rates over longer periods of time caused by high light, but a positive carbon balance was kept in sun plants. High light leaves have higher photosynthetic capacities adjusted to higher light loads than shade leaves or 'self-shaded' leaves (Turnbull *et al*., 1993). But the danger of damaging effects via photoinhibition is higher in leaves, which are exposed to high radiation loads, which then can reduce the net carbon assimilation. Further field research is needed to understand the adaptations of high light leaves and their response to high light loads and the acclimatisation of shade leaves, which were newly exposed to high-light conditions.

Long *et al*. (1994) specify that the rate of thermal dissipation and xanthophyll de-epoxidation are both increased when diurnal photoinhibition occurs. Even with a

recovery span of a few hours, diurnal photoinhibition can affect plants negatively. Long *et al*. (1994) highlighted that such recovery times might be still too short to avoid significant losses in potential carbon gain of up to 10%. Species with no photosynthetic down-regulation and extra carbon gain are proposed to have an increased capacity for repair mechanisms (Long *et al*., 1993). *Zea* genotypes from habitats of high altitudes, where the potential for chilling-dependent photoinhibition was greatest, expressed the highest resistance towards photoinhibition. The same findings were observed for $C_4$ grasses of *Cyperus longus* in northern versus southern Europe (Gravett & Long, 1990). The lower sensitivity towards photoinhibitory events correlated with a higher recovery rate from photoinhibitory events, implying increased repair ability. It is arguable, whether the plants in my study were not experiencing enough drought-stress to express photoinhibiton or alternatively avoided a down-regulation via other mechanisms, such as a higher activity of the xanthophyll cycle. Obviously, the divaricate and non-divaricate plants in the glasshouse expressed no sign of diurnal photoinhibition. The divaricate plants in the field showed diurnal changes in $F_v/F_m$ values, but the midday depressions were rather small and short-lived.

As seen above, the divaricate shrubs are influenced by frost events in winter (Howell *et al*., 2002), but apparently rather little by drought in summer. So far, there has been no comparison of the survival of divaricate and non-divaricate seedlings in a sub-alpine area after winter. Knowledge about different growth rates in plants of a divaricate growth form versus an open architecture as seen in non-divaricate shrubs would have enriched the predictions of my study. R. Christian *et al*. (unpubl.) investigated architectural traits in divaricate and non-divaricate shrubs grown on a lowland experimental site.

The influence of excessive light on rainforest understorey plants experiencing sunflecks was observed via $F_v/F_m$ and xanthophyll cycle activity estimation by Watling *et al*. (1997). Short exposure to high light resulted in rapid, large decreases in photochemical quenching, more open PSII centres compared to the plants not exposed to high light, as well as slow and continuing declines in quantum yields, detected by $F_v/F_m$ decreases. The decline in $F_v/F_m$ persisted for up to 90 minutes after a sunfleck. Light saturation points for the plants investigated by Watling *et al.* (1997) were below

500 µmol quanta $m^{-2}$ $s^{-1}$. The conversion of violaxanthin to antheraxanthin and zeaxanthin was fast and occurred continuously during exposure to sunflecks, with recovery periods of up to an hour. So far, the effect of sunflecks occurring in the interior of divaricate shrubs has not been investigated. Nevertheless, the field experiment showed fast recoveries in most plants after the midday decrease of $F_v/F_m$. As Watling *et al*. (1997) found for their understorey plants, the 'self-shaded' divaricate shrubs in the field displayed reduced photosynthetic rates while exposed to PFDs above 500 µmol quanta $m^{-2}$ $s^{-1}$. The concentrations of photoprotective pigments such as antheraxanthin and zeaxanthin in my study plants were also high under high light and in experimentally exposed leaves (Section 3.2.4). This could indicate a high number of sunflecks occurring in the interior of divaricate shrubs or an insufficient 'self-shading' of leaves by the divaricate growth form and therefore a lesser prevention of photodamage in divaricate shrubs than hypothesised.

<u>High Light and High Temperature</u>

In summer, high temperatures and high radiation loads often occur at the same time. When temperatures rise above optimum, decreases in photosynthesis occur first reversibly, but above a critical temperature irreversibly (Berry & Björkman, 1980). Several *Eucalyptus* species showed a 30% reduction in $F_v/F_m$ under high light and high temperatures; the leaves with the highest exposure to sunlight had a reduction up to 40% (Ögren & Evan, 1992). In my study all plants displayed decreases in their water potentials and $F_v/F_m$ ratios under high light conditions at noon. In contrast to my hypothesis (Figure 1.2), increased concentrations of photoprotective pigments were found in divaricate leaves in the field, as well as high concentrations of a-tocopherol in divaricate leaves in the glasshouse. The results of the fluorescence measurements did not indicate drastic differences between divaricate and non-divaricate growth forms. The reduction of the sunlight by 25% provoked different water potentials and $F_v/F_m$ ratios between *Corokia* and *Coprosma*, but the different growth forms were only significantly different in the evening measurements. Genus had significant effects on water potential and $F_v/F_m$ predawn, noon and in the evening in the field and glasshouse experiments (Figure 5.1). That genus had a significant effect but growth habit generally did not in these summer measurements provides no support for the notion that the divaricate growth form gives general benefits as a mechanism to offset the effects of high light and high temperature. It would appear that the physiological

responses of these plants had evolved prior to the split between the divaricate and non-divaricate growth forms. This supports the hypothesis that divaricates are of a very recent origin (Went 1971).

Leaf and air temperatures are likely to vary within the canopies of plants. Jifon & Syvertsen (2003) found a temperature difference of up to 9?C when they compared the inner and outer canopy of grapefruit trees (*Citrus paradisi*). Jifon & Syvertsen (2003) argued that xanthophyll cycle pigments helped protect sun leaves from the reversible photoinhibitory effects of excessive radiation and temperatures. Shaded leaves had less pronounced photoinhibition and maintained higher $CO_2$ assimilation rates, thought to be due to the reduction in direct sun light and higher quantities of diffuse light received in the inner canopy. The air temperature in the canopy of divaricate shrubs has been shown to have only minor variation (Kelly & Ogle 1990), but no further investigations of this were carried out in my study under different light and water exposures. Howell *et al*. (2002) showed gradual changes in $F_v/F_m$ within the canopy; the inner leaves displaying the lowest amount of photoinhibition. Based on this, my study only investigated the interior leaves of mature divaricate shrubs. High light intensities and/ or mild drought provoked photoinhibitory events in all plants, and were manifested in higher concentrations of photoprotective pigments or lowered $F_v/F_m$ ratios, from which most plants recovered in the evening. In agreement with my original hypothesis, the leaves of young non-divaricate plants in the glasshouse trial expressed higher concentrations of photoprotective pigments and a-tocopherol than young divaricate leaves. However, in contrast to this, mature divaricate leaves in the field trial had the highest concentrations of photoprotective pigments (Figure 5.1). It could be concluded that the leaves of mature divaricate shrubs in the field experiment recovered from photoinhibition and possible oxidative stress via photoprotective pigments and a-tocopherol.

Obviously, the high concentrations of photoprotective pigments and a-tocopherol provided some protection against photoinhibitory events in divaricates with the mature ‘self-shading’ growth form. However, the pigments were not enough to avoid photoinhibition completely as seen in the decreases of $F_v/F_m$ at noon, the high daytime respiration rates and low quantum efficiencies. Therefore, the hypothesis that the

'self-shading' divaricate habit would provide the advantage of avoiding photoinhibition (Section 1.3 and 3.1.1) was not supported by these experimental findings (Section 3.1.3). This conclusion was further reinforced by the differences between genera being more pronounced than the differences between the divaricate and non-divaricate growth forms (Figure 5.1).

High Light and Water Stress

It is often assumed that drought stress occurs together with heat stress, but high light conditions also lead to water deficits. Taiz & Zeiger (1998) describe the effects of water stress on stomatal closure and photosynthetic activity in leaves. The closure of stomata also affects the availability of $CO_2$. The combined occurrence of water deficits, high light and high temperatures in summer increases the potential for the occurrence of photoinhibition and photo-oxidation (Powles, 1984).

Effects of the combination of high light and drought were observed by Gauhl (1979). Sun-adapted *Solanum dulcamara* plants did show signs of photoinhibition under water restricted conditions, but photosynthesised well under well-irrigated conditions. Plants grown in the shade showed severe effects of photoinhibition when transferred to full sunlight and the photoinhibitory effects increased with increasing water deficit. Osmond (1983) argues that these effects could be affected by nutritional stress and, by implication by water stress. Nutritional aspects were not investigated in this study, but might have an important role in the distribution of divaricate versus non-divaricate plants (see below).

The present study showed decreases in water potential, $F_v/F_m$ ratios and maximum photosynthetic rates in divaricate plants in the field and divaricate and non-divaricate plants in the glasshouse during the summer months (Figure 5.1). Dowton (1983) also found a decrease in grapevine leaf fluorescence when the plants experienced water stress over short temporal scales. Drought stresses applied over longer temporal scales did not initiate losses in variable or minimal fluorescence, due to the maintenance of turgor via osmotic adjustments, until the point where the leaves were morphologically damaged. As discussed above, fluorescence measurements in the present study did not show prominent differences between the different light or water treatments. This could be due in part to mildness of the stress applied or to the long adaptation time

given before the measurements were recorded (three months in both the field and the glasshouse). This long term adaptation might have diminished mild heat or high light effects as the plants adapted to the changed environment.

In contrast to my hypothesis that divaricate leaves should maintain high photosynthetic rates in adverse conditions, the lowest maximum photosynthetic rates were found in divaricate leaves under shade in the streambed in the field; and under sunlight and water stressed conditions in the glasshouse (Section 4.3). Summarising the findings, divaricate leaves do not show a consistent avoidance of photoinhibition. Also, strong genera effects were found in these investigations, whereas the growth form only displayed significant effects in terms of water potential and a-tocopherol concentrations. Divaricate leaves showed the highest a-tocopherol concentrations, indicating active mechanisms of photoprotection. In contrast to my hypothesis (Figure 1.2), divaricate leaves obviously are not protected against high light and drought stress solely by their 'self-shading' and water-conserving growth form (Figure 5.1). The costs of photoprotection by physiological mechanisms were still incurred by divaricate plants. This finding does not support the hypothesis that the evolution of the divaricate growth form was an adaptation to habitats with high radiation loads and seasonal drought conditions.

As discussed by Anderson & Osmond (1987), leaves acclimated to shade have a higher susceptibility to photoinhibition when exposed to high light. In contrast, the carbon gain is higher in shaded leaves than in sun exposed leaves, because less photoprotection is needed and fewer photoinhibitory events occur. In *Quercus ilex* and *Q. coccifera* (Valladares *et al.*, 2004) lower carbon gain was found in leaves in high light. Obviously, the shaded parts of the crown make an important contribution to whole-plant carbon gain, in particular because these Mediterranean shrubs have a low leaf area index. The divaricate leaves used in the present study have very small leaf areas compared to non-divaricate leaves (Section 2.1). Additionally, the calculations of R. Christian (unpublished data) of the costs to maintain the branch layer, which is typical for divaricate shrubs, showed that divaricate leaves would need to have nearly twice the photosynthetic rates per unit area of non-divaricate leaves to match their whole-plant carbon gain. Although divaricate leaves had higher maximum photosynthetic rates than non-divaricate leaves in the glasshouse and the highest

maximum photosynthetic rates in the field, the difference did not approach this magnitude. Also, inner leaves which were exposed to the exterior climate showed the most pronounced decreases in most parameters (consistent with Kelly & Ogle, 1990, and Howell *et al*., 2002), indicating that 'self-shading' increases the susceptibility to photoinhibitory events in the event that incident light suddenly increases. Howell *et al*. (2002) found cold-induced photoinhibition in exposed leaves of three divaricate shrub species and most leaves showed no recovery from these photoinhibitory events, concluding that the 'self-shading' growth of divaricate shrubs prevent photodamage. This study investigated two divaricate species and their susceptibility to drought-induced photoinhibition in leaves under different sun exposures during the day. Although photoinhibitory events were found in exposed leaves, the results were inconclusive, as there was a greater difference in photoinhibitory events between genera than growth forms. From both results of the gas exchange measurements and pigment analyses there seems to be little evidence that the 'self-shading' growth form of divaricate shrubs is an adaptation to prevent photodamage.

<u>High Light and Low Temperatures</u>

Low temperatures have adverse effects on the metabolic functions of plants and photosynthesis is often the first influenced. The combination of high light intensities and low temperatures are especially damaging and often result in photo-oxidative damage to long-term exposed leaves (Hendrey *et al*., 1987). In contrast, low temperatures which only occur at night have little or no effect on photosynthesis and the PSII complex in particular (Ottander *et al*., 1995).

My study did not investigate low temperature effects, although the divaricate plants in the sub-alpine and alpine areas can experience sudden temperature reductions for hours or days even during the summer. For testing the evolutionary hypotheses for the effect of the divaricate growth form on photosynthesis, it may be particularly important to collect data concerning photosynthetic responses and the amount of cold-induced photoinhibition seen in these plants during such low temperature events. Additionally, it could be interesting to determine the length of the recovery period after such events and if photosynthetic carbon gain would be influenced. All these factors could help us to understand why divaricate plants are found in adverse habitats and non-divaricate plants are not (McGlone & Webb, 1981 and McGlone, 1985). It

could verify or refute assumptions made in the past (Chapter 1) or even lead to new insights and theories of how and why the divaricate growth form developed in New Zealand. Divaricate leaves which experienced atypical high light loads in the experimental set up of Howell *et al*. (2002), displayed the highest reductions in the photochemical efficiency of PSII, and had only partial recovery, compared to shaded or control plants. Also, the light response curves of those exposed leaves showed reduced photosynthetic rates, with reductions in maximum photosynthetic rates of up to 50%. Increased shading by branches in the interior not only decreased the amount of light but also the amount of photoinhibition, as measured by chlorophyll fluorescence.

Biochemical Adaptation

Secondary compounds are known to play important roles in the adaptation of species in different habitats. So far, no research has been undertaken on divaricate plants and their biochemical adaptation to adverse environments. Dungan *et al*. (2003) compared the photosynthetic response to different seasonal and temperature conditions in two New Zealand plants with contrasting leaf phenologies. In spring and summer, maximum rates of carboxylation and electron transport were more than 60% higher than in autumn/ winter and were significantly related to leaf nitrogen concentration per area in both leaf phenologies. Investigations of the nutrient supply in the different habitats of divaricate and non-divaricate shrubs as well as a comparison of the possible different usages of biochemical resources, such as nitrogen or potassium formate, would help to understand the distribution of divaricate and non-divaricate plants in different niches. Nitrogen may be a good predictor of the photosynthetic capacity in leaves because high leaf nitrogen concentrations have been correlated with high photosynthetic rates and dry matter accumulation (Evan, 1989). Potassium formate is known to reduce oxidative damage to photosystems by scavenging radicals and supplying $CO_2$ which may also be an important factor (Shiraishi *et al*., 2000). The observation of enzyme activities under different stress situations in divaricate versus non-divaricate leaves would give further insights into their adaptation to certain habitats and into the evolutionary development of divaricate and non-divaricate shrubs.

Morphological Adaptation

To use morphological adaptations to reduce the risk of photoinhibition, the total area of exposed chloroplasts has to be reduced. This can be achieved by moving and aggregating chloroplasts inside the cells (Long *et al*., 1994), or changing the angle of the whole leaf to the sun and therefore changing the amount of light absorbed (Powles, 1984; Long *et al*., 1994). The divaricate growth form was hypothesised to prevent photoinhibitory events through its self-shading growth form as well as by locating most of the small leaves in the shaded interior. As seen in my study, and in the study of Howell *et al*. (2002), photoinhibitory events occur in divaricate leaves even when they are 'self-shaded'. But both studies also found an increase in photoinhibition, when the outer branch layer was removed and interior leaves were exposed to exterior climate. This could indicate that the 'self-shading' branch layer on the outside of divaricate shrubs reduces photoinhibition to some extent. In contrast, both studies found that increased stress also increased the amount of photoinhibition, measured by a decrease in $F_v/F_m$. Conversely, divaricate leaves often displayed fast recoveries from these photoinhibitory events. Although the divaricate growth form did show high maximum photosynthetic rates, photoinhibitory events were often observed in divaricate and non-divaricate shrubs as a response to stressful treatments. These findings do not support the hypothesis that the 'self-shading' growth form of divaricate plants would decrease the frequency of photoinhibitory events.

## 5.2 Climate versus Moa Browsing Theories

Two main theories for the evolution of divaricate shrubs have been proposed to date. Environmental conditions provide an alternative to the theory of moa browsing as selective pressures for the development and distribution of divaricate shrubs. In this study, environmental conditions such as high radiation loads and mild drought were used to observe different adaptations of divaricate and non-divaricate leaves, such as water potentials of leaves and shoots, photochemistry through chlorophyll fluorescence measurements, pigment and a-tocopherol concentrations and gas exchange parameters. My hypothesis was that the evolution of divaricate shrubs was driven by adaptations to adverse environmental conditions, such as high radiation loads and/ or seasonal drought. As seen in Section 5.1, this hypothesis was not

generally supported by measurements of the $F_v/F_m$ ratio and the concentrations of photoprotective pigments and a-tocopherol.

Greenwood & Atkinson proposed in 1977 that the evolution of divaricate shrubs was a response to repetitive moa browsing. In an attempt to test this idea, Bond *et al*. (2004) observed emus and ostriches feeding on divaricate shrubs. Although the ratites did not preferentially browse divaricates, feeding was possible on this growth form. Given the stronger bone structure and therefore higher potential shear force of a moa bill (R. Holdaway, pers. comm.); the divaricate growth form obviously did not completely obliviate browsing on this species (see also Burrows 1980a, 1980b, Burrows *et al*., 1981). Additionally, the divaricate growth form is also found in other parts of the world. In Patagonia, spiny divaricates can be found in open habitats with low precipitation (McQueen, 2000). Cooper & Ginnet (1998) found limitations in browsing on the foliage of spiny woody plants compared to spineless species by large mammals. In contrast, small herbivores were able to manoeuvre around the spines, and no limitation in their browsing was found. Furthermore, their browsing had significant effects on the plant community (Gutierrez *et al*., 1997).

Divaricate shrubs of New Zealand, which are mostly spineless, are often found in open habitats where lizards are found. Lord & Marshall (2001) found a significant relationship between the occurrence of small fruits in pale colours, shrub-like growth forms, open habitats, and higher altitudes. A possibility could be that the evolution of divaricate shrubs in New Zealand is also influenced by limitations to seed dispersal and the presence or absence of seed dispersers.

Divaricate shrubs were hypothesised to use their outer branch layer as a form of umbrella (pers. comm., M. Turnbull) to shade their inner leaves and protect them from a harsh exterior climate. It was argued as either an advantage in past glacial periods or to more recent conditions, providing protection against high light, frost, or drought; subsequently reducing photoinhibitory events (Chapter 1). Therefore, shaded or 'self-shaded' leaves, which were hypothesised to avoid the costs of photoinhibition and photoprotection, should therefore maintain higher carbon gains than sun leaves during periods of adverse conditions. As described in Chapter 4, the balance between the benefits to carbon gain by avoiding photoinhibition versus the costs of

photoprotection to develop and maintain such branch umbrellas as found in divaricate shrubs is only starting to be investigated. The data from the present study do not support this hypothesis because of the observed high daytime respiration rates and low quantum efficiencies. McGlone & Webb (1981) argued that the costs for the establishment of the divaricate habit would be great. In contrast, the reduced light quantity in the interior would allow the majority of the leaves to photosynthesise without the costs of photoinhibition and/ or photoprotection throughout the year. This was hypothesised to be a benefit to the divaricate growth form, as net carbon gain under this scenario should be higher. The higher maximum photosynthetic rates observed in divaricate leaves support this notion. In contrast, the same leaves had higher daytime respiration rates and often lower quantum efficiencies than their non-divaricate congeners. Also, the decreases in water potential and $F_v/F_m$ seen in divaricate leaves tends to negate a benefit of divaricates in high light and drought in summer. Howell *et al*. (2002) did find cold-induced photoinhibition in divaricate leaves in the field, which suggests a winter, but not necessarily a year-round, benefit in the carbon gain in divaricate versus non-divaricate leaves.

Differences in energy dissipation via the xanthophyll cycle have previously been investigated for several species in understorey and fully exposed light conditions by Demmig-Adams *et al*. (1995). Shaded leaves reached much higher concentrations of antheraxanthin and zeaxanthin than sun leaves after a short exposure to high light. The plants used in my glasshouse trial were still very young and the divaricate shrubs had not completely developed their typical growth form. In contrast, the plants in the field experiment were mature divaricates, and these displayed the highest concentrations of photoprotective pigments and vitamin in my study. It could be possible that the ‘self-shading’ of divaricate leaves is not substantial enough and therefore, high light events do occur during the day in the interior of divaricate shrubs. This hypothesis would be supported by my findings of photoinhibitory events in experimentally unchanged interior leaves and the high concentrations of photoprotective pigments and a-tocopherol. Again, it does not support my main hypothesis that divaricate shrubs could have evolved as an adaptation to high radiation and drought.

The differences between divaricate and non-divaricate leaf responses to different light and water treatments in this study were mostly consistent but small. To support my hypothesis that the divaricate habit evolved to protect interior leaves from photoinhibitory events and the costs of photoprotection (Figure 1.2), substantial differences reflecting strong evolutionary pressure would have been expected. In contrast, the main differences were found mostly between the genera of *Corokia* and *Coprosma* (Figure 5.1). This tends to indicate that the divaricate growth form developed independently and after these genera developed the physiological characteristics required for photoprotection. It also indicates that the divaricate habit is not uniquely suited to the habitats in which divaricates are found at present, even though their evolution is of recent origin. It would be interesting to compare estimates of the time of divergence of divaricate and non-divaricate species within the different genera, most likely by using molecular-clock measurements. If it could be shown that the divaricate species all diverged from their non-divaricate congeners synchronously, it would be interesting to try to correlate this with historical climatic events. If it could be shown that speciation within different genera might have co-occurred with major climate events, such as glaciations or warming of the mean temperatures, this could be good evidence for the climate theory. This could reveal that these genera evolved the divaricate form in response to the same climatic conditions. Although this would indicate that climate played an important role in the evolution of this growth form, it would still remain unclear what the ultimate selection pressure was. A change in climate may also alter, for example, herbivore (moa) abundance, so that the actual selection pressure was not climate *per se*.

# APPENDICIE'S

## A1 Comparison between *Corokia* Divaricate, Non-Divaricate and Intermediate Hybrids in a Glasshouse trial in 2003

This appendix section describes an attempt to determine physiological differences between hybrid plants of *Corokia cotoneaster* x *buddleioides* and the divaricate *C. cotoneaster* (Figure 2.4) and non-divariate *C. buddeioides*. The intermediate hybrid plants were purchased as putative divaricate (*C. cotoneaster*) plants and used in the glasshouse experiments in 2002. After recognition of more intermediate morphological traits of the hybrid plants, non-hybrid divaricate plants were purchased (Section 2.1.3). Comparative measurements of all three taxa were performed to determine the differences in the physiological response of divaricate and hybrid versus non-divaricate leaves. It was expected that the most pronounced difference would be found between divaricate and non-divaricate leaves, whereas the intermediate hybrid with its relatively small leaves would have a physiological response to the treatments close to those for the divaricate leaves and therefore could be regarded as comparable to divaricate plants for the purposes of statistical analysis of the glasshouse experiments.

The physiological response of the divaricate *C. cotoneaster*, non-divaricate *C. buddleioides* and intermediate hybrid *C. cotoneaster* x *buddleioides* leaves were compared in the glasshouse experiment in 2003 (Section 2.1.3). Water potential measurements (Section 2.2) and fluorescence measurements (Section 3.1) were carried out in January and December 2003. Analysis of pigments and vitamin E (Section 3.2) and gas exchange measurements (Chapter 4) were carried out in December 2003. All plants were grown for at least six months under the treatments described in Section 2.1.3. Due to insect infestation in the glasshouse, some young plants did not survive until the measurements were taken. When only one plant or result was available for a certain treatment combination, data are marked as '#' in the graphs.

Response to Drought

The three *Corokia* taxa, including divaricate, intermediate hybrid and non-divaricate shrubs, were measured and compared for their water potential values. The non-divaricate leaves were measured directly in the Scholander pressure chamber, divaricate and hybrid water potentials were taken from small shoots with at least 3 healthy leaves on them. It was expected that divaricate and non-divaricate shrubs would possess different water usage patterns due to their varying growth forms. The divaricate growth form was hypothesised to prevent water losses via transpiration due to the self-shading growth form (Chapter 1). Therefore, the divaricate shoots were predicted to have less negative water potentials at noon, when transpiration in plants is highest. Additionally, the recovery in the evening towards the predawn water potential values should be more complete in divaricate leave because of their water conserving growth form. The non-divaricate leaves should express the lowest water potential values at noon, as they have the largest leaf area (Section 2.1.3) and therefore a large transpiration surface with high potential water loss in high temperatures. The intermediate hybrid had a leaf area intermediate between the non-divaricate and the divaricate, although the number of leaves per branch was more similar to non-divaricate than divaricate plants. An intermediate response between the divaricate and non-divaricate plants was expected in these plants. A water potential response to the light and water treatments close to the response seen for divaricate shoots would support the inclusion of the hybrid plants in for the analysis for divaricate leaves.

In January, measurements were made predawn, noon and evening (Figure A1.1); and in December predawn only (Figure A1.2). In January (Figure A1.1a), plants under water stressed conditions had low predawn water potentials. All habits under water stress conditions had lower water potentials at noon (Figure A1.1b) and did not re-establish predawn water potential values in the evening measurements compared to well-watered plants. Interestingly, the intermediate hybrid under water stressed conditions had low values at noon, but did not recover as well as the divaricate or non-divaricate plants in the evening (Figure A1.1c). Divaricate leaves displayed lower predawn water potential under water stressed conditions than under well-watered conditions.

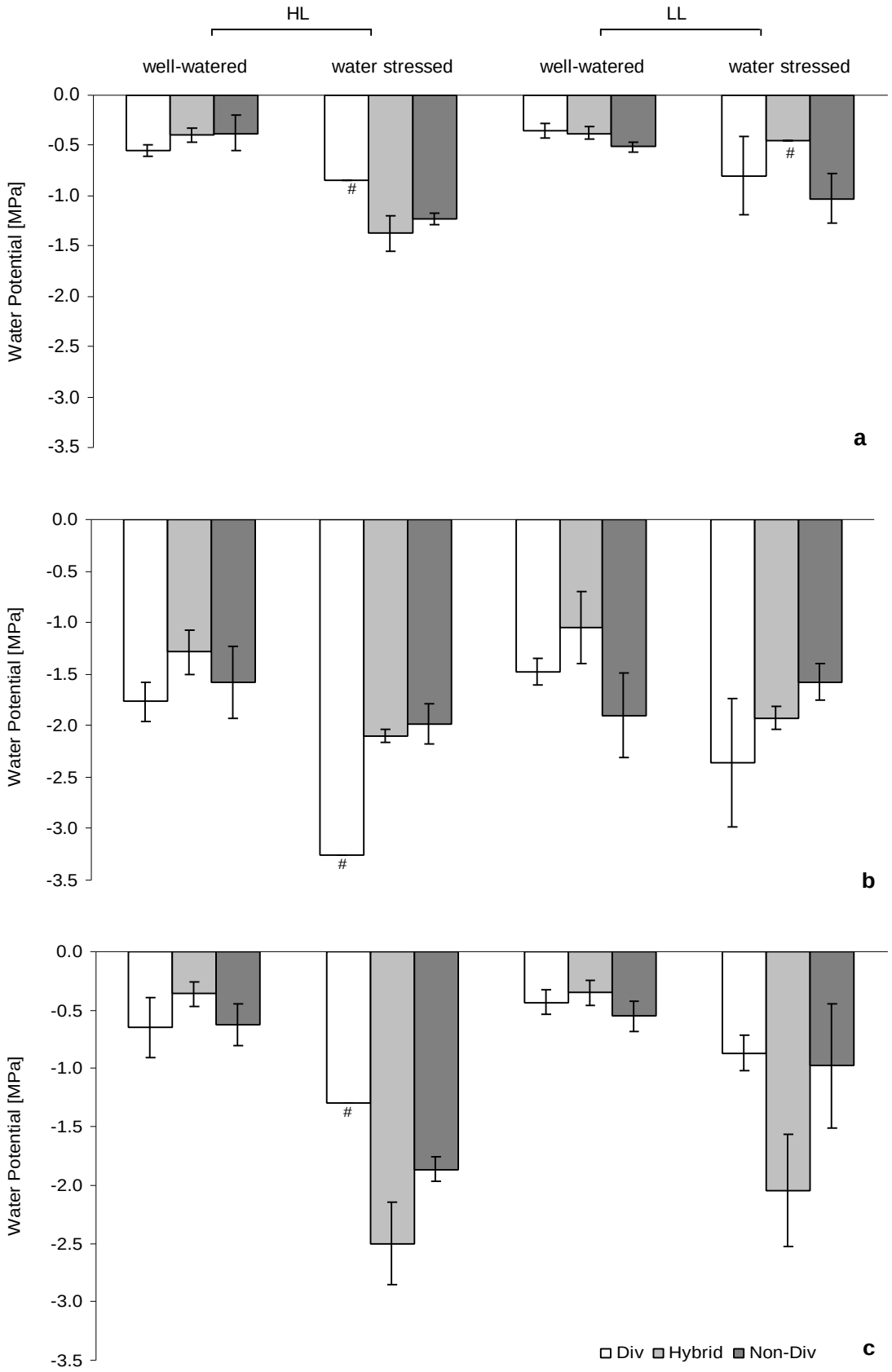



Figure A1.1 Water potential measurements for *Corokia cotoneaster* (Div), *Corokia* hybrid (Hybrid) and *Corokia buddleioides* (Non-Div); measurements taken at (a) predawn, (b) noon, and (c) evening, in the glasshouse, January 2003. Recorded under well-watered and water stressed conditions, and two different light treatments (HL = sun light, LL = shaded) [n = 4].

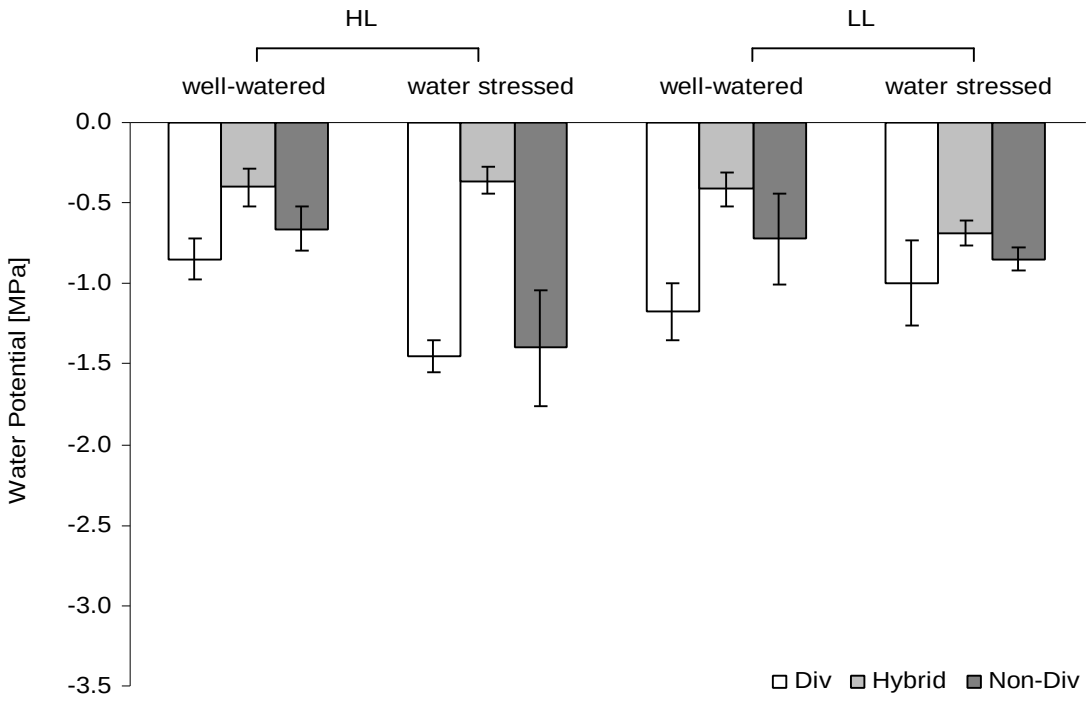



Figure A1.2 Water potential measurements for *Corokia cotoneaster* (Div), *Corokia* hybrid (Hybrid) and *Corokia buddleioides* (Non-Div); measurements taken predawn in the glasshouse, December 2003. Recorded under well-watered and water stressed conditions, and two different light treatments (HL = sun light, LL = shaded) [n = 4].

At noon, divaricate shoots had the lowest water potentials of the three growth forms, but had a faster recovery towards predawn water potential values in the evening than non-divaricate or hybrid leaves. During December, the divaricate and non-divaricate plants had the lowest water potentials, whereas the hybrid plants had less negative water potentials. In the ANOVA, habit had a significant effect at the noon and evening measurements in January, and also for the predawn measurements in December (Table A3.1). The interaction of water availability* habit had significant effects on all measurements taken in January. Under mild drought (water stressed) all plants expressed significantly lower water potentials than under well watered conditions.

In January, the water potential values of divaricate shoots in the evening were not as negative as those of the hybrid or non-divaricate plants under water stressed conditions in both light treatments. The water potential values in December were more negative than in January. This is likely due to hotter days with less overcast skies in December than in January. Possibly, transpiration was less extensive in

January as less PFDs would reduce light and temperature stress. This could also indicate a more economical use of available water sources in divaricate leaves in diffuse light, which could be caused by their unique self-shading and water conserving growth form. The comparison between divaricate, non-divaricate and hybrid *Corokias* showed that although divaricates and non-divaricates respond differently to the treatments, the fastest response to changed water potentials was shown by divaricate shoots. As shown in Figure 2.1, the hybrid morphology is closer to the non-divaricate growth form than the divaricate growth form. Under well-watered conditions, the least negative water potential values were seen during the day in the hybrid shoots. In water stressed conditions, the noon measurements show low values for hybrid shoots, and in the evening these shoots still expressed the most negative water potential values. The hybrid leaf and stem traits could offer some protection against water loss at noon, but did not support the recovery toward predawn values better than non-divaricate leaf and growth traits.

Fluorescence

$F_v/F_m$ was measured for divaricate, intermediate hybrid and non-divaricate *Corokia* leaves. As described in Section 3.1.2, chlorophyll fluorescence was estimated at the same time as shoot and leaf water potentials. In January, $F_v/F_m$ was measured predawn, noon and in the evening, and in December predawn only. As argued in Section 3.1, $F_v/F_m$ is influenced by light intensity, and over-saturation can induce photoinhibition. Healthy plants have $F_v/F_m$ ratios around 0.8. As the light load changes during the day, the amount of emitted fluorescence and therefore $F_v/F_m$ changes in leaves. As seen before, water shortages can also increase photoinhibition due to the inactivation of PSII, which also reduces $F_v/F_m$. Therefore, leaves which are exposed to high light and mild drought stress should display decreased $F_v/F_m$ values, in particular at noon, when light intensities are highest. Non-divaricate leaves are without a sheltered outer branch layer, in contrast to divaricate leaves. The large non-divaricate leaves were hypothesised to express low predawn $F_v/F_m$ values, a substantial decrease in $F_v/F_m$ at noon and an incomplete recovery of the $F_v/F_m$ towards predawn values in the evening measurements. The ‘self-shading’ and water conserving growth form should protect the small divaricate leaves against photoinhibitory events and the $F_v/F_m$ ratio should remain high during the day. The

small leaves of the intermediate hybrid were expected to express similar $F_v/F_m$ values as divaricate leaves during the day.

In all leaves, diurnal changes in $F_v/F_m$ were found (Figure A1.3), indicating photoinhibitory effects to some extent. In particular, hybrid leaves in the shade showed low values in well-watered conditions. The better recovery of the $F_v/F_m$ values towards the predawn values in divaricate leaves indicates a potential advantage of the self-shading and water conserving growth form. A faster recovery from photoinhibitory effects could either indicate a lesser amount of inactivated PSII or a faster re-activation of inactivated PSII via the mechanisms described in Section 3.1.1 (D1 repair, thermal dissipation of energy via xanthophyll cycle). Significant effects of habit were only found for the evening measurements in January. Divaricate and non-divaricate leaves showed a similar response to the light and water treatments, whereas the hybrid leaves responded differently. The intermediate hybrid displayed the lowest $F_v/F_m$ values under shade and well-watered conditions in the evening. In December, the predawn $F_v/F_m$ values of the hybrid leaves were comparable with those of divaricate leaves when observed under water stressed conditions, but comparable with non-divaricate leaves when observed under well-watered conditions.

In summary, a significant effect of habit on the $F_v/F_m$ values were only found in the evening, where the hybrid leaves showed a response different from divaricate and non-divaricate leaves. The interactions of light level* habit, water availability* habit and light level* water availability* habit had also significant effects on the $F_v/F_m$ ratios. This inconsistent response of the hybrid leaves made it hard to include the hybrid plants in the analysis of divaricate leaves in this experiment. The small leaf size alone was obviously not sufficient for hybrid plants to express the higher $F_v/F_m$ values in the more adverse treatments, as seen in the divaricate leaves. The shelter by branches on the outside of the plant might have had a positive influence on the water and light conditions received by the interior divaricate leaves, explaining the discrepancy between the results for the hybrids and divaricates.

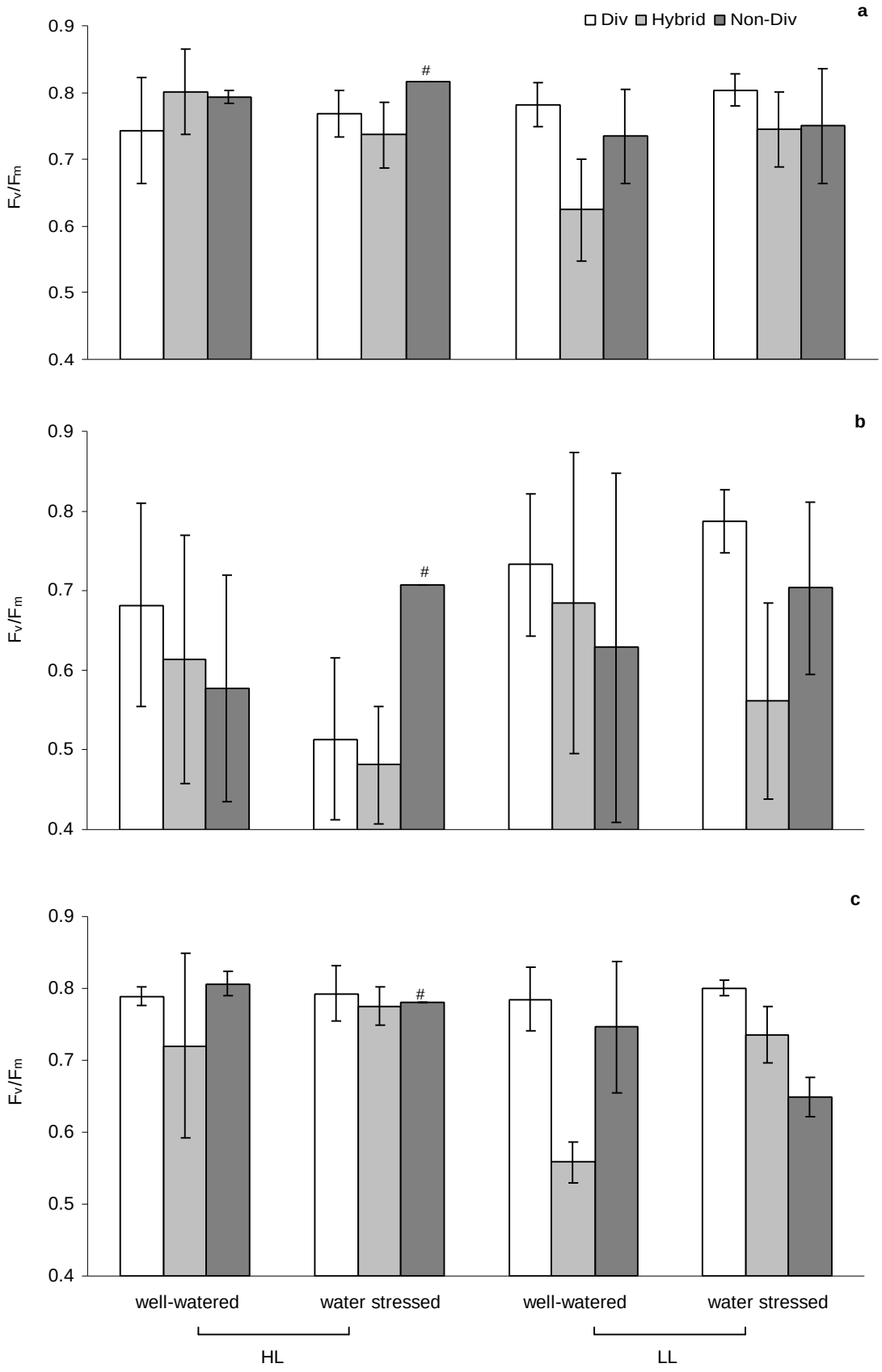



Figure A1.3 Photochemical efficiency of PS II (Fv/Fm) in the Glasshouse at (a) predawn, (b) noon and (c) evening in January 2003 for *Corokia cotoneaster* (Div), a *Corokia* hybrid (Hybrid) and *Corokia buddleioides* (Non-Div.) in well-watered and water stressed conditions and two different light treatments (HL = sun light, LL=shaded) [n = 4].

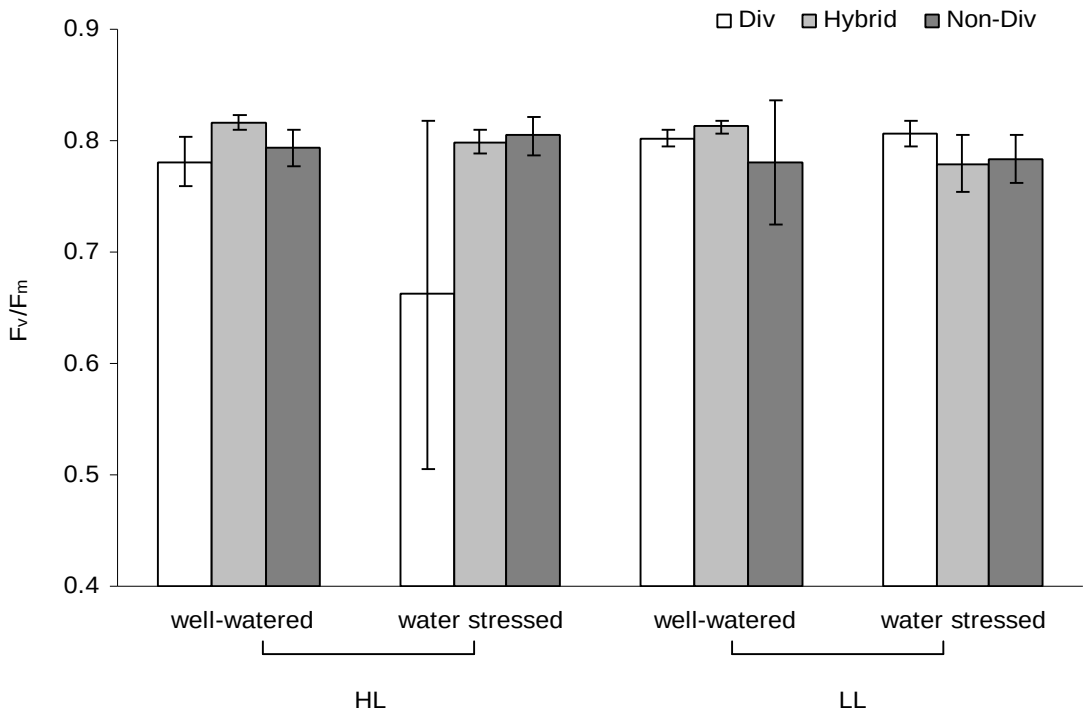



Figure A1.4 Photochemical efficiency of PS II (Fv/Fm) in the Glasshouse predawn in December 2003 for *Corokia cotoneaster* (Div), a *Corokia* hybrid (Hybrid) and *Corokia buddleioides* (Non-Div.) in well-watered and water stressed conditions and two different light treatments (HL = sun light, LL=shaded) [n = 4].

Pigment and ?-Tocopherol Compositions

In December 2003, leaf samples for pigment and ?-tocopherol analysis were taken from *C. cotoneaster*, the *C. cotoneaster x buddleioides* hybrid and *C. buddleioides*. Due to technical difficulties during the HPLC analysis (Section 3.2.2) samples were lost and there was insufficient replication to interpret the effect of light level on the pigment composition, but there were enough samples for the analysis of ?-tocopherol. As demonstrated in Section 3.2.1, the amounts of photoprotective pigments increase with increasing light intensities, air temperature and water stress. Divaricate leaves were hypothesised to use water conservatively and shade their interior leaves via their highly interlaced branches on the outer crown (Chapter 1). This could offer these interior leaves enough protection against high light loads and therefore high amounts of photoprotective pigments and antioxidants such as ?-tocopherol would not be required in the same levels as in the unshaded non-divaricate leaves. As mentioned above, the intermediate hybrid was hypothesised to respond in a manner similar to divaricate leaves, and therefore concentrations of photoprotective pigments and the

antioxidant ?-tocopherol were expected to be similar to the values in divaricate leaves or at least intermediate to the values of divaricate and non-divaricate leaves.

The concentrations of violaxanthin, antheraxanthin and zeaxanthin per unit total chlorophyll were higher in divaricate leaves than in hybrid and non-divaricate leaves (Figure A1.5). The relation of violaxanthin, antheraxanthin and zeaxanthin to unit leaf area produced an even more striking pattern (Figure A1.6). The relation of each single pigment and ?-tocopherol on a leaf area basis showed that there were higher concentrations for all pigments and for ?-tocopherol in divaricate leaves (examples shown in Figures A1.6, A1.12). Relating pigment concentration to leaf fresh weight also showed that divaricates had the highest relative amounts of violaxanthin, antheraxanthin and zeaxanthin (Figure A1.7). Hybrid and non-divaricate leaves showed similar concentrations in violaxanthin, antheraxanthin and zeaxanthin, which were substantially lower than in the divaricate leaves (Figures A1.5 to A1.7).

As discussed in Section 3.2, neoxanthin and lutein have structural functions and therefore normally do not vary greatly in their concentrations. As seen in Section 3.2.3 and 3.2.4, this study found differences in the concentrations between the growth forms. The highest concentrations of neoxanthin and lutein were found in divaricate leaves, only the concentration of neoxanthin per unit fresh weight was shown (Figure A1.8). The concentrations for hybrid and non-divaricate leaves were similar for neoxanthin and lutein per unit total chlorophyll, leaf area and fresh weight (data only shown for neoxanthin per unit fresh weight, Figure A1.8).

The concentrations of chlorophyll a, chlorophyll b and chlorophyll a+b per unit fresh weight were highest in divaricate leaves (Figure A1.9). Again, the concentrations for chlorophyll a, chlorophyll b and chlorophyll a+b in hybrid and non-divaricate leaves were similar and substantially lower than in the divaricate leaves. The chlorophyll a:b ratio was found to be around 3.0 in the divaricate and the non-divaricate leaves, whereas the intermediate hybrid leaves exhibited a ratio of 2.7 (Figure A1.10).

As was found for violaxanthin, antheraxanthin and zeaxanthin, chlorophyll a and chlorophyll b, the ?-carotene concentrations were highest in divaricate leaves.

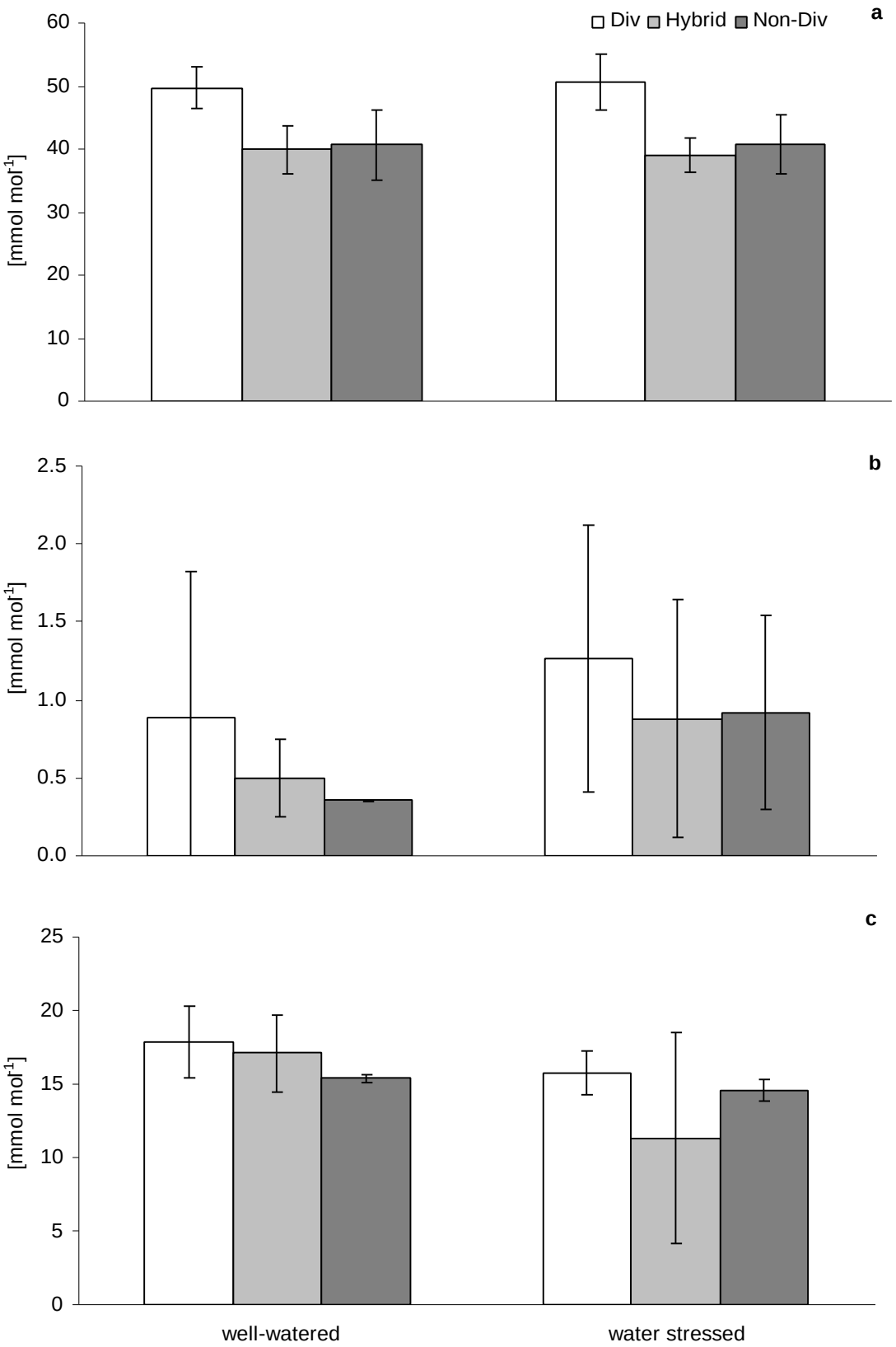



Figure A1.5 Contents of (a) violaxanthin, (b) antheraxanthin and (c) zeaxanthin per unit total chlorophyll in the Glasshouse in 2003 for divaricate (Div), intermediate hybrid (Hybrid) and non-divaricate leaves (Non-Div) in well-watered and water stressed conditions [n = 4].

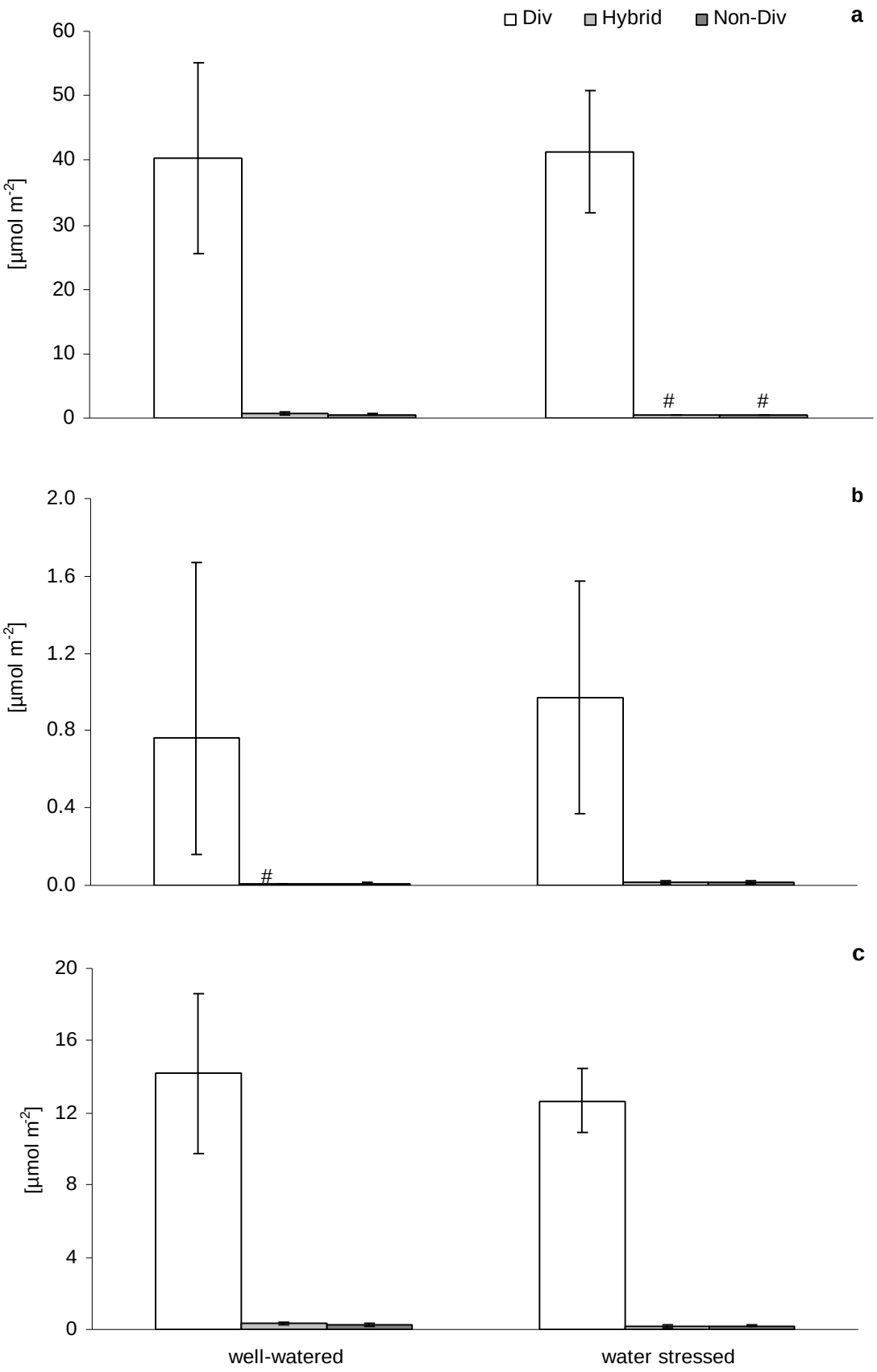



Figure A1.6 Contents of (a) violaxanthin, (b) antheraxanthin and (c) zeaxanthin per unit leaf area in the Glasshouse in 2003 for divaricate (Div), intermediate hybrid (Hybrid) and non-divaricate leaves (Non-Div) in well-watered and water stressed conditions [n = 4].

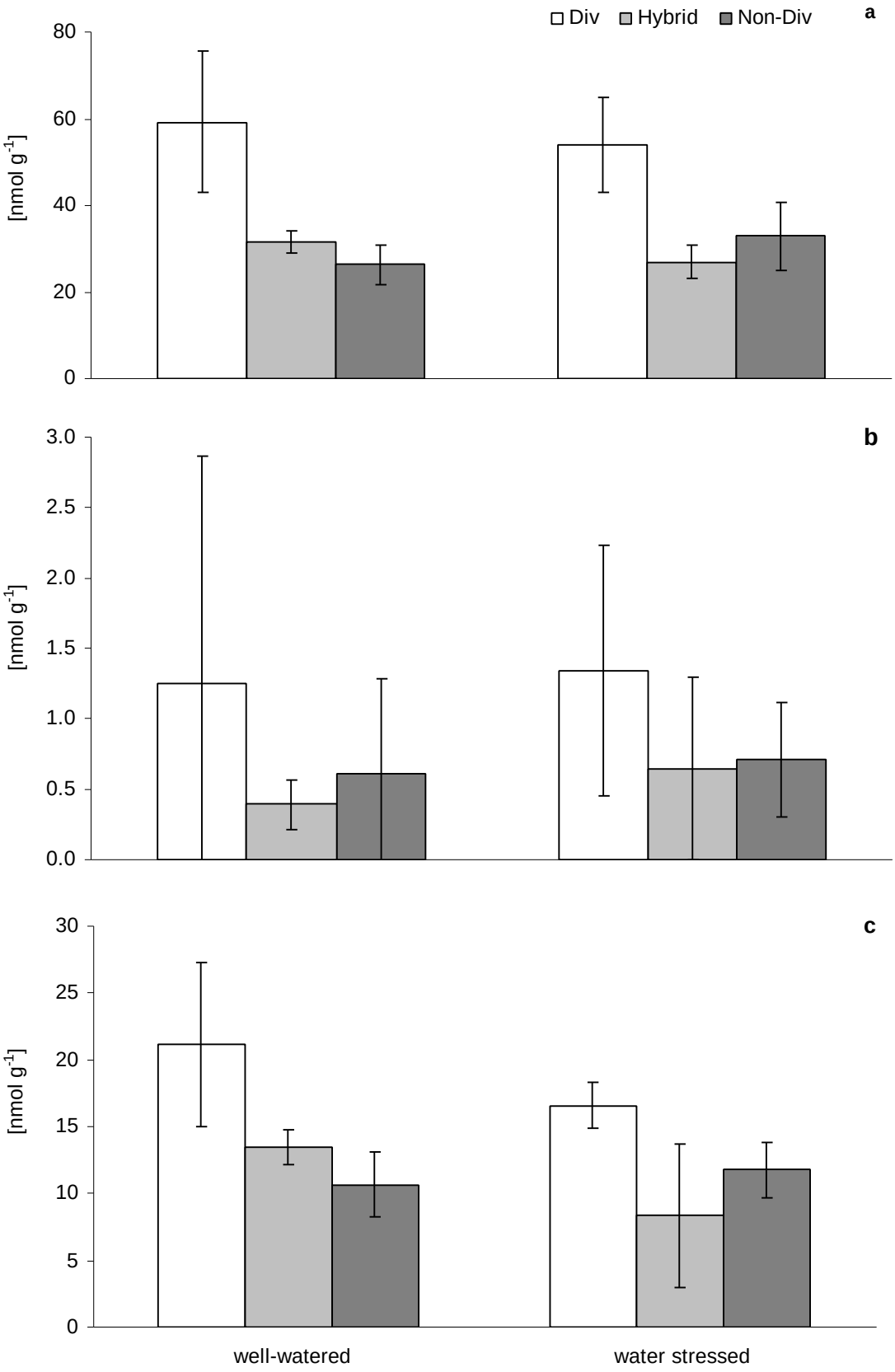



Figure A1.7 Contents of (a) violaxanthin, (b) antheraxanthin and (c) zeaxanthin per unit fresh weight in the Glasshouse in 2003 for divaricate (Div), intermediate hybrid (Hybrid) and non-divaricate leaves (Non-Div) in well-watered and water stressed conditions [n = 4].

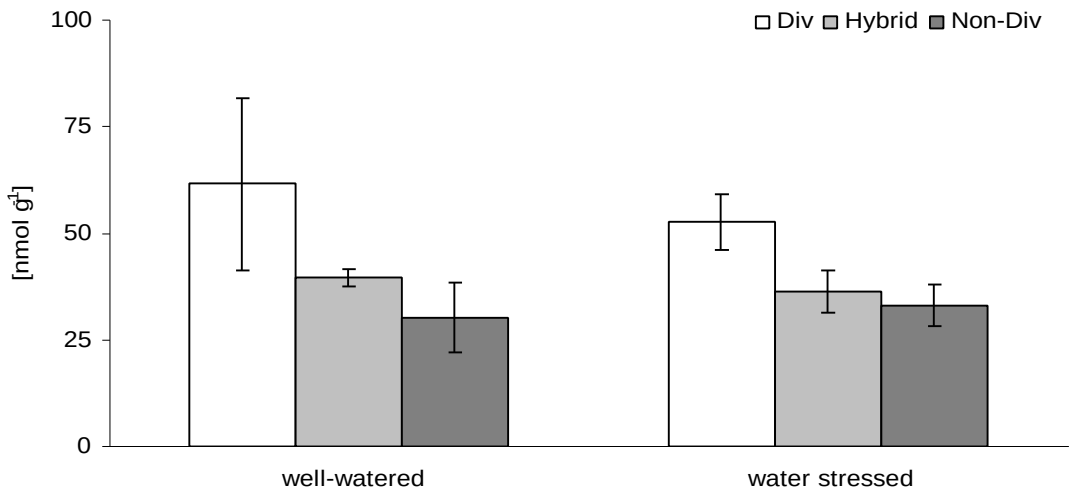



Figure A1.8 Contents of neoxanthin per unit fresh weight in the Glasshouse in 2003 for divaricate (Div), intermediate hybrid (Hybrid) and non-divaricate (Non-Div) in well-watered and water-stressed conditions [n = 4].

When calculated on a fresh weight basis (Figure A1.11), ?-carotene concentrations in hybrid leaves were more similar to non-divaricate than to divaricate leaves.

The concentrations of ?-tocopherol per unit total chlorophyll, leaf area and fresh weight are displayed in Figure A1.12. The content of ?-tocopherol was still high in divaricate leaves, but when calculated per unit of total chlorophyll and fresh weight, intermediate hybrid and non-divaricate leaves displayed higher concentrations. The content of ?-tocopherol per unit total chlorophyll was highest in non-divaricate leaves under high light and well-watered conditions. In this treatment, hybrid leaves displayed their lowest concentrations per unit leaf area and fresh weight. When related to total chlorophyll, the lowest ?-tocopherol concentrations in all growth forms were found for plants in shaded conditions.

The ANOVA analysis of the pigment and ?-tocopherol concentrations were presented per unit total chlorophyll (Table A3.3 to A3.10), unit leaf area (Table A3.11 to A3.21) and unit fresh weight (Table A3.22 to A3.32). The relation to unit total chlorophyll displayed significant effects of habit on the concentrations of violaxanthin, neoxanthin, ?-carotene and ?-tocopherol (Table A3.3, A3.7, A3.9 and A3.10).

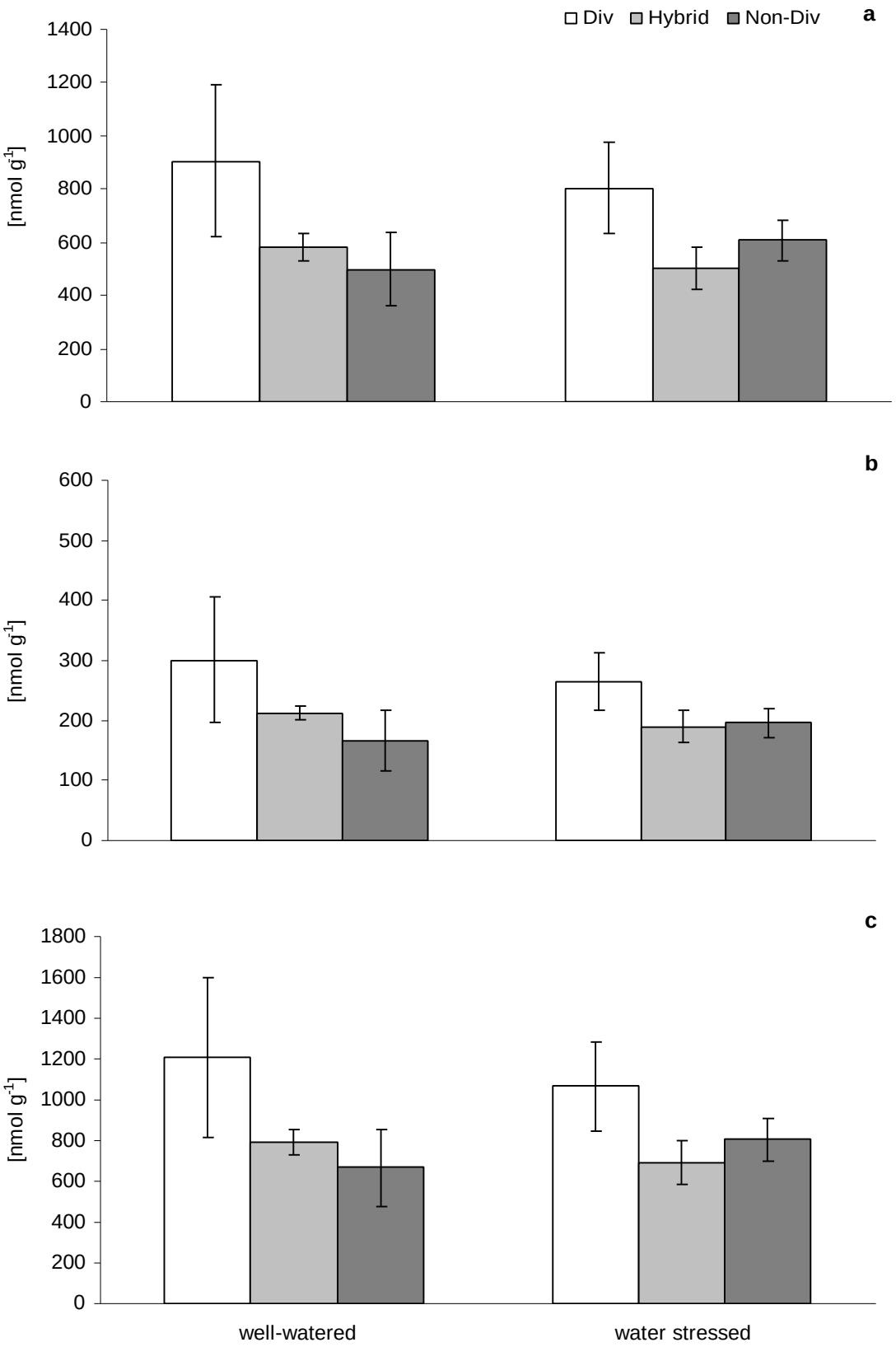



Figure A1.9 Contents of (a) chlorophyll a, (b) chlorophyll b and (c) chlorophyll a+b per unit fresh weight in the Glasshouse in 2003 for divaricate (Div), intermediate hybrid (Hybrid) and non-divaricate leaves (Non-Div) in well-watered and water stressed conditions [n = 4].

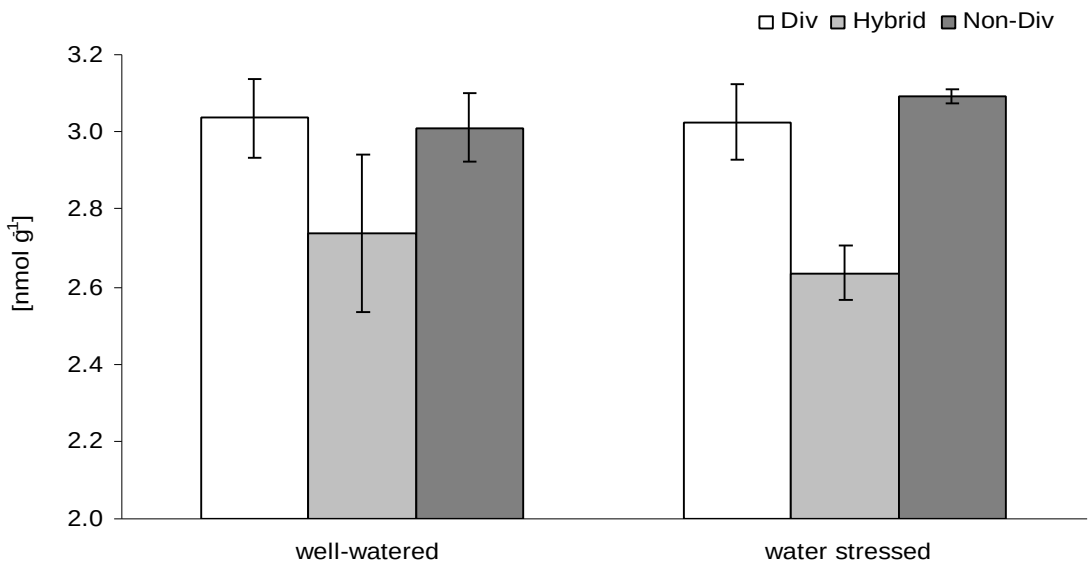



Figure A1.10 Ratio of chlorophyll a:b in the Glasshouse in 2003 for divaricate (Div), intermediate hybrid (Hybrid) and non-divaricate (Non-Div) in well-watered and water-stressed conditions [n = 4].

The ?-tocopherol concentrations were also significantly affected by light level, light level* habit and water availability* habit (A3.10). The relation to unit leaf area showed significant effects of habit for all pigments and ?-tocopherol (A3.11 to A3.21), which was also significantly affected by light level and light level* habit (A3.20 and A3.21). The relation to unit fresh weight also displayed significant effects of habit for all pigments, except for antheraxanthin (A3.22, A3.24 to A3.31). ?-Tocopherol per unit fresh weight was significantly affected by light level, light level* habit and water availability* habit (A3.30 and A3.31). For all pigment analyses, divaricate leaves showed the highest concentrations, whereas hybrid and non-divaricate leaves had similarly low concentrations. This result is in contrast to my hypothesis that divaricate and hybrid leaves would have similar pigment concentrations. The concentrations of ?-tocopherol per unit leaf area displayed the same result. However, the hybrid leaves had significantly different responses to the water and light treatments as seen in the ?-tocopherol concentration per unit chlorophyll or per unit fresh weight. Due to technical difficulties, samples were lost and the pigment analysis for light level became impossible; only samples of shaded leaves were analysed. The water availability and the interaction of water availability* habit did not significantly affect any pigment concentrations per unit total chlorophyll, leaf area or fresh weight (A3.3 to A3.31).

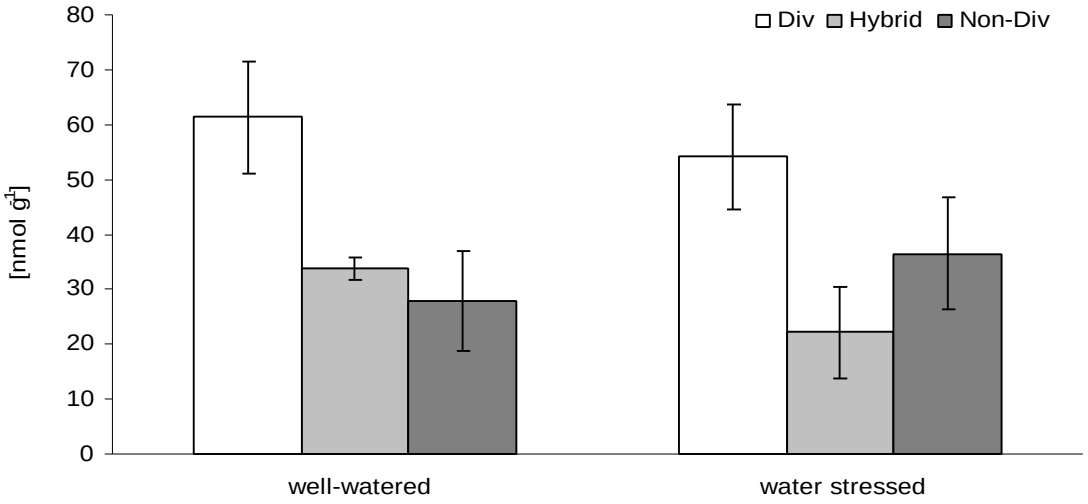



Figure A1.11 Contents of ß-carotene per unit fresh weight in the Glasshouse in 2003 for divaricate (Div), intermediate hybrid (Hybrid) and non-divaricate leaves (Non-Div) in well-watered and water stressed conditions [n = 4].

Divaricate leaves, which were expected to have low contents of photoprotective pigments due to their self-shading growth form, expressed high concentrations of xanthophyll cycle pigments, chlorophyll a and chlorophyll b, a high chlorophyll a:b ratio and high concentrations of ?-carotene and ?-tocopherol. All those pigments have at least to some extent photoprotective functions (Section 3.2.1) and concentrations are usually highest in leaves with full sun exposure. ?-Tocopherol functions as an antioxidant in plant cells, and concentrations normally increase as radiation loads increase. Therefore, the analysis of the pigment and antioxidant contents in divaricate leaves brought results contradicting the hypothesis that shading, and here in particular 'self-shading', reduces the concentration of photoprotective pigments. As discussed in chapters 2 to 4, the degree of 'self-shading' in divaricate plants in the glasshouse was not as great as seen in fully-grown plants of the experimental species, or in other species, such as in beech (*Fagus sylvatica*; Hansen *et al.*, 2002b). The plants were still very young and the typical crown structure not fully developed, which could have influenced the results. Comparing the ?-tocopherol concentrations in high light and shaded plants, nearly all samples expressed higher concentrations of ?-tocopherol in high light. In the shade, divaricate leaves had much lower concentrations of vitamin E than hybrid or non-divaricate leaves. Here, there is some indication that divaricate plants have a lower requirement for photoprotection than the other growth forms.

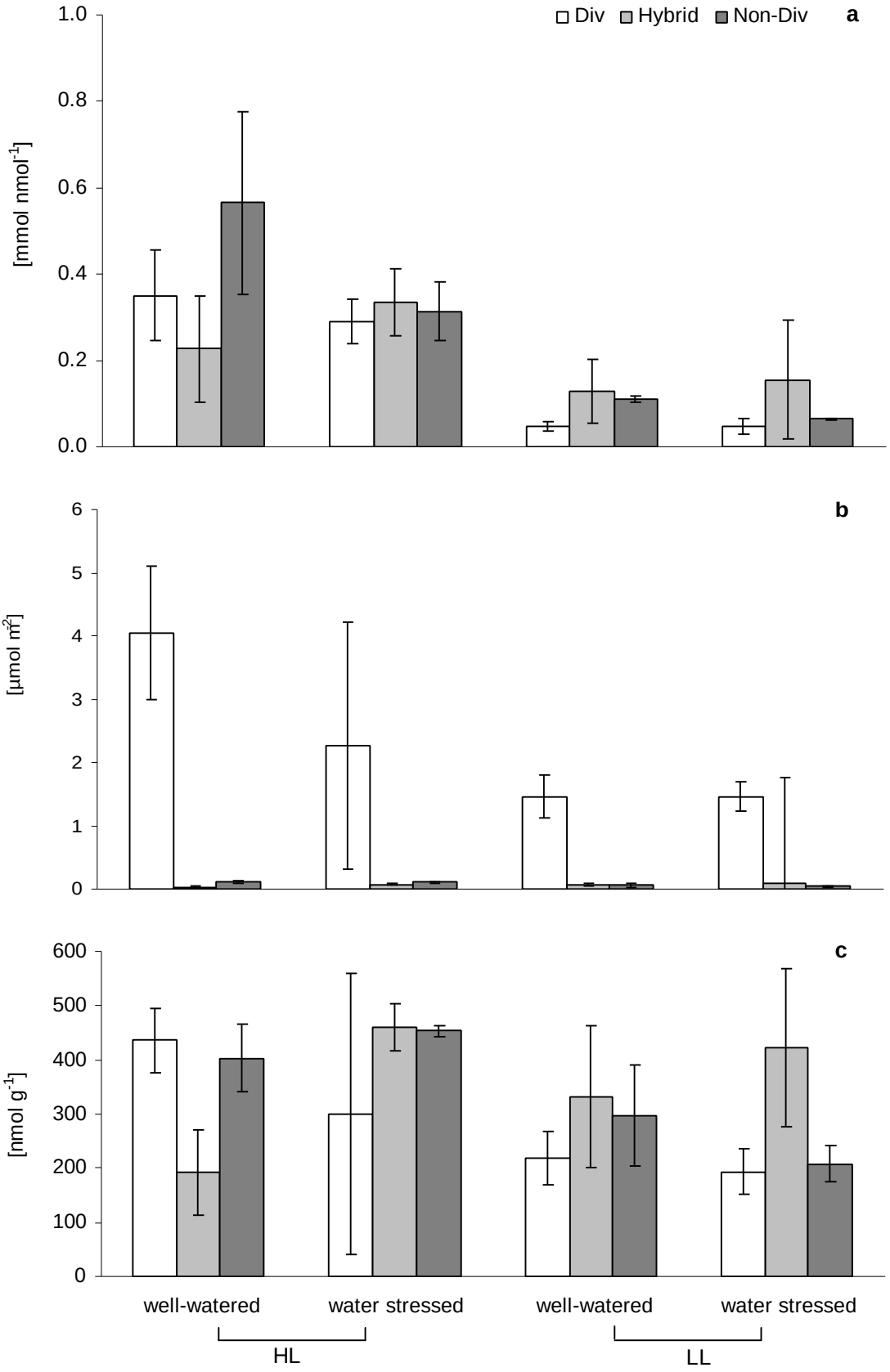



Figure A1.12 Contents of ?-tocopherol per unit (a) total chlorophyll, (b) leaf area and (c) fresh weight in the Glasshouse in 2003 for divaricate (Div), intermediate hybrid (Hybrid) and non-divaricate leaves (Non-Div) in well-watered and water stressed conditions and two different light treatments (HL = sun light, LL=shaded) [n = 4].

Lower water availability decreased the concentrations ?-tocopherol in divaricate leaves in particular. The naturally unshaded leaves of the intermediate hybrid and the non-divaricate plants displayed much lower concentrations of all the analysed pigments and of ?-tocopherol. The concentrations of each pigment and ?-tocopherol were similar in hybrid and non-divaricate leaves. Therefore, the hybrid leaf trait did not represent divaricate leaf traits and should not be used for modelling the response of divaricate leaves in regard to pigment and antioxidant concentrations.

Gas Exchange

In 2003, light response curves were determined for leaves of the divaricate *Corokia cotoneaster*, the non-divaricate *Corokia buddleioides* and a hybrid of both of them. Parameters, such as daytime respiration (Rd), quantum efficiency (QE) and maximum photosynthetic rate ($A_{max}$) were subsequently calculated from these light response curves (Section 4.2). Chapter 4 described in detail the influence of water deficit and high light load on plants and divaricate and non-divaricate leaves in particular. As argued by R. Christian (pers. comm.), divaricate leaves should have a much higher photosynthetic rate than non-divaricates to produce a positive carbon gain at the whole-plant level, due to the costs of the greater biomass of branches required to form the typical 'self-shading' growth form. The photosynthetic rates of divaricate, intermediate hybrid and non-divaricate leaves were compared to test the hypothesis that non-divaricate leaves have lower photosynthetic rates due to them being unshaded, and therefore more susceptible to photoinhibition under high light and under water stress. Also, these measurements of photosynthetic rates further test the hypothesis that the intermediate hybrid is functionally similar to the divaricate form. Comparable values for Rd, QE and $A_{max}$ in divaricate and hybrid leaves were expected, whereas a contrasting picture between divaricate and non-divaricate leaves was expected to emerge. High Rd, low QE and low photosynthetic rates in unshaded non-divaricate leaves would lead to a lower carbon gain under adverse environmental conditions.

Daytime respiration, shown in Figure A1.13a, displayed a pronounced difference in the response to water treatments between leaves of divaricate and hybrid plants versus

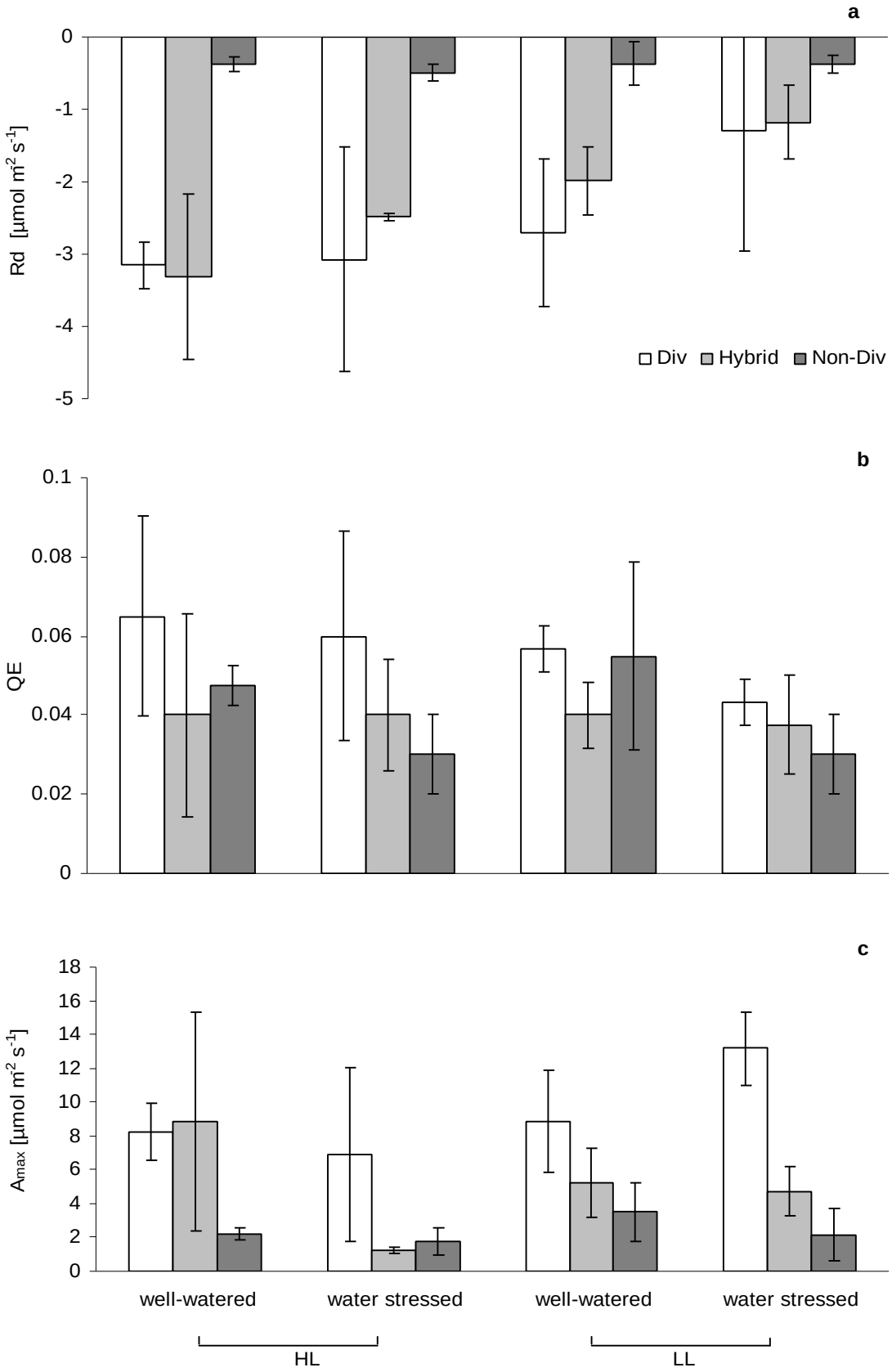



Figure A1.13 Gas exchange measurements in the Glasshouse, summer 2003 for (a) daytime respiration (Rd), (b) quantum efficiency (QE) and (c) maximum photosynthetic rate (Amax) in well-watered and water stressed conditions and two different light treatments (HL = sun light, LL = shaded) for *Corokia cotoneaster* (Div), a *Corokia* hybrid (Hybrid) and *Corokia buddleioides* (Non-Div.).

non-divaricate plants. Divaricate and hybrid leaves had Rd's between 1.2 and 3.3 µmol $CO_2$ $m^{-2}$ $s^{-1}$. The non-divaricate leaves had Rd's between 0.37 and 0.5 µmol $CO_2$ $m^{-2}$ $s^{-1}$. In well-watered conditions, the leaves of divaricate and hybrid plants show higher Rd's than under water-stressed conditions. The non-divaricate leaves did not show variations in different water treatments. The divaricate *Corokia* had the highest Rd's. Significant effects of water availability and habit on Rd's were found (Table A3.33). The hybrid leaves displayed values which were similar to the values for divaricate leaves.

QE was highest, around 0.06 (Figure A1.13b), in divaricate leaves in all treatments. Leaves of the hybrid did not show variations in QE, with the values around 0.04. Non-divaricate leaves had high QEs in well-watered conditions, around 0.05. However in water-stressed conditions they reached values around 0.03. Habit was found to have significant effects on QE (Table A3.34). The values of QE of the true divaricate leaves were higher than for the intermediate hybrid and non-divaricate leaves.

In contrast to my findings in the glasshouse experiment in 2002, *C. cotoneaster* expressed a high $CO_2$ uptake (photosynthetic rate) over the range of light intensities investigated. The highest value of 13 µmol $CO_2$ $m^{-2}$ $s^{-1}$ for $A_{max}$ in divaricate *Corokia* leaves was found under shade and water stressed conditions (Figure A1.13c). The lowest value of 7 µmol $CO_2$ $m^{-2}$ $s^{-1}$ was recorded under natural light and water stressed conditions. Hybrid leaves showed their highest $A_{max}$ of 9 µmol $CO_2$ $m^{-2}$ $s^{-1}$ in high light and well-watered conditions, but reached only 1 µmol $CO_2$ $m^{-2}$ $s^{-1}$ under water-stressed conditions. The non-divaricate leaves expressed $A_{max}$ in all treatments, and reached only 3.5 µmol $CO_2$ $m^{-2}$ $s^{-1}$ in well-watered and shaded conditions. The ANOVA analysis showed significant effects of habit and light level* water availability on maximum photosynthetic rate (Table A3.35). $A_{max}$ was highest in divaricate leaves in all treatments and lowest in non-divaricate leaves, the hybrid leaves expressed intermediate values. Therefore, it seems that the 'self-shading' and water conserving growth form of the divaricate shrubs was better able to maintain a high photosynthetic rate under the experimental conditions than the hybrid or non-divaricate plants. The hybrid, therefore, was not found to be equivalent to the divaricate form.

It has been clearly shown that hybrid leaves are not a suitable model for divariacte leaves, as is shown by the different responses of the hybrid leaves seen in Rd, QE and $A_{max}$.

The transpiration rates (E) were significantly effected by habit, light level* water availability, light level* habit and water availability* habit. Plants grown under good water availability had higher E than plants grown under water stress divaricate and non-divaricate leaves in particular. The hybrid leaves showed higher E than divaricate leaves, non-divaricate leaves had the lowest E. In contrast to divaricate and non-divaricate leaves, hybrid leaves showed the highest E when grown under water stress. Habit and water availability* habit had a significant effect on stomatal conductance ($g_s$). Plants under good water availability had higher values of $g_s$ than plants under water stress, hybrid plants had the highest and non-divaricate plants the lowest values. Overall, hybrid plants grown under water stress showed the highest values of $g_s$, the lowest values were found in non-divaricate plants grown under water stress.

Summary

Divaricate shrubs have interesting features in their architecture as well as in their physiological response to different light and water availabilities. As discussed above, the divaricate growth form maintains less negative water potentials under water stressed conditions than hybrid or non-divaricate leaves predawn and in the evening, but can express very low water potentials at noon. This greater diurnal change of water potentials in divaricate leaves than in non-divarciate or hybrid leaves could indicate a higher drought tolerance than shown in non-divaricate or hybrid leaves. The water potential values of hybrid and non-divaricate leaves were often closer to each other than divaricate to hybrid leaves. The fluorescence parameter $F_v/F_m$ of divaricate, hybrid and non-divaricate plants was more influenced by light and water treatments than by the leaf trait. Only for the evening measurements, were significant effects of habit on $F_v/F_m$ found.

The concentrations of photoprotective pigments were hypothesised to be lowest in divaricate leaves, caused by their self-shading growth form, which also should have been water conserving. In contrast to these predictions, the highest pigment and ?-

tocopherol concentrations were found in divaricate leaves, with only minor differences between hybrid and non-divaricate leaves. Pigment concentrations were not affected by water availability. Determinations of light level effects were only possible for ?-tocopherol, and here the lowest concentrations of ?-tocopherol were found in shaded divaricate leaves as it was hypothesised. Also, water stressed conditions tended to reduce the concentrations of ?-tocopherol in divaricate leaves in particular. The findings for ?-tocopherol supported the hypothesis that the divaricate growth form reduces the amount of photoprotection via self-shading and water conservation.

High Rd's were found in divaricate leaves and in hybrid leaves. All leaf traits showed the lowest Rd under water stressed conditions. Low Rd and $A_{max}$ in shade and water stressed conditions were hypothesised for divaricate leaves and the results supported this hypothesis. The divaricate 'self-shading' growth form, combined with particularly small leaves, seems to be advantageous in these conditions. In contrast to my predictions, the hybrid leaves did not mimic the divaricate behaviour for all gas exchange parameters. Rd and QE values in certain treatments were similar, but $A_{max}$ of hybrid leaves were closer to non-divaricate than divaricate leaves.

Although the use of hybrid plants as a model for divaricates was shown to be possible for some parameters, the two were not equivalent for all parameters. The young age of plants and problems with insect infestation could have had a negative influence on this result. Measurements on older, more mature plants would possibly provide a better comparison, particularly on plants in the field where both growth forms occur naturally.

## A2 Anova Tables of Field Data 2001-2003 and Glasshouse Data 2002

Table A2.1 Analysis of variance table for $F_v/F_m$ at the Cass field site, (a) predawn, (b) noon and (c) evening measurements taken during November 2001 and January 2002. Month, light level (natural daylight, shaded and exposed plants), water availability (streambed and N-facing slope) and genus (*Corokia cotoneaster* and *Coprosma propinqua*) as treatments [significant results in bold].

| a | Df | Sum of Sq | Mean Sq | F Value | Pr(F) |
|---|---|---|---|---|---|
| Month | 1 | 0.0116163 | 0.01161633 | 3.63 | 0.061 |
| Light level | 2 | 0.0185221 | 0.00926104 | 2.89 | 0.062 |
| **Water availability** | 1 | 0.0230288 | 0.02302879 | 7.20 | **0.009** |
| **Genus** | 1 | 0.0274033 | 0.02740326 | 8.56 | **0.005** |
| Month*Light level | 2 | 0.0088606 | 0.00443030 | 1.38 | 0.257 |
| Month*Water availability | 1 | 0.0105005 | 0.01050051 | 3.28 | 0.074 |
| Light level*Water availability | 2 | 0.0009790 | 0.00048952 | 0.15 | 0.858 |
| Month*Genus | 1 | 0.0040000 | 0.00400003 | 1.25 | 0.267 |
| Light level*Genus | 2 | 0.0063282 | 0.00316411 | 0.99 | 0.377 |
| Water availability*Genus | 1 | 0.0021733 | 0.00217331 | 0.68 | 0.413 |
| Month*Light level*Water availability | 2 | 0.0174429 | 0.00872147 | 2.73 | 0.072 |
| Month*Light level*Genus | 2 | 0.0019205 | 0.00096025 | 0.30 | 0.742 |
| **Month*Water availability*Genus** | 1 | 0.0182285 | 0.01822851 | 5.70 | **0.020** |
| Light level*Water availability*Genus | 2 | 0.0089595 | 0.00447975 | 1.40 | 0.253 |
| Month*Light level*Water availability*Genus | 2 | 0.0153964 | 0.00769821 | 2.41 | 0.098 |
| Residuals | 71 | 0.2271754 | 0.00319965 | | |

| b | Df | Sum of Sq | Mean Sq | F Value | Pr(F) |
|---|---|---|---|---|---|
| **Month** | 1 | 0.02080612 | 0.02080612 | 24.38 | **0.000** |
| **Light level** | 2 | 0.01692470 | 0.00846235 | 9.92 | **0.000** |
| Water availability | 1 | 0.00024265 | 0.00024265 | 0.28 | 0.596 |
| **Genus** | 1 | 0.03077842 | 0.03077842 | 36.07 | **0.000** |
| Month*Light level | 2 | 0.00250358 | 0.00125179 | 1.47 | 0.238 |
| Month*Water availability | 1 | 0.00000273 | 0.00000273 | 0.00 | 0.955 |
| Light level*Water availability | 2 | 0.00066675 | 0.00033338 | 0.39 | 0.678 |
| Month*Genus | 1 | 0.00158039 | 0.00158039 | 1.85 | 0.178 |
| Light level*Genus | 2 | 0.00299398 | 0.00149699 | 1.75 | 0.181 |
| Water availability*Genus | 1 | 0.00003198 | 0.00003198 | 0.04 | 0.847 |
| Month*Light level*Water availability | 2 | 0.00078066 | 0.00039033 | 0.46 | 0.635 |
| Month*Light level*Genus | 2 | 0.00206764 | 0.00103382 | 1.21 | 0.304 |
| Month*Water availability*Genus | 1 | 0.00214849 | 0.00214849 | 2.52 | 0.117 |
| Light level*Water availability*Genus | 2 | 0.00129367 | 0.00064683 | 0.76 | 0.472 |
| Month*Light level*Water availability*Genus | 2 | 0.00135986 | 0.00067993 | 0.80 | 0.455 |
| Residuals | 71 | 0.06059167 | 0.00085340 | | |

| c | Df | Sum of Sq | Mean Sq | F Value | Pr(F) |
|---|---|---|---|---|---|
| **Month** | 1 | 0.01078776 | 0.01078776 | 10.37 | **0.002** |
| **Light level** | 2 | 0.02390181 | 0.01195090 | 11.48 | **0.000** |
| Water availability | 1 | 0.00338558 | 0.00338558 | 3.25 | 0.076 |
| **Genus** | 1 | 0.03273933 | 0.03273933 | 31.5 | **0.000** |
| Month*Light level | 2 | 0.00317074 | 0.00158537 | 1.52 | 0.225 |
| Month*Water availability | 1 | 0.00005008 | 0.00005008 | 0.05 | 0.827 |
| Light level*Water availability | 2 | 0.00002357 | 0.00001178 | 0.01 | 0.989 |
| Month*Genus | 1 | 0.00026292 | 0.00026292 | 0.25 | 0.617 |
| Light level*Genus | 2 | 0.00374913 | 0.00187456 | 1.80 | 0.173 |
| Water availability*Genus | 1 | 0.00151890 | 0.00151890 | 1.46 | 0.231 |
| Month*Light level*Water availability | 2 | 0.00089195 | 0.00044597 | 0.43 | 0.653 |
| Month*Light level*Genus | 2 | 0.00275427 | 0.00137714 | 1.32 | 0.273 |
| **Month*Water availability*Genus** | 1 | 0.00434323 | 0.00434323 | 4.17 | **0.045** |
| Light level*Water availability*Genus | 2 | 0.00229631 | 0.00114815 | 1.10 | 0.338 |
| Month*Light level*Water availability*Genus | 2 | 0.00350234 | 0.00175117 | 1.68 | 0.193 |
| Residuals | 70 | 0.07284933 | 0.00104070 | | |

Table A2.2 Analysis of variance table for $F_v/F_m$ at the Cass field site, (a) predawn, (b) noon and (c) evening measurements taken during January and March 2003. Month, light level (natural daylight, shaded and exposed plants), water availability (streambed and N-facing slope) and genus (*Corokia cotoneaster* and *Coprosma propinqua*) as treatments [significant results in bold].

| a | Df | Sum of Sq | Mean Sq | F Value | Pr(F) |
|---|---|---|---|---|---|
| Month | 1 | 0.00158640 | 0.001586400 | 1.83 | 0.180 |
| Light level | 2 | 0.00022085 | 0.000110427 | 0.13 | 0.881 |
| Water availability | 1 | 0.00048568 | 0.000485682 | 0.56 | 0.457 |
| **Genus** | 1 | 0.00644023 | 0.006440230 | 7.44 | **0.008** |
| Month*Light level | 2 | 0.00074323 | 0.000371613 | 0.43 | 0.653 |
| Month*Water availability | 1 | 0.00279008 | 0.002790081 | 3.22 | 0.077 |
| Light level*Water availability | 2 | 0.00118653 | 0.000593263 | 0.69 | 0.508 |
| Month*Genus | 1 | 0.00013080 | 0.000130801 | 0.15 | 0.699 |
| Light level*Genus | 2 | 0.00024609 | 0.000123043 | 0.14 | 0.868 |
| Water availability*Genus | 1 | 0.00011009 | 0.000110090 | 0.13 | 0.722 |
| Month*Light level*Water availability | 2 | 0.00075953 | 0.000379764 | 0.44 | 0.647 |
| Month*Light level*Genus | 2 | 0.00071793 | 0.000358967 | 0.41 | 0.662 |
| Month*Water availability*Genus | 1 | 0.00111000 | 0.001110000 | 1.28 | 0.262 |
| Light level*Water availability*Genus | 2 | 0.00038577 | 0.000192884 | 0.22 | 0.801 |
| Month*Light level*Water availability*Genus | 2 | 0.00036601 | 0.000183004 | 0.21 | 0.810 |
| Residuals | 69 | 0.05975519 | 0.000866017 | | |

| b | Df | Sum of Sq | Mean Sq | F Value | Pr(F) |
|---|---|---|---|---|---|
| **Month** | 1 | 0.01410261 | 0.01410261 | 15.39 | **0.001** |
| **Light level** | 2 | 0.01332947 | 0.00666474 | 7.28 | **0.001** |
| **Water availability** | 1 | 0.04510070 | 0.04510070 | 49.23 | **0.000** |
| **Genus** | 1 | 0.00493034 | 0.00493034 | 5.38 | **0.023** |
| Month*Light level | 2 | 0.00058489 | 0.00029245 | 0.32 | 0.728 |
| **Month*Water availability** | 1 | 0.01910637 | 0.01910637 | 20.86 | **0.000** |
| Light level*Water availability | 2 | 0.00456795 | 0.00228397 | 2.49 | 0.090 |
| Month*Genus | 1 | 0.00003826 | 0.00003826 | 0.04 | 0.839 |
| **Light level*Genus** | 2 | 0.00789023 | 0.00394512 | 4.31 | **0.017** |
| Water availability*Genus | 1 | 0.00035386 | 0.00035386 | 0.39 | 0.536 |
| Month*Light level*Water availability | 2 | 0.00080840 | 0.00040420 | 0.44 | 0.645 |
| Month*Light level*Genus | 2 | 0.00457979 | 0.00228989 | 2.50 | 0.089 |
| Month*Water availability*Genus | 1 | 0.00005314 | 0.00005314 | 0.06 | 0.810 |
| Light level*Water availability*Genus | 2 | 0.00517621 | 0.00258810 | 2.83 | 0.066 |
| Month*Light level*Water availability*Genus | 2 | 0.00173352 | 0.00086676 | 0.95 | 0.393 |
| Residuals | 71 | 0.06504225 | 0.00091609 | | |

| c | Df | Sum of Sq | Mean Sq | F Value | Pr(F) |
|---|---|---|---|---|---|
| Month | 1 | 0.000056691 | 0.000056691 | 0.46 | 0.502 |
| **Light level** | 2 | 0.002127137 | 0.001063569 | 8.54 | **0.001** |
| **Water availability** | 1 | 0.000575590 | 0.000575590 | 4.62 | **0.035** |
| **Genus** | 1 | 0.007500350 | 0.007500350 | 60.22 | **0.000** |
| Month*Light level | 2 | 0.000185071 | 0.000092535 | 0.74 | 0.480 |
| **Month*Water availability** | 1 | 0.004313337 | 0.004313337 | 34.63 | **0.000** |
| **Light level*Water availability** | 2 | 0.001168416 | 0.000584208 | 4.69 | **0.012** |
| Month*Genus | 1 | 0.000317784 | 0.000317784 | 2.55 | 0.115 |
| Light level*Genus | 2 | 0.000728015 | 0.000364007 | 2.92 | 0.060 |
| Water availability*Genus | 1 | 0.000000491 | 0.000000491 | 0.00 | 0.950 |
| Month*Light level*Water availability | 2 | 0.000089368 | 0.000044684 | 0.36 | 0.700 |
| Month*Light level*Genus | 2 | 0.000390410 | 0.000195205 | 1.57 | 0.216 |
| Month*Water availability*Genus | 1 | 0.000249758 | 0.000249758 | 2.01 | 0.161 |
| Light level*Water availability*Genus | 2 | 0.000095807 | 0.000047904 | 0.38 | 0.682 |
| Month*Light level*Water availability*Genus | 2 | 0.000033774 | 0.000016887 | 0.14 | 0.873 |
| Residuals | 70 | 0.008718479 | 0.000124550 | | |

Table A2.3 Analysis of variance table for $F_v/F_m$ in the glasshouse, (a) predawn, (b) noon and (c) evening measurements during March and June 2002. Month, light level (100% vs 75% of natural daylight), water availability (200 mls every 2 days and 50mls every 3 days) and genus (*Corokia cotoneaster, Coprosma propinqua, Corokia buddleioide* and *Coprosma robusta*) as treatments. Measurements recorded from shoots of *Corokia cotoneaster* and *Coprosma propinqua*; leaves of *Corokia buddleioides* and *Coprosma robusta*. [significant results in bold].

| a | Df | Sum of Sq | Mean Sq | F Value | Pr(F) |
|---|---|---|---|---|---|
| **Month** | 1 | 0.002714364 | 0.002714364 | 42.66 | **0.000** |
| Light level | 1 | 0.000023211 | 0.000023211 | 0.36 | 0.547 |
| **Water availability** | 1 | 0.000417522 | 0.000417522 | 6.56 | **0.012** |
| **Genus** | 1 | 0.000667455 | 0.000667455 | 10.49 | **0.002** |
| Habit | 1 | 0.000089309 | 0.000089309 | 1.40 | 0.239 |
| **Month*Light level** | 1 | 0.001210837 | 0.001210837 | 19.03 | **0.000** |
| Month*Water availability | 1 | 0.000057571 | 0.000057571 | 0.91 | 0.344 |
| **Light level*Water availability** | 1 | 0.000398556 | 0.000398556 | 6.26 | **0.014** |
| Month*Genus | 1 | 0.000059881 | 0.000059881 | 0.94 | 0.335 |
| Light level*Genus | 1 | 0.000006085 | 0.000006085 | 0.10 | 0.758 |
| **Water availability* Genus** | 1 | 0.001046789 | 0.001046789 | 16.45 | **0.000** |
| Month*Habit | 1 | 0.000245867 | 0.000245867 | 3.86 | 0.052 |
| Light level*Habit | 1 | 0.000212879 | 0.000212879 | 3.35 | 0.071 |
| Water availability*Habit | 1 | 0.000008204 | 0.000008204 | 0.13 | 0.720 |
| Genus*Habit | 1 | 0.000169991 | 0.000169991 | 2.67 | 0.106 |
| Month*Light level*Water availability | 1 | 0.000007907 | 0.000007907 | 0.12 | 0.7250 |
| Month*Light level*Genus | 1 | 0.000058163 | 0.000058163 | 0.91 | 0.342 |
| Month*Water availability*Genus | 1 | 0.000094882 | 0.000094882 | 1.49 | 0.225 |
| Light level*Water availability*Genus | 1 | 0.000009421 | 0.000009421 | 0.15 | 0.701 |
| Month*Light level*Habit | 1 | 0.000022995 | 0.000022995 | 0.36 | 0.549 |
| Month*Water availability*Habit | 1 | 0.000000622 | 0.000000622 | 0.01 | 0.921 |
| Light level*Water availability*Habit | 1 | 0.000036369 | 0.000036369 | 0.57 | 0.452 |
| Month*Genus*Habit | 1 | 0.000004966 | 0.000004966 | 0.08 | 0.781 |
| Light level*Genus*Habit | 1 | 0.000001475 | 0.000001475 | 0.02 | 0.879 |
| **Water availability* Genus* Habit** | 1 | 0.000971656 | 0.000971656 | 15.27 | **0.000** |
| Month*Light level*Water availability*Genus | 1 | 0.000016095 | 0.000016095 | 0.25 | 0.616 |
| Month*Light level*Water availability*Habit | 1 | 0.000009088 | 0.000009088 | 0.14 | 0.706 |
| **Month*Light level*Genus*Habit** | 1 | 0.000249053 | 0.000249053 | 3.92 | **0.051** |
| Month*Water availability*Genus*Habit | 1 | 0.000031319 | 0.000031319 | 0.49 | 0.485 |
| Light level*Water availability*Genus*Habit | 1 | 0.000096996 | 0.000096996 | 1.53 | 0.220 |
| Month*Light level*Water availability*Genus*Habit | 1 | 0.000000021 | 0.000000021 | 0.00 | 0.986 |
| Residuals | 92 | 0.005853250 | 0.000063622 | | |

| b | Df | Sum of Sq | Mean Sq | F Value | Pr(F) |
|---|---|---|---|---|---|
| **Month** | 1 | 0.00189760 | 0.001897596 | 16.26 | **0.000** |
| Light level | 1 | 0.00002984 | 0.000029844 | 0.26 | 0.614 |
| Water availability | 1 | 0.00001412 | 0.000014124 | 0.12 | 0.729 |
| **Genus** | 1 | 0.00233665 | 0.002336653 | 20.03 | **0.000** |
| Habit | 1 | 0.00039320 | 0.000393198 | 3.37 | 0.070 |
| Month*Light level | 1 | 0.00003563 | 0.000035630 | 0.31 | 0.582 |
| Month*Water availability | 1 | 0.00005648 | 0.000056482 | 0.48 | 0.488 |
| Light level*Water availability | 1 | 0.00002852 | 0.000028515 | 0.24 | 0.622 |
| **Month*Genus** | 1 | 0.00065157 | 0.000651565 | 5.58 | **0.020** |
| Light level*Genus | 1 | 0.00001372 | 0.000013724 | 0.12 | 0.732 |
| Water availability*Genus | 1 | 0.00002177 | 0.000021769 | 0.19 | 0.667 |
| Month*Habit | 1 | 0.00023838 | 0.000238378 | 2.04 | 0.156 |
| Light level*Habit | 1 | 0.00003896 | 0.000038964 | 0.33 | 0.565 |
| Water availability*Habit | 1 | 0.00000020 | 0.000000203 | 0.00 | 0.967 |
| Genus*Habit | 1 | 0.00007666 | 0.000076660 | 0.66 | 0.420 |
| Month*Light level*Water availability | 1 | 0.00010470 | 0.000104705 | 0.90 | 0.346 |
| Month*Light level*Genus | 1 | 0.00000003 | 0.000000031 | 0.00 | 0.987 |
| Month*Water availability*Genus | 1 | 0.00015670 | 0.000156696 | 1.34 | 0.250 |
| Light level*Water availability*Genus | 1 | 0.00040459 | 0.000404592 | 3.47 | 0.066 |
| Month*Light level*Habit | 1 | 0.00021801 | 0.000218015 | 1.87 | 0.175 |
| Month*Water availability*Habit | 1 | 0.00000002 | 0.000000017 | 0.00 | 0.990 |
| Light level*Water availability*Habit | 1 | 0.00004291 | 0.000042909 | 0.37 | 0.546 |
| Month*Genus*Habit | 1 | 0.00002901 | 0.000029007 | 0.25 | 0.619 |
| Light level*Genus*Habit | 1 | 0.00027612 | 0.000276116 | 2.37 | 0.127 |
| **Water availability* Genus*Habit** | 1 | 0.00128919 | 0.001289186 | 11.05 | **0.001** |
| Month*Light level*Water availability*Genus | 1 | 0.00002354 | 0.000023542 | 0.20 | 0.654 |
| Month*Light level*Water availability*Habit | 1 | 0.00003639 | 0.000036387 | 0.31 | 0.578 |
| Month*Light level*Genus*Habit | 1 | 0.00024721 | 0.000247210 | 2.12 | 0.149 |
| Month*Water availability*Genus*Habit | 1 | 0.00001441 | 0.000014408 | 0.12 | 0.726 |
| Light level*Water availability*Genus*Habit | 1 | 0.00004145 | 0.000041450 | 0.36 | 0.553 |
| Month*Light level*Water availability*Genus*Habit | 1 | 0.00013400 | 0.000134001 | 1.15 | 0.287 |
| Residuals | 92 | 0.01073542 | 0.000116689 | | |

| c | Df | Sum of Sq | Mean Sq | F Value | Pr(F) |
|---|---|---|---|---|---|
| **Month** | 1 | 0.000500262 | 0.000500262 | 4.85 | **0.030** |
| Light level | 1 | 0.000020351 | 0.000020351 | 0.20 | 0.658 |
| Water availability | 1 | 0.000248753 | 0.000248753 | 2.41 | 0.124 |
| **Genus** | 1 | 0.003030518 | 0.003030518 | 29.37 | **0.000** |
| **Habit** | 1 | 0.001265018 | 0.001265018 | 12.26 | **0.001** |
| **Month*Light level** | 1 | 0.001390049 | 0.001390049 | 13.47 | **0.000** |
| **Month*Water availability** | 1 | 0.001194682 | 0.001194682 | 11.58 | **0.001** |
| Light level*Water availability | 1 | 0.000113278 | 0.000113278 | 1.10 | 0.298 |
| Month*Genus | 1 | 0.000018569 | 0.000018569 | 0.18 | 0.672 |
| Light level*Genus | 1 | 0.000312996 | 0.000312996 | 3.03 | 0.085 |
| Water availability*Genus | 1 | 0.000102293 | 0.000102293 | 0.99 | 0.322 |
| Month*Habit | 1 | 0.000000143 | 0.000000143 | 0.00 | 0.970 |
| Light level*Habit | 1 | 0.000007944 | 0.000007944 | 0.08 | 0.782 |
| Water availability*Habit | 1 | 0.000013918 | 0.000013918 | 0.14 | 0.714 |
| **Genus*Habit** | 1 | 0.001669726 | 0.001669726 | 16.18 | **0.000** |
| Month*Light level*Water availability | 1 | 0.000067820 | 0.000067820 | 0.66 | 0.420 |
| Month*Light level*Genus | 1 | 0.000031185 | 0.000031185 | 0.30 | 0.584 |
| Month*Water availability*Genus | 1 | 0.000048455 | 0.000048455 | 0.47 | 0.495 |
| Light level*Water availability*Genus | 1 | 0.000342098 | 0.000342098 | 3.32 | 0.072 |
| **Month*Light level*Habit** | 1 | 0.000951797 | 0.000951797 | 9.22 | **0.003** |
| Month*Water availability*Habit | 1 | 0.000169746 | 0.000169746 | 1.65 | 0.203 |
| **Light level*Water availability*Habit** | 1 | 0.000665283 | 0.000665283 | 6.45 | **0.013** |
| Month*Genus*Habit | 1 | 0.000245688 | 0.000245688 | 2.38 | 0.126 |
| Light level*Genus*Habit | 1 | 0.000093023 | 0.000093023 | 0.90 | 0.345 |
| **Water availability* Genus*Habit** | 1 | 0.001037534 | 0.001037534 | 10.05 | **0.002** |
| Month*Light level*Water availability*Genus | 1 | 0.000001416 | 0.000001416 | 0.01 | 0.907 |
| Month*Light level*Water availability*Habit | 1 | 0.000106226 | 0.000106226 | 1.03 | 0.313 |
| Month*Light level*Genus*Habit | 1 | 0.000056089 | 0.000056089 | 0.54 | 0.463 |
| Month*Water availability*Genus*Habit | 1 | 0.000253835 | 0.000253835 | 2.46 | 0.120 |
| Light level*Water availability*Genus*Habit | 1 | 0.000013432 | 0.000013432 | 0.13 | 0.719 |
| Month*Light level*Water availability*Genus*Habit | 1 | 0.000006915 | 0.000006915 | 0.07 | 0.796 |
| Residuals | 91 | 0.009390667 | 0.000103194 | | |

Table A2.4 Analysis of variance table for the pigment ***Violaxanthin in mmol per mol total Chlorophyll***, samples taken at the Cass field site during late afternoon in March 2003. Light level (natural daylight, shaded and exposed plants), water availability (streambed and N-facing slope) and genus (*Corokia cotoneaster* and *Coprosma propinqua*) as treatments [significant results in bold].

| | Df | Sum of Sq | Mean Sq | F Value | Pr(F) |
|---|---|---|---|---|---|
| Light level | 2 | 167.7220 | 83.8610 | 1.87 | 0.186 |
| **Water availability** | 1 | 301.4358 | 301.4358 | 6.73 | **0.020** |
| **Genus** | 1 | 723.2145 | 723.2145 | 16.14 | **0.001** |
| **Light level*Water availability** | 2 | 544.7336 | 272.3668 | 6.08 | **0.011** |
| Light level*Genus | 2 | 220.6615 | 110.3308 | 2.46 | 0.117 |
| **Water availability*Genus** | 1 | 238.9681 | 238.9681 | 5.33 | **0.035** |
| Light level*Water availability*Genus | 2 | 309.4638 | 154.7319 | 3.45 | 0.057 |
| Residuals | 16 | 716.9332 | 44.8083 | | |

Table A2.5 Analysis of variance table for the pigment ***Antheraxanthin in mmol per mol total Chlorophyl*** , samples taken at the Cass field site during late afternoon in March 2003. Light level (natural daylight, shaded and exposed plants), water availability (streambed and N-facing slope) and genus (*Corokia cotoneaster* and *Coprosma propinqua*) as treatments [significant results in bold].

| | Df | Sum of Sq | Mean Sq | F Value | Pr(F) |
|---|---|---|---|---|---|
| **Light level** | 2 | 707.2548 | 353.6274 | 10.72 | **0.001** |
| Water availability | 1 | 50.0529 | 50.0529 | 1.52 | 0.236 |
| Genus | 1 | 119.2319 | 119.2319 | 3.61 | 0.075 |
| Light level*Water availability | 2 | 43.9469 | 21.9734 | 0.67 | 0.527 |
| Light level*Genus | 2 | 113.8501 | 56.9250 | 1.73 | 0.210 |
| Water availability*Genus | 1 | 96.0904 | 96.0904 | 2.91 | 0.107 |
| Light level*Water availability*Genus | 2 | 172.4628 | 86.2314 | 2.61 | 0.104 |
| Residuals | 16 | 527.7845 | 32.9865 | | |

Table A2.6 Analysis of variance table for the pigment ***Zeaxanthin in mmol per mol total Chlorophyll***, samples taken at the Cass field site during late afternoon in March 2003. Light level (natural daylight, shaded and exposed plants), water availability (streambed and N-facing slope) and genus (*Corokia cotoneaster* and *Coprosma propinqua*) as treatments [significant results in bold].

| | Df | Sum of Sq | Mean Sq | F Value | Pr(F) |
|---|---|---|---|---|---|
| **Light level** | 2 | 3002.937 | 1501.468 | 11.77 | **0.001** |
| Water availability | 1 | 449.378 | 449.378 | 3.52 | 0.079 |
| Genus | 1 | 270.444 | 270.444 | 2.12 | 0.165 |
| **Light level*Water availability** | 2 | 1088.467 | 544.233 | 4.27 | **0.033** |
| Light level*Genus | 2 | 22.376 | 11.188 | 0.09 | 0.917 |
| **Water availability*Genus** | 1 | 781.758 | 781.758 | 6.13 | **0.025** |
| Light level*Water availability*Genus | 2 | 815.905 | 407.952 | 3.20 | 0.068 |
| Residuals | 16 | 2041.869 | 127.617 | | |

Table A2.7 Analysis of variance table for the pigments in the ***Deepoxidation state in mmol per mol sum Xanthophyll cycle pigments***, samples taken at the Cass field site during late afternoon in March 2003. Light level (natural daylight, shaded and exposed plants), water availability (streambed and N-facing slope) and genus (*Corokia cotoneaster* and *Coprosma propinqua*) as treatments [significant results in bold].

| | Df | Sum of Sq | Mean Sq | F Value | Pr(F) |
|---|---|---|---|---|---|
| **Light level** | 2 | 4162.879 | 2081.440 | 10.91 | **0.001** |
| Water availability | 1 | 7.768 | 7.768 | 0.04 | 0.843 |
| **Genus** | 1 | 1171.298 | 1171.298 | 6.13 | **0.025** |
| **Light level*Water availability** | 2 | 1464.718 | 732.359 | 3.84 | **0.043** |
| Light level*Genus | 2 | 80.582 | 40.291 | 0.21 | 0.812 |
| Water availability*Genus | 1 | 535.320 | 535.320 | 2.81 | 0.1132 |
| Light level*Water availability*Genus | 2 | 583.193 | 291.596 | 1.53 | 0.247 |
| Residuals | 16 | 3052.584 | 190.787 | | |

Table A2.8 Analysis of variance table for the pigment ***Neoxanthin in mmol per mol total Chlorophyll***, samples taken at the Cass field site during late afternoon in March 2003. Light level (natural daylight, shaded and exposed plants), water availability (streambed and N-facing slope) and genus (*Corokia cotoneaster* and *Coprosma propinqua*) as treatments [significant results in bold].

| | Df | Sum of Sq | Mean Sq | F Value | Pr(F) |
|---|---|---|---|---|---|
| Light level | 2 | 59.6820 | 29.84100 | 0.76 | 0.484 |
| Water availability | 1 | 0.4981 | 0.49813 | 0.01 | 0.912 |
| Genus | 1 | 32.5673 | 32.56732 | 0.83 | 0.376 |
| Light level*Water availability | 2 | 45.6108 | 22.80540 | 0.58 | 0.572 |
| Light level*Genus | 2 | 52.4498 | 26.22489 | 0.67 | 0.527 |
| Water availability*Genus | 1 | 12.8043 | 12.80431 | 0.33 | 0.576 |
| Light level*Water availability*Genus | 2 | 53.2687 | 26.63433 | 0.68 | 0.522 |
| Residuals | 17 | 670.5317 | 39.44304 | | |

Table A2.9 Analysis of variance table for the pigment ***Lutein in mmol per mol total Chlorophyll***, samples taken at the Cass field site during late afternoon in March 2003. Light level (natural daylight, shaded and exposed plants), water availability (streambed and N-facing slope) and genus (*Corokia cotoneaster* and *Coprosma propinqua*) as treatments [significant results in bold].

| | Df | Sum of Sq | Mean Sq | F Value | Pr(F) |
|---|---|---|---|---|---|
| **Light level** | 2 | 1704.338 | 852.1689 | 4.99 | **0.021** |
| Water availability | 1 | 6.206 | 6.2064 | 0.04 | 0.851 |
| Genus | 1 | 26.458 | 26.4579 | 0.16 | 0.699 |
| Light level*Water availability | 2 | 219.716 | 109.8581 | 0.64 | 0.539 |
| Light level*Genus | 2 | 62.650 | 31.3251 | 0.18 | 0.834 |
| Water availability*Genus | 1 | 21.614 | 21.6140 | 0.13 | 0.727 |
| Light level*Water availability*Genus | 2 | 364.947 | 182.4735 | 1.07 | 0.367 |
| Residuals | 16 | 2733.597 | 170.8498 | | |

Table A2.10 Analysis of variance table for the pigment ***ß-carotene in mmol per mol total Chlorophyll***, samples taken at the Cass field site during late afternoon in March 2003. Light level (natural daylight, shaded and exposed plants), water availability (streambed and N-facing slope) and genus (*Corokia cotoneaster* and *Coprosma propinqua*) as treatments [significant results in bold].

| | Df | Sum of Sq | Mean Sq | F Value | Pr(F) |
|---|---|---|---|---|---|
| Light level | 2 | 510.87 | 255.435 | 0.539330 | 0.5877768 |
| Water availability | 1 | 4.63 | 4.625 | 0.009766 | 0.9218257 |
| **Genus** | 1 | 2804.06 | 2804.061 | 5.920534 | **0.0200605** |
| Light level*Water availability | 2 | 810.18 | 405.091 | 0.855316 | 0.4336072 |
| **Light level*Genus** | 2 | 3915.20 | 1957.601 | 4.133307 | **0.0242143** |
| Water availability*Genus | 1 | 468.71 | 468.708 | 0.989636 | 0.3264713 |
| Light level*Water availability*Genus | 2 | 1253.56 | 626.778 | 1.323387 | 0.2788731 |
| Residuals | 36 | 17050.18 | 473.616 | | |

Table A2.11 Analysis of variance table for ***vitamin E in mol per mol total Chlorophyll***, samples taken at the Cass field site during late afternoon in February 2002. Light level (natural daylight, shaded and exposed plants), water availability (streambed and N-facing slope) and genus (*Corokia cotoneaster* and *Coprosma propinqua*) as treatments [significant results in bold].

| | Df | Sum of Sq | Mean Sq | F Value | Pr(F) |
|---|---|---|---|---|---|
| Light level | 2 | 0.011 | 0.0056 | 0.15 | 0.862 |
| Water availability | 1 | 0.048 | 0.0477 | 1.27 | 0.267 |
| **Genus** | 1 | 0.229 | 0.2289 | 6.10 | **0.018** |
| Light level*Water availability | 2 | 0.131 | 0.0654 | 1.74 | 0.189 |
| Light level*Genus | 2 | 0.031 | 0.0153 | 0.41 | 0.668 |
| Water availability*Genus | 1 | 0.023 | 0.0229 | 0.61 | 0.439 |
| Light level*Water availability*Genus | 2 | 0.106 | 0.0530 | 1.41 | 0.257 |
| Residuals | 38 | 1.426 | 0.0375 | | |

Table A2.12 Analysis of variance table for ***vitamin E in mol per mol total Chlorophyll***, samples taken at the Cass field site during late afternoon in March 2003. Light level (natural daylight, shaded and exposed plants), water availability (streambed and N-facing slope) and genus (*Corokia cotoneaster* and *Coprosma propinqua*) as treatments [significant results in bold].

| | Df | Sum of Sq | Mean Sq | F Value | Pr(F) |
|---|---|---|---|---|---|
| Light level | 2 | 0.0225 | 0.01126 | 0.73 | 0.488 |
| Water availability | 1 | 0.0242 | 0.02422 | 1.58 | 0.218 |
| Genus | 1 | 0.0260 | 0.02600 | 1.69 | 0.202 |
| Light level*Water availability | 2 | 0.0016 | 0.00082 | 0.05 | 0.948 |
| Light level*Genus | 2 | 0.0030 | 0.00150 | 0.10 | 0.907 |
| Water availability*Genus | 1 | 0.0396 | 0.03960 | 2.58 | 0.118 |
| Light level*Water availability*Genus | 2 | 0.0091 | 0.00454 | 0.30 | 0.746 |
| Residuals | 34 | 0.5229 | 0.01538 | | |

Table A2.13 Analysis of variance table for the pigment ***Violaxanthin in µmol per m$^{-2}$ leaf area***, samples taken at the Cass field site during late afternoon in March 2003. Light level (natural daylight, shaded and exposed plants), water availability (streambed and N-facing slope) and genus (*Corokia cotoneaster* and *Coprosma propinqua*) as treatments [significant results in bold].

| | Df | Sum of Sq | Mean Sq | F Value | Pr(F) |
|---|---|---|---|---|---|
| Light level | 2 | 4225.46 | 2112.73 | 2.63 | 0.103 |
| **Water availability** | 1 | 3774.23 | 3774.23 | 4.69 | **0.046** |
| **Genus** | 1 | 17647.13 | 17647.13 | 21.94 | **0.000** |
| **Light level*Water availability** | 2 | 7552.61 | 3776.31 | 4.69 | **0.025** |
| Light level*Genus | 2 | 3920.30 | 1960.15 | 2.44 | 0.119 |
| **Water availability*Genus** | 1 | 4425.49 | 4425.49 | 5.50 | **0.032** |
| **Light level*Water availability*Genus** | 2 | 9327.77 | 4663.88 | 5.80 | **0.013** |
| Residuals | 16 | 12871.34 | 804.46 | | |

Table A2.14 Analysis of variance table for the pigment ***Antheraxanthin in µmol per m$^{-2}$ leaf area***, samples taken at the Cass field site during late afternoon in March 2003. Light level (natural daylight, shaded and exposed plants), water availability (streambed and N-facing slope) and genus (*Corokia cotoneaster* and *Coprosma propinqua*) as treatments [significant results in bold].

| | Df | Sum of Sq | Mean Sq | F Value | Pr(F) |
|---|---|---|---|---|---|
| **Light level** | 2 | 3406.699 | 1703.349 | 5.05 | **0.020** |
| Water availability | 1 | 297.838 | 297.838 | 0.88 | 0.362 |
| Genus | 1 | 96.585 | 96.585 | 0.29 | 0.600 |
| Light level*Water availability | 2 | 290.305 | 145.152 | 0.43 | 0.658 |
| Light level*Genus | 2 | 498.516 | 249.258 | 0.74 | 0.494 |
| Water availability*Genus | 1 | 313.538 | 313.538 | 0.93 | 0.350 |
| Light level*Water availability*Genus | 2 | 804.681 | 402.340 | 1.19 | 0.329 |
| Residuals | 16 | 5402.048 | 337.628 | | |

Table A2.15 Analysis of variance table for the pigment ***Zeaxanthin in µmol per m$^{-2}$ leaf area***, samples taken at the Cass field site during late afternoon in March 2003. Light level (natural daylight, shaded and exposed plants), water availability (streambed and N-facing slope) and genus (*Corokia cotoneaster* and *Coprosma propinqua*) as treatments [significant results in bold].

| | Df | Sum of Sq | Mean Sq | F Value | Pr(F) |
|---|---|---|---|---|---|
| **Light level** | 2 | 19863.10 | 9931.552 | 9.53 | **0.002** |
| Water availability | 1 | 3422.03 | 422.030 | 3.28 | 0.089 |
| Genus | 1 | 969.09 | 969.094 | 0.93 | 0.349 |
| Light level*Water availability | 2 | 7450.34 | 3725.171 | 3.58 | 0.052 |
| Light level*Genus | 2 | 444.83 | 222.413 | 0.21 | 0.810 |
| Water availability*Genus | 1 | 3002.39 | 3002.393 | 2.88 | 0.109 |
| Light level*Water availability*Genus | 2 | 4516.65 | 2258.327 | 2.17 | 0.147 |
| Residuals | 16 | 16673.79 | 1042.112 | | |

Table A2.16 Analysis of variance table for the pigment ***Neoxanthin in µmol per m$^{-2}$ leaf area***, samples taken at the Cass field site during late afternoon in March 2003. Light level (natural daylight, shaded and exposed plants), water availability (streambed and N-facing slope) and genus (*Corokia cotoneaster* and *Coprosma propinqua*) as treatments [significant results in bold].

| | Df | Sum of Sq | Mean Sq | F Value | Pr(F) |
|---|---|---|---|---|---|
| Light level | 2 | 13566.8 | 6783.41 | 0.88 | 0.435 |
| Water availability | 1 | 72.6 | 72.57 | 0.01 | 0.924 |
| **Genus** | 1 | 43186.7 | 43186.67 | 5.58 | **0.031** |
| Light level*Water availability | 2 | 6878.5 | 3439.27 | 0.44 | 0.649 |
| Light level*Genus | 2 | 28045.3 | 14022.67 | 1.81 | 0.195 |
| Water availability*Genus | 1 | 11364.3 | 11364.26 | 1.47 | 0.243 |
| Light level*Water availability*Genus | 2 | 10107.4 | 5053.69 | 0.65 | 0.534 |
| Residuals | 16 | 123858.9 | 7741.18 | | |

Table A2.17 Analysis of variance table for the pigment ***Lutein in µmol per m$^{-2}$ leaf area***, samples taken at the Cass field site during late afternoon in March 2003. Light level (natural daylight, shaded and exposed plants), water availability (streambed and N-facing slope) and genus (*Corokia cotoneaster* and *Coprosma propinqua*) as treatments [significant results in bold].

| | Df | Sum of Sq | Mean Sq | F Value | Pr(F) |
|---|---|---|---|---|---|
| Light level | 2 | 13566.8 | 6783.41 | 0.88 | 0.435 |
| Water availability | 1 | 72.6 | 72.57 | 0.01 | 0.924 |
| **Genus** | 1 | 43186.7 | 43186.67 | 5.58 | **0.031** |
| Light level*Water availability | 2 | 6878.5 | 3439.27 | 0.44 | 0.649 |
| Light level*Genus | 2 | 28045.3 | 14022.67 | 1.81 | 0.195 |
| Water availability*Genus | 1 | 11364.3 | 11364.26 | 1.47 | 0.243 |
| Light level*Water availability*Genus | 2 | 10107.4 | 5053.69 | 0.65 | 0.534 |
| Residuals | 16 | 123858.9 | 7741.18 | | |

Table A2.18 Analysis of variance table for the pigment ***Chlorophyll a in µmol per m$^{-2}$ leaf area***, samples taken at the Cass field site during late afternoon in March 2003. Light level (natural daylight, shaded and exposed plants), water availability (streambed and N-facing slope) and genus (*Corokia cotoneaster* and *Coprosma propinqua*) as treatments [significant results in bold].

| | Df | Sum of Sq | Mean Sq | F Value | Pr(F) |
|---|---|---|---|---|---|
| **Light level** | 2 | 983859 | 491929.4 | 3.83 | **0.044** |
| Water availability | 1 | 143 | 143.0 | 0.00 | 0.974 |
| **Genus** | 1 | 891683 | 891683.0 | 6.93 | **0.018** |
| Light level*Water availability | 2 | 63223 | 31611.4 | 0.25 | 0.785 |
| Light level*Genus | 2 | 384412 | 192206.2 | 1.50 | 0.254 |
| Water availability*Genus | 1 | 117641 | 117640.8 | 0.92 | 0.353 |
| Light level*Water availability*Genus | 2 | 499536 | 249768.0 | 1.94 | 0.176 |
| Residuals | 16 | 2057549 | 128596.8 | | |

Table A2.19 Analysis of variance table for the pigment ***Chlorophyll b in µmol per m$^{-2}$ leaf area***, samples taken at the Cass field site during late afternoon in March 2003. Light level (natural daylight, shaded and exposed plants), water availability (streambed and N-facing slope) and genus (*Corokia cotoneaster* and *Coprosma propinqua*) as treatments [significant results in bold].

| | Df | Sum of Sq | Mean Sq | F Value | Pr(F) |
|---|---|---|---|---|---|
| Light level | 2 | 109701.7 | 54850.86 | 3.13 | 0.071 |
| Water availability | 1 | 519.0 | 519.01 | 0.03 | 0.866 |
| Genus | 1 | 50515.7 | 50515.66 | 2.883 | 0.109 |
| Light level*Water availability | 2 | 6888.2 | 3444.09 | 0.20 | 0.824 |
| Light level*Genus | 2 | 65087.8 | 32543.91 | 1.86 | 0.188 |
| Water availability*Genus | 1 | 15756.7 | 15756.68 | 0.90 | 0.357 |
| Light level*Water availability*Genus | 2 | 56899.0 | 28449.48 | 1.62 | 0.228 |
| Residuals | 16 | 280321.9 | 17520.12 | | |

Table A2.20 Analysis of variance table for the pigment ***Chlorophyll a+b in µmol per m$^{-2}$ leaf area***, samples taken at the Cass field site during late afternoon in March 2003. Light level (natural daylight, shaded and exposed plants), water availability (streambed and N-facing slope) and genus (*Corokia cotoneaster* and *Coprosma propinqua*) as treatments [significant results in bold].

| | Df | Sum of Sq | Mean Sq | F Value | Pr(F) |
|---|---|---|---|---|---|
| **Light level** | 2 | 1745246 | 872623 | 3.71 | **0.048** |
| Water availability | 1 | 117 | 117 | 0.00 | 0.983 |
| **Genus** | 1 | 1366680 | 1366680 | 5.81 | **0.028** |
| Light level*Water availability | 2 | 111532 | 55766 | 0.24 | 0.792 |
| Light level*Genus | 2 | 740432 | 370216 | 1.57 | 0.238 |
| Water availability*Genus | 1 | 219499 | 219499 | 0.93 | 0.348 |
| Light level*Water availability*Genus | 2 | 879840 | 439920 | 1.87 | 0.186 |
| Residuals | 16 | 3763752 | 235235 | | |

Table A2.21 Analysis of variance table for the pigment ***Chlorophyll a:b in µmol per m$^{-2}$ leaf area***, samples taken at the Cass field site during late afternoon in March 2003. Light level (natural daylight, shaded and exposed plants), water availability (streambed and N-facing slope) and genus (*Corokia cotoneaster* and *Coprosma propinqua*) as treatments [significant results in bold].

| | Df | Sum of Sq | Mean Sq | F Value | Pr(F) |
|---|---|---|---|---|---|
| Light level | 2 | 0.0340329 | 0.01701643 | 0.51 | 0.611 |
| Water availability | 1 | 0.0080036 | 0.00800362 | 0.24 | 0.632 |
| Genus | 1 | 0.0952891 | 0.09528909 | 2.84 | 0.111 |
| Light level*Water availability | 2 | 0.0020084 | 0.00100418 | 0.03 | 0.971 |
| Light level*Genus | 2 | 0.1336930 | 0.06684649 | 1.99 | 0.169 |
| Water availability*Genus | 1 | 0.0082780 | 0.00827798 | 0.25 | 0.626 |
| Light level*Water availability*Genus | 2 | 0.0528796 | 0.02643982 | 0.79 | 0.471 |
| Residuals | 16 | 0.5364583 | 0.03352865 | | |

Table A2.22 Analysis of variance table for the pigment ***ß-Carotene in µmol per m$^{-2}$ leaf area***, samples taken at the Cass field site during late afternoon in March 2003. Light level (natural daylight, shaded and exposed plants), water availability (streambed and N-facing slope) and genus (*Corokia cotoneaster* and *Coprosma propinqua*) as treatments [significant results in bold].

| | Df | Sum of Sq | Mean Sq | F Value | Pr(F) |
|---|---|---|---|---|---|
| Light level | 2 | 2995.84 | 1497.92 | 2.41 | 0.122 |
| Water availability | 1 | 234.21 | 234.21 | 0.38 | 0.548 |
| **Genus** | 1 | 10405.67 | 10405.67 | 16.74 | **0.001** |
| Light level*Water availability | 2 | 566.63 | 283.31 | 0.46 | 0.642 |
| Light level*Genus | 2 | 1577.86 | 788.93 | 1.27 | 0.308 |
| Water availability*Genus | 1 | 659.20 | 659.20 | 1.06 | 0.318 |
| Light level*Water availability*Genus | 2 | 503.22 | 251.61 | 0.41 | 0.674 |
| Residuals | 16 | 9943.73 | 621.48 | | |

Table A2.23 Analysis of variance table for ***vitamin E in µmol per cm$^{-2}$ leaf area***, samples taken at the Cass field site during late afternoon in February 2002. Light level (natural daylight, shaded and exposed plants), water availability (streambed and N-facing slope) and genus (*Corokia cotoneaster* and *Coprosma propinqua*) as treatments [significant results in bold].

| | Df | Sum of Sq | Mean Sq | F Value | Pr(F) |
|---|---|---|---|---|---|
| Light level | 2 | 58 | 28.9 | 0.37 | 0.692 |
| Water availability | 1 | 106 | 105.9 | 1.36 | 0.251 |
| **Genus** | 1 | 317 | 317.4 | 4.08 | **0.050** |
| Light level*Water availability | 2 | 304 | 151.8 | 1.95 | 0.156 |
| Light level*Genus | 2 | 223 | 111.3 | 1.43 | 0.252 |
| Water availability*Genus | 1 | 92 | 91.6 | 1.18 | 0.285 |
| Light level*Water availability*Genus | 2 | 300 | 149.8 | 1.93 | 0.160 |
| Residuals | 39 | 3035 | 77.8 | | |

Table A2.24 Analysis of variance table for ***vitamin E in µmol per m$^{-2}$ leaf area***, samples taken at the Cass field site during late afternoon in March 2003. Light level (natural daylight, shaded and exposed plants), water availability (streambed and N-facing slope) and genus (*Corokia cotoneaster* and *Coprosma propinqua*) as treatments [significant results in bold].

| | Df | Sum of Sq | Mean Sq | F Value | Pr(F) |
|---|---|---|---|---|---|
| Light level | 2 | 7.7 | 3.83 | 0.50 | 0.609 |
| **Water availability** | 1 | 85.7 | 85.72 | 11.29 | **0.002** |
| **Genus** | 1 | 75.8 | 75.80 | 9.99 | **0.003** |
| Light level*Water availability | 2 | 21.1 | 10.56 | 1.39 | 0.263 |
| Light level*Genus | 2 | 18.5 | 9.26 | 1.22 | 0.308 |
| Water availability*Genus | 1 | 5.4 | 5.42 | 0.71 | 0.404 |
| Light level*Water availability*Genus | 2 | 10.5 | 5.23 | 0.69 | 0.509 |
| Residuals | 258.1 | 7.59 | | | |

Table A2.25 Analysis of variance table for the pigment ***Violaxanthin in nmol per g fresh weight***, samples taken at the Cass field site during late afternoon in March 2003. Light level (natural daylight, shaded and exposed plants), water availability (streambed and N-facing slope) and genus (*Corokia cotoneaster* and *Coprosma propinqua*) as treatments [significant results in bold].

| | Df | Sum of Sq | Mean Sq | F Value | Pr(F) |
|---|---|---|---|---|---|
| Light level | 2 | 519.324 | 259.662 | 1.78 | 0.194 |
| **Water availability** | 1 | 1027.691 | 1027.691 | 7.04 | **0.015** |
| **Genus** | 1 | 2050.288 | 2050.288 | 14.04 | **0.001** |
| Light level*Water availability | 2 | 883.476 | 441.738 | 3.03 | 0.070 |
| Light level*Genus | 2 | 659.187 | 329.593 | 2.26 | 0.130 |
| **Water availability*Genus** | 1 | 655.295 | 655.295 | 4.49 | **0.046** |
| **Light level*Water availability*Genus** | 2 | 1366.486 | 683.243 | 4.68 | **0.021** |
| Residuals | 21 | 3066.948 | 146.045 | | |

Table A2.26 Analysis of variance table for the pigment ***Antheraxanthin in nmol per g fresh weight***, samples taken at the Cass field site during late afternoon in March 2003. Light level (natural daylight, shaded and exposed plants), water availability (streambed and N-facing slope) and genus (*Corokia cotoneaster* and *Coprosma propinqua*) as treatments [significant results in bold].

| | Df | Sum of Sq | Mean Sq | F Value | Pr(F) |
|---|---|---|---|---|---|
| Light level | 2 | 321.170 | 160.5851 | 2.64 | 0.095 |
| Water availability | 1 | 47.273 | 47.2731 | 0.78 | 0.388 |
| Genus | 1 | 27.462 | 27.4624 | 0.45 | 0.509 |
| Light level*Water availability | 2 | 1.665 | 0.8327 | 0.01 | 0.986 |
| Light level*Genus | 2 | 3.004 | 1.5021 | 0.03 | 0.976 |
| Water availability*Genus | 1 | 42.550 | 42.5505 | 0.70 | 0.412 |
| Light level*Water availability*Genus | 2 | 37.640 | 18.8199 | 0.31 | 0.737 |
| Residuals | 21 | 1276.666 | 60.7936 | | |

Table A2.27 Analysis of variance table for the pigment ***Zeaxanthin in nmol per g fresh weight***, samples taken at the Cass field site during late afternoon in March 2003. Light level (natural daylight, shaded and exposed plants), water availability (streambed and N-facing slope) and genus (*Corokia cotoneaster* and *Coprosma propinqua*) as treatments [significant results in bold].

| | Df | Sum of Sq | Mean Sq | F Value | Pr(F) |
|---|---|---|---|---|---|
| **Light level** | 2 | 2477.940 | 1238.970 | 5.70 | **0.011** |
| Water availability | 1 | 186.837 | 186.837 | 0.86 | 0.364 |
| Genus | 1 | 183.999 | 183.999 | 0.85 | 0.368 |
| Light level*Water availability | 2 | 273.269 | 136.634 | 0.63 | 0.543 |
| Light level*Genus | 2 | 101.924 | 50.962 | 0.24 | 0.793 |
| Water availability*Genus | 1 | 344.093 | 344.093 | 1.58 | 0.222 |
| Light level*Water availability*Genus | 2 | 979.297 | 489.649 | 2.25 | 0.130 |
| Residuals | 21 | 4563.596 | 217.314 | | |

Table A2.28 Analysis of variance table for the pigment ***Neoxanthin in nmol per g fresh weight***, samples taken at the Cass field site during late afternoon in March 2003. Light level (natural daylight, shaded and exposed plants), water availability (streambed and N-facing slope) and genus (*Corokia cotoneaster* and *Coprosma propinqua*) as treatments [significant results in bold].

| | Df | Sum of Sq | Mean Sq | F Value | Pr(F) |
|---|---|---|---|---|---|
| **Light level** | 2 | 1004.539 | 502.2693 | 5.98 | **0.009** |
| Water availability | 1 | 36.342 | 36.3417 | 0.43 | 0.518 |
| **Genus** | 1 | 806.353 | 806.3533 | 9.60 | **0.005** |
| Light level*Water availability | 2 | 15.812 | 7.9058 | 0.09 | 0.911 |
| Light level*Genus | 2 | 253.610 | 126.8052 | 1.51 | 0.244 |
| Water availability*Genus | 1 | 33.287 | 33.2873 | 0.40 | 0.536 |
| **Light level*Water availability*Genus** | 2 | 824.982 | 412.4908 | 4.91 | **0.018** |
| Residuals | 21 | 1764.204 | 84.0097 | | |

Table A2.29 Analysis of variance table for the pigment ***Lutein in nmol per g fresh weight***, samples taken at the Cass field site during late afternoon in March 2003. Light level (natural daylight, shaded and exposed plants), water availability (streambed and N-facing slope) and genus (*Corokia cotoneaster* and *Coprosma propinqua*) as treatments [significant results in bold].

| | Df | Sum of Sq | Mean Sq | F Value | Pr(F) |
|---|---|---|---|---|---|
| Light level | 2 | 5674.91 | 2837.457 | 2.67 | 0.093 |
| Water availability | 1 | 716.12 | 716.123 | 0.67 | 0.421 |
| **Genus** | 1 | 5759.24 | 5759.236 | 5.41 | **0.030** |
| Light level*Water availability | 2 | 775.36 | 387.682 | 0.36 | 0.699 |
| Light level*Genus | 2 | 3950.44 | 1975.221 | 1.86 | 0.181 |
| Water availability*Genus | 1 | 268.31 | 268.313 | 0.25 | 0.621 |
| Light level*Water availability*Genus | 2 | 5129.71 | 2564.853 | 2.41 | 0.114 |
| Residuals | 21 | 22349.17 | 1064.246 | | |

Table A2.30 Analysis of variance table for the pigment ***Chlorophyll a in nmol per g fresh weight***, samples taken at the Cass field site during late afternoon in March 2003. Light level (natural daylight, shaded and exposed plants), water availability (streambed and N-facing slope) and genus (*Corokia cotoneaster* and *Coprosma propinqua*) as treatments [significant results in bold].

| | Df | Sum of Sq | Mean Sq | F Value | Pr(F) |
|---|---|---|---|---|---|
| **Light level** | 2 | 204877.2 | 102438.6 | 4.47 | **0.024** |
| Water availability | 1 | 18927.0 | 18927.0 | 0.83 | 0.374 |
| Genus | 1 | 82772.9 | 82772.9 | 3.62 | 0.071 |
| Light level*Water availability | 2 | 3336.0 | 1668.0 | 0.07 | 0.930 |
| Light level*Genus | 2 | 134457.7 | 67228.9 | 2.94 | 0.075 |
| Water availability*Genus | 1 | 201.9 | 201.9 | 0.01 | 0.926 |
| **Light level*Water availability*Genus** | 2 | 162658.0 | 81329.0 | 3.55 | **0.047** |
| Residuals | 21 | 480898.8 | 22899.9 | | |

Table A2.31 Analysis of variance table for the pigment ***Chlorophyll b in nmol per g fresh weight***, samples taken at the Cass field site during late afternoon in March 2003. Light level (natural daylight, shaded and exposed plants), water availability (streambed and N-facing slope) and genus (*Corokia cotoneaster* and *Coprosma propinqua*) as treatments [significant results in bold].

| | Df | Sum of Sq | Mean Sq | F Value | Pr(F) |
|---|---|---|---|---|---|
| **Light level** | 2 | 27229.13 | 13614.57 | 4.91 | **0.018** |
| Water availability | 1 | 1074.89 | 1074.89 | 0.39 | 0.540 |
| Genus | 1 | 2580.95 | 2580.95 | 0.93 | 0.345 |
| Light level*Water availability | 2 | 1095.58 | 547.79 | 0.20 | 0.822 |
| Light level*Genus | 2 | 13067.50 | 6533.75 | 2.36 | 0.119 |
| Water availability*Genus | 1 | 291.46 | 291.46 | 0.11 | 0.749 |
| **Light level*Water availability*Genus** | 2 | 25518.43 | 12759.22 | 4.60 | **0.022** |
| Residuals | 21 | 58192.79 | 2771.09 | | |

Table A2.32 Analysis of variance table for the pigment ***Chlorophyll a+b in nmol per g fresh weight***, samples taken at the Cass field site during late afternoon in March 2003. Light level (natural daylight, shaded and exposed plants), water availability (streambed and N-facing slope) and genus (*Corokia cotoneaster* and *Coprosma propinqua*) as treatments [significant results in bold].

| | Df | Sum of Sq | Mean Sq | F Value | Pr(F) |
|---|---|---|---|---|---|
| **Light level** | 2 | 381231.7 | 190615.9 | 4.71 | **0.020** |
| Water availability | 1 | 29022.9 | 29022.9 | 0.72 | 0.407 |
| Genus | 1 | 114586.2 | 114586.2 | 2.83 | 0.107 |
| Light level*Water availability | 2 | 6946.2 | 3473.1 | 0.09 | 0.918 |
| Light level*Genus | 2 | 227762.6 | 113881.3 | 2.81 | 0.083 |
| Water availability*Genus | 1 | 978.5 | 978.5 | 0.02 | 0.878 |
| **Light level*Water availability*Genus** | 2 | 316550.7 | 158275.3 | 3.91 | **0.036** |
| Residuals | 21 | 849892.6 | 40471.1 | | |

Table A2.33 Analysis of variance table for the pigment ***Chlorophyll a:b in nmol per g fresh weight***, samples taken at the Cass field site during late afternoon in March 2003. Light level (natural daylight, shaded and exposed plants), water availability (streambed and N-facing slope) and genus (*Corokia cotoneaster* and *Coprosma propinqua*) as treatments [significant results in bold].

| | Df | Sum of Sq | Mean Sq | F Value | Pr(F) |
|---|---|---|---|---|---|
| Light level | 2 | 0.0402656 | 0.0201328 | 0.53 | 0.594 |
| Water availability | 1 | 0.0069805 | 0.0069805 | 0.19 | 0.672 |
| **Genus** | 1 | 0.1971796 | 0.1971796 | 5.22 | **0.033** |
| Light level*Water availability | 2 | 0.0796592 | 0.0398296 | 1.06 | 0.366 |
| Light level*Genus | 2 | 0.1142760 | 0.0571380 | 1.51 | 0.243 |
| Water availability*Genus | 1 | 0.0352442 | 0.0352442 | 0.93 | 0.345 |
| Light level*Water availability*Genus | 2 | 0.1115657 | 0.0557829 | 1.48 | 0.251 |
| Residuals | 21 | 0.7926950 | 0.0377474 | | |

Table A2.34 Analysis of variance table for the pigment ***ß-carotene in nmol per g fresh weight***, samples taken at the Cass field site during late afternoon in March 2003. Light level (natural daylight, shaded and exposed plants), water availability (streambed and N-facing slope) and genus (*Corokia cotoneaster* and *Coprosma propinqua*) as treatments [significant results in bold].

| | Df | Sum of Sq | Mean Sq | F Value | Pr(F) |
|---|---|---|---|---|---|
| Light level | 2 | 539.826 | 269.913 | 2.78 | 0.085 |
| Water availability | 1 | 0.150 | 0.150 | 0.00 | 0.969 |
| **Genus** | 1 | 1122.355 | 1122.355 | 11.55 | **0.003** |
| Light level*Water availability | 2 | 113.274 | 56.637 | 0.58 | 0.567 |
| Light level*Genus | 2 | 264.753 | 132.376 | 1.36 | 0.278 |
| Water availability*Genus | 1 | 0.258 | 0.258 | 0.00 | 0.959 |
| Light level*Water availability*Genus | 2 | 438.688 | 219.344 | 2.26 | 0.130 |
| Residuals | 21 | 2041.418 | 97.210 | | |

Table A2.35 Analysis of variance table for ***vitamin E in µmol per g fresh weight***, samples taken at the Cass field site during late afternoon in February 2002. Light level (natural daylight, shaded and exposed plants), water availability (streambed and N-facing slope) and genus (*Corokia cotoneaster* and *Coprosma propinqua*) as treatments [significant results in bold].

| | Df | Sum of Sq | Mean Sq | F Value | Pr(F) |
|---|---|---|---|---|---|
| Light level | 2 | 93344 | 46672 | 0.41 | 0.666 |
| Water availability | 1 | 136809 | 136809 | 1.20 | 0.280 |
| **Genus** | 1 | 483955 | 483955 | 4.26 | **0.046** |
| Light level*Water availability | 2 | 398010 | 199005 | 1.75 | 0.188 |
| Light level*Genus | 2 | 332417 | 166208 | 1.46 | 0.245 |
| Water availability*Genus | 1 | 131360 | 131360 | 1.16 | 0.289 |
| Light level*Water availability*Genus | 2 | 405507 | 202753 | 1.78 | 0.182 |
| Residuals | 38 | 4321351 | 113720 | | |

Table A2.36 Analysis of variance table for ***vitamin E in µmol per g fresh weight***, samples taken at the Cass field site during late afternoon in March 2003. Light level (natural daylight, shaded and exposed plants), water availability (streambed and N-facing slope) and genus (*Corokia cotoneaster* and *Coprosma propinqua*) as treatments [significant results in bold].

| | Df | Sum of Sq | Mean Sq | F Value | Pr(F) |
|---|---|---|---|---|---|
| Light level | 2 | 7167 | 3583 | 0.39 | 0.678 |
| **Water availability** | 1 | 122767 | 122767 | 13.48 | **0.001** |
| **Genus** | 1 | 131121 | 131121 | 14.39 | **0.001** |
| Light level*Water availability | 2 | 16594 | 8297 | 0.91 | 0.412 |
| Light level*Genus | 2 | 25800 | 12900 | 1.42 | 0.257 |
| Water availability*Genus | 1 | 8757 | 8757 | 0.96 | 0.334 |
| Light level*Water availability*Genus | 2 | 9760 | 4880 | 0.54 | 0.590 |
| Residuals | 34 | 309749 | 9110 | | |

Table A2.37 Analysis of variance table for the pigment ***Violaxanthin in mmol per mol total Chlorophyll,*** samples taken in the glasshouse during late afternoon in June 2002. Light level (100% vs 75% of natural daylight), water availability (200 mls every 2 days and 50mls every 3 days) and genus (*Corokia cotoneaster, Coprosma propinqua, Corokia buddleioide* and *Coprosma robusta*) as treatments. Leaves taken from divaricates *Corokia cotoneaster* and *Coprosma propinqua* and non-divaricates *Corokia buddleioides* and *Coprosma robusta*. [significant results in bold].

| | Df | Sum of Sq | Mean Sq | F Value | Pr(F) |
|---|---|---|---|---|---|
| Light level | 1 | 22.024 | 22.0245 | 0.31 | 0.588 |
| Water availability | 1 | 41.127 | 41.1269 | 0.57 | 0.460 |
| Genus | 1 | 223.506 | 223.5065 | 3.10 | 0.096 |
| Habit | 1 | 62.368 | 62.3684 | 0.87 | 0.365 |
| Light level*Water availability | 1 | 179.996 | 179.9961 | 2.50 | 0.132 |
| Light level*Genus | 1 | 8.000 | 7.9997 | 0.11 | 0.743 |
| Water availability*Genus | 1 | 0.043 | 0.0428 | 0.00 | 0.981 |
| Light level*Habit | 1 | 138.296 | 138.2956 | 1.92 | 0.184 |
| Water availability*Habit | 1 | 0.856 | 0.8564 | 0.01 | 0.915 |
| Genus*Habit | 1 | 68.748 | 68.7482 | 0.95 | 0.342 |
| Light level*Water availability*Genus | 1 | 13.437 | 13.4374 | 0.19 | 0.671 |
| Light level*Genus*Habit | 1 | 5.866 | 5.8660 | 0.08 | 0.779 |
| Water availability*Genus*Habit | 1 | 250.066 | 250.0660 | 3.47 | 0.080 |
| Residuals | 17 | 1224.694 | 72.0408 | | |

Table A2.38 Analysis of variance table for the pigment ***Antheraxanthin in mmol per mol total Chlorophyll***, samples taken in the glasshouse during late afternoon in June 2002. Light level (100% vs 75% of natural daylight), water availability (200 mls every 2 days and 50mls every 3 days) and genus (*Corokia cotoneaster, Coprosma propinqua, Corokia buddleioide* and *Coprosma robusta*) as treatments. Leaves taken from divaricates *Corokia cotoneaster* and *Coprosma propinqua* and non-divaricates *Corokia buddleioides* and *Coprosma robusta*. [significant results in bold].

| | Df | Sum of Sq | Mean Sq | F Value | Pr(F) |
|---|---|---|---|---|---|
| Light level | 1 | 26.5416 | 26.54162 | 1.24 | 0.282 |
| Water availability | 1 | 0.8183 | 0.81827 | 0.04 | 0.848 |
| Genus | 1 | 31.8193 | 31.81930 | 1.48 | 0.240 |
| Habit | 1 | 23.6715 | 23.67154 | 1.10 | 0.309 |
| Light level*Water availability | 1 | 4.1546 | 4.15455 | 0.19 | 0.666 |
| Light level*Genus | 1 | 23.1540 | 23.15402 | 1.08 | 0.314 |
| Water availability*Genus | 1 | 0.0742 | 0.07417 | 0.00 | 0.954 |
| Light level*Habit | 1 | 7.4607 | 7.46072 | 0.35 | 0.563 |
| Water availability*Habit | 1 | 0.0912 | 0.09123 | 0.00 | 0.949 |
| Genus*Habit | 1 | 18.7906 | 18.79056 | 0.88 | 0.363 |
| Light level*Water availability*Genus | 1 | 5.3185 | 5.31848 | 0.25 | 0.625 |
| Light level*Genus*Habit | 1 | 4.8627 | 4.86272 | 0.23 | 0.640 |
| Water availability*Genus*Habit | 1 | 0.8308 | 0.83081 | 0.04 | 0.846 |
| Residuals | 17 | 365.1226 | 21.47780 | | |

Table A2.39 Analysis of variance table for the pigment ***Zeaxanthin in mmol per mol total Chlorophyll***, samples taken in the glasshouse during late afternoon in June 2002. Light level (100% vs 75% of natural daylight), water availability (200 mls every 2 days and 50mls every 3 days) and genus (*Corokia cotoneaster*, *Coprosma propinqua*, *Corokia buddleioide* and *Coprosma robusta*) as treatments. Leaves taken from divaricates *Corokia cotoneaster* and *Coprosma propinqua* and non-divaricates *Corokia buddleioides* and *Coprosma robusta*. [significant results in bold].

| | Df | Sum of Sq | Mean Sq | F Value | Pr(F) |
|---|---|---|---|---|---|
| Light level | 1 | 487.733 | 487.7333 | 0.86 | 0.368 |
| Water availability | 1 | 342.976 | 342.9755 | 0.60 | 0.448 |
| Genus | 1 | 513.823 | 513.8230 | 0.90 | 0.356 |
| Habit | 1 | 418.283 | 418.2828 | 0.73 | 0.403 |
| Light level*Water availability | 1 | 67.486 | 67.4864 | 0.12 | 0.735 |
| Light level*Genus | 1 | 350.090 | 350.0899 | 0.61 | 0.444 |
| Water availability*Genus | 1 | 569.551 | 569.5514 | 1.00 | 0.331 |
| Light level*Habit | 1 | 136.935 | 136.9351 | 0.24 | 0.630 |
| Water availability*Habit | 1 | 219.886 | 219.8864 | 0.39 | 0.543 |
| Genus*Habit | 1 | 429.584 | 429.5842 | 0.75 | 0.397 |
| Light level*Water availability*Genus | 1 | 152.794 | 152.7941 | 0.27 | 0.611 |
| Light level*Genus*Habit | 1 | 79.212 | 79.2117 | 0.14 | 0.714 |
| Water availability*Genus*Habit | 1 | 368.774 | 368.7745 | 0.65 | 0.432 |
| Residuals | 17 | 9682.675 | 569.5691 | | |

Table A2.40 Analysis of variance table for the pigments in the ***Deepoxidation state in mmol per mol sum Xanthophyll cycle pigments***, samples taken in the glasshouse during late afternoon in June 2002. Light level (100% vs 75% of natural daylight), water availability (200 mls every 2 days and 50mls every 3 days) and genus (*Corokia cotoneaster*, *Coprosma propinqua*, *Corokia buddleioide* and *Coprosma robusta*) as treatments. Leaves taken from divaricates *Corokia cotoneaster* and *Coprosma propinqua* and non-divaricates *Corokia buddleioides* and *Coprosma robusta*. [significant results in bold].

| | Df | Sum of Sq | Mean Sq | F Value | Pr(F) |
|---|---|---|---|---|---|
| Light level | 1 | 521.539 | 521.5393 | 2.89 | 0.096 |
| Water availability | 1 | 82.783 | 82.7830 | 0.46 | 0.501 |
| Genus | 1 | 32.566 | 32.5658 | 0.18 | 0.673 |
| Habit | 1 | 283.048 | 283.0476 | 1.57 | 0.217 |
| Light level*Water availability | 1 | 110.377 | 110.3772 | 0.61 | 0.438 |
| Light level*Genus | 1 | 32.075 | 32.0750 | 0.18 | 0.675 |
| Water availability*Genus | 1 | 3.133 | 3.1331 | 0.02 | 0.896 |
| Light level*Habit | 1 | 246.622 | 246.6224 | 1.37 | 0.248 |
| Water availability*Habit | 1 | 163.938 | 163.9376 | 0.91 | 0.346 |
| Genus*Habit | 1 | 1.829 | 1.8293 | 0.01 | 0.920 |
| Light level*Water availability*Genus | 1 | 18.879 | 18.8792 | 0.11 | 0.748 |
| Light level*Water availability*Habit | 1 | 145.304 | 145.3041 | 0.81 | 0.374 |
| Light level*Genus*Habit | 1 | 1.510 | 1.5098 | 0.01 | 0.927 |
| Water availability*Genus*Habit | 1 | 109.005 | 109.0046 | 0.61 | 0.441 |
| Light level*Water availability*Genus*Habit | 1 | 38.451 | 38.4510 | 0.21 | 0.646 |
| Residuals | 44 | 7931.536 | 180.2622 | | |

Table A2.41 Analysis of variance table for the pigment ***Neoxanthin in mmol per mol total Chlorophyll***, samples taken in the glasshouse during late afternoon in June 2002. Light level (100% vs 75% of natural daylight), water availability (200 mls every 2 days and 50mls every 3 days) and genus (*Corokia cotoneaster*, *Coprosma propinqua*, *Corokia buddleioide* and *Coprosma robusta*) as treatments. Leaves taken from divaricates *Corokia cotoneaster* and *Coprosma propinqua* and non-divaricates *Corokia buddleioides* and *Coprosma robusta*. [significant results in bold].

| | Df | Sum of Sq | Mean Sq | F Value | Pr(F) |
|---|---|---|---|---|---|
| Light level | 1 | 108.519 | 108.5194 | 0.97 | 0.340 |
| Water availability | 1 | 146.385 | 146.3847 | 1.30 | 0.270 |
| Genus | 1 | 16.939 | 16.9393 | 0.15 | 0.703 |
| Habit | 1 | 60.489 | 60.4892 | 0.54 | 0.473 |
| Light level*Water availability | 1 | 200.576 | 200.5761 | 1.78 | 0.199 |
| Light level*Genus | 1 | 87.687 | 87.6871 | 0.78 | 0.390 |
| Water availability*Genus | 1 | 87.351 | 87.3510 | 0.78 | 0.390 |
| Light level*Habit | 1 | 1.475 | 1.4751 | 0.01 | 0.910 |
| Water availability*Habit | 1 | 0.040 | 0.0403 | 0.00 | 0.985 |
| Genus*Habit | 1 | 1.165 | 1.1647 | 0.01 | 0.920 |
| Light level*Water availability*Genus | 1 | 85.394 | 85.3945 | 0.76 | 0.396 |
| Light level*Genus*Habit | 1 | 0.028 | 0.0278 | 0.00 | 0.988 |
| Water availability*Genus*Habit | 1 | 1.982 | 1.9816 | 0.02 | 0.896 |
| Residuals | 17 | 1911.643 | 112.4496 | | |

Table A2.42 Analysis of variance table for the pigment ***Lutein in mmol per mol total Chlorophyll***, samples taken in the glasshouse during late afternoon in June 2002. Light level (100% vs 75% of natural daylight), water availability (200 mls every 2 days and 50mls every 3 days) and genus (*Corokia cotoneaster*, *Coprosma propinqua*, *Corokia buddleioide* and *Coprosma robusta*) as treatments. Leaves taken from divaricates *Corokia cotoneaster* and *Coprosma propinqua* and non-divaricates *Corokia buddleioides* and *Coprosma robusta*. [significant results in bold].

| | Df | Sum of Sq | Mean Sq | F Value | Pr(F) |
|---|---|---|---|---|---|
| Light level | 1 | 545.715 | 545.715 | 1.45 | 0.245 |
| Water availability | 1 | 3.558 | 3.558 | 0.01 | 0.924 |
| **Genus** | 1 | 2951.109 | 2951.109 | 7.83 | **0.012** |
| **Habit** | 1 | 1670.402 | 1670.402 | 4.43 | **0.050** |
| **Light level*Water availability** | 1 | 1679.452 | 1679.452 | 4.46 | **0.050** |
| Light level*Genus | 1 | 317.214 | 317.214 | 0.84 | 0.372 |
| Water availability*Genus | 1 | 84.951 | 84.951 | 0.23 | 0.641 |
| Light level*Habit | 1 | 227.820 | 227.820 | 0.60 | 0.448 |
| Water availability*Habit | 1 | 480.277 | 480.277 | 1.27 | 0.275 |
| Genus*Habit | 1 | 119.728 | 119.728 | 0.32 | 0.580 |
| Light level*Water availability*Genus | 1 | 481.663 | 481.663 | 1.28 | 0.274 |
| Light level*Genus*Habit | 1 | 146.409 | 146.409 | 0.39 | 0.541 |
| Water availability*Genus*Habit | 1 | 308.584 | 308.584 | 0.82 | 0.378 |
| Residuals | 17 | 6407.596 | 376.917 | | |

Table A2.43 Analysis of variance table for the pigment ***ß-carotene in mmol per mol total Chlorophyll***, samples taken in the glasshouse during late afternoon in June 2002. Light level (100% vs 75% of natural daylight), water availability (200 mls every 2 days and 50mls every 3 days) and genus (*Corokia cotoneaster, Coprosma propinqua, Corokia buddleioide* and *Coprosma robusta*) as treatments. Leaves taken from divaricates *Corokia cotoneaster* and *Coprosma propinqua* and non-divaricates *Corokia buddleioides* and *Coprosma robusta*. [significant results in bold].

| | Df | Sum of Sq | Mean Sq | F Value | Pr(F) |
|---|---|---|---|---|---|
| Light level | 1 | 6.471 | 6.471 | 0.046 | 0.831 |
| Water availability | 1 | 557.994 | 557.994 | 3.96 | 0.052 |
| **Genus** | 1 | 3771.929 | 3771.929 | 26.74 | **0.000** |
| Light level*Water availability | 1 | 32.471 | 32.471 | 0.23 | 0.633 |
| Light level*Genus | 1 | 7.225 | 7.225 | 0.05 | 0.822 |
| Water availability*Genus | 1 | 401.428 | 401.428 | 2.85 | 0.098 |
| Light level*Water availability*Genus | 1 | 9.190 | 9.190 | 0.07 | 0.800 |
| Residuals | 51 | 7194.092 | 141.061 | | |

Table A2.44 Analysis of variance table for ***vitamin E in mol per mol total Chlorophyll***, samples taken in the glasshouse during late afternoon in June 2002. Light level (100% vs 75% of natural daylight), water availability (200 mls every 2 days and 50mls every 3 days) and genus (*Corokia cotoneaster, Coprosma propinqua, Corokia buddleioide* and *Coprosma robusta*) as treatments. Leaves taken from divaricates *Corokia cotoneaster* and *Coprosma propinqua* and non-divaricates *Corokia buddleioides* and *Coprosma robusta.* [significant results in bold].

| | Df | Sum of Sq | Mean Sq | F Value | Pr(F) |
|---|---|---|---|---|---|
| Light level | 1 | 0.00105 | 0.001048 | 3.81 | 0.057 |
| Water availability | 1 | 0.00035 | 0.000345 | 1.25 | 0.269 |
| Genus | 1 | 0.00000 | 0.000004 | 0.02 | 0.902 |
| **Habit** | 1 | 0.00711 | 0.007107 | 25.84 | **0.000** |
| Light level*Water availability | 1 | 0.00000 | 0.000000 | 0.00 | 0.966 |
| Light level*Genus | 1 | 0.00008 | 0.000080 | 0.29 | 0.592 |
| Water availability*Genus | 1 | 0.00085 | 0.000849 | 3.09 | 0.086 |
| Light level*Habit | 1 | 0.00008 | 0.000082 | 0.30 | 0.587 |
| Water availability*Habit | 1 | 0.00003 | 0.000029 | 0.11 | 0.747 |
| Genus*Habit | 1 | 0.00019 | 0.000190 | 0.69 | 0.411 |
| Light level*Water availability*Genus | 1 | 0.00000 | 0.000000 | 0.00 | 0.974 |
| Light level*Water availability*Habit | 1 | 0.00039 | 0.000394 | 1.43 | 0.238 |
| Light level*Genus*Habit | 1 | 0.00034 | 0.000341 | 1.24 | 0.272 |
| Water availability*Genus*Habit | 1 | 0.00016 | 0.000156 | 0.57 | 0.456 |
| Light level*Water availability*Genus*Habit | 1 | 0.00030 | 0.000299 | 1.09 | 0.303 |
| Residuals | 43 | 0.01183 | 0.000275 | | |

Table A2.45 Analysis of variance table for the pigment ***Violaxanthin in µmol per m$^{-2}$ leaf area***, samples taken in the glasshouse during late afternoon in June 2002. Light level (100% vs 75% of natural daylight), water availability (200 mls every 2 days and 50mls every 3 days) and genus (*Corokia cotoneaster, Coprosma propinqua, Corokia buddleioide* and *Coprosma robusta*) as treatments. Leaves taken from divaricates *Corokia cotoneaster* and *Coprosma propinqua* and non-divaricates *Corokia buddleioides* and *Coprosma robusta*. [significant results in bold].

| | Df | Sum of Sq | Mean Sq | F Value | Pr(F) |
|---|---|---|---|---|---|
| Light level | 1 | 84835 | 84835 | 1.59 | 0.225 |
| Water availability | 1 | 197875 | 197875 | 3.71 | 0.071 |
| **Genus** | 1 | 467981 | 467981 | 8.77 | **0.009** |
| **Habit** | 1 | 1463820 | 1463820 | 27.42 | **0.000** |
| Light level*Water availability | 1 | 26996 | 26996 | 0.51 | 0.487 |
| Light level*Genus | 1 | 45200 | 45200 | 0.85 | 0.370 |
| Water availability*Genus | 1 | 98299 | 98299 | 1.84 | 0.193 |
| Light level*Habit | 1 | 85938 | 85938 | 1.61 | 0.222 |
| Water availability*Habit | 1 | 9232 | 9232 | 0.17 | 0.683 |
| **Genus*Habit** | 1 | 743450 | 743450 | 13.93 | **0.002** |
| Light level*Water availability*Genus | 1 | 836 | 836 | 0.02 | 0.902 |
| Light level*Genus*Habit | 1 | 7830 | 7830 | 0.15 | 0.707 |
| Water availability*Genus*Habit | 1 | 219 | 219 | 0.00 | 0.950 |
| Residuals | 17 | 907444 | 53379 | | |

Table A2.46 Analysis of variance table for the pigment ***Antheraxanthin in µmol per m$^{-2}$ leaf area***, samples taken in the glasshouse during late afternoon in June 2002. Light level (100% vs 75% of natural daylight), water availability (200 mls every 2 days and 50mls every 3 days) and genus (*Corokia cotoneaster, Coprosma propinqua, Corokia buddleioide* and *Coprosma robusta*) as treatments. Leaves taken from divaricates *Corokia cotoneaster* and *Coprosma propinqua* and non-divaricates *Corokia buddleioides* and *Coprosma robusta*. [significant results in bold].

| | Df | Sum of Sq | Mean Sq | F Value | Pr(F) |
|---|---|---|---|---|---|
| Light level | 1 | 84.783 | 84.7827 | 1.00 | 0.331 |
| Water availability | 1 | 58.125 | 58.1253 | 0.69 | 0.419 |
| **Genus** | 1 | 388.155 | 388.1546 | 4.59 | **0.047** |
| Habit | 1 | 90.720 | 90.7204 | 1.07 | 0.315 |
| Light level*Water availability | 1 | 0.852 | 0.8522 | 0.01 | 0.921 |
| Light level*Genus | 1 | 65.054 | 65.0538 | 0.77 | 0.393 |
| Water availability*Genus | 1 | 28.332 | 28.3317 | 0.33 | 0.570 |
| Light level*Habit | 1 | 1.628 | 1.6275 | 0.02 | 0.891 |
| Water availability*Habit | 1 | 28.888 | 28.8884 | 0.34 | 0.567 |
| Genus*Habit | 1 | 38.271 | 38.2710 | 0.45 | 0.510 |
| Light level*Water availability*Genus | 1 | 0.244 | 0.2444 | 0.00 | 0.958 |
| Light level*Genus*Habit | 1 | 5.075 | 5.0751 | 0.06 | 0.809 |
| Water availability*Genus*Habit | 1 | 14.948 | 14.9479 | 0.18 | 0.680 |
| Residuals | 17 | 1438.197 | 84.5998 | | |

Table A2.47 Analysis of variance table for the pigment ***Zeaxanthin in µmol per m$^{-2}$ leaf area***, samples taken in the glasshouse during late afternoon in June 2002. Light level (100% vs 75% of natural daylight), water availability (200 mls every 2 days and 50mls every 3 days) and genus (*Corokia cotoneaster, Coprosma propinqua, Corokia buddleioide* and *Coprosma robusta*) as treatments. Leaves taken from divaricates *Corokia cotoneaster* and *Coprosma propinqua* and non-divaricates *Corokia buddleioides* and *Coprosma robusta*. [significant results in bold].

| | Df | Sum of Sq | Mean Sq | F Value | Pr(F) |
|---|---|---|---|---|---|
| Light level | 1 | 786.21 | 786.210 | 0.24 | 0.633 |
| Water availability | 1 | 3135.34 | 3135.338 | 0.94 | 0.345 |
| Genus | 1 | 1483.12 | 1483.118 | 0.45 | 0.513 |
| Habit | 1 | 303.07 | 303.075 | 0.09 | 0.766 |
| Light level*Water availability | 1 | 65.62 | 65.622 | 0.02 | 0.890 |
| Light level*Genus | 1 | 3954.55 | 3954.551 | 1.19 | 0.290 |
| Water availability*Genus | 1 | 1786.34 | 1786.340 | 0.54 | 0.473 |
| Light level*Habit | 1 | 2174.87 | 2174.870 | 0.66 | 0.430 |
| Water availability*Habit | 1 | 1396.79 | 1396.792 | 0.42 | 0.525 |
| Genus*Habit | 1 | 5199.47 | 5199.475 | 1.57 | 0.228 |
| Light level*Water availability*Genus | 1 | 1136.28 | 1136.283 | 0.34 | 0.566 |
| Light level*Genus*Habit | 1 | 48.65 | 48.654 | 0.02 | 0.905 |
| Water availability*Genus*Habit | 1 | 1615.15 | 1615.151 | 0.49 | 0.495 |
| Residuals | 17 | 56445.34 | 3320.314 | | |

Table A2.48 Analysis of variance table for the pigment ***Neoaxanthin in µmol per m$^{-2}$ leaf area***, samples taken in the glasshouse during late afternoon in June 2002. Light level (100% vs 75% of natural daylight), water availability (200 mls every 2 days and 50mls every 3 days) and genus (*Corokia cotoneaster, Coprosma propinqua, Corokia buddleioide* and *Coprosma robusta*) as treatments. Leaves taken from divaricates *Corokia cotoneaster* and *Coprosma propinqua* and non-divaricates *Corokia buddleioides* and *Coprosma robusta*. [significant results in bold].

| | Df | Sum of Sq | Mean Sq | F Value | Pr(F) |
|---|---|---|---|---|---|
| Light level | 1 | 1372.50 | 1372.500 | 1.50 | 0.237 |
| Water availability | 1 | 515.69 | 515.688 | 0.57 | 0.463 |
| Genus | 1 | 889.73 | 889.733 | 0.97 | 0.338 |
| Habit | 1 | 26.31 | 26.311 | 0.03 | 0.867 |
| Light level*Water availability | 1 | 1158.43 | 1158.428 | 1.27 | 0.276 |
| Light level*Genus | 1 | 1066.07 | 1066.074 | 1.17 | 0.295 |
| Water availability*Genus | 1 | 379.98 | 379.976 | 0.42 | 0.528 |
| Light level*Habit | 1 | 103.67 | 103.671 | 0.11 | 0.740 |
| Water availability*Habit | 1 | 12.87 | 12.868 | 0.01 | 0.907 |
| Genus*Habit | 1 | 198.54 | 198.535 | 0.22 | 0.647 |
| Light level*Water availability*Genus | 1 | 701.55 | 701.547 | 0.77 | 0.393 |
| Light level*Genus*Habit | 1 | 22.95 | 22.947 | 0.03 | 0.876 |
| Water availability*Genus*Habit | 1 | 11.35 | 11.350 | 0.01 | 0.913 |
| Residuals | 17 | 15530.33 | 913.549 | | |

Table A2.49 Analysis of variance table for the pigment ***Lutein in µmol per m$^{-2}$ leaf area***, samples taken in the glasshouse during late afternoon in June 2002. Light level (100% vs 75% of natural daylight), water availability (200 mls every 2 days and 50mls every 3 days) and genus (*Corokia cotoneaster, Coprosma propinqua, Corokia buddleioide* and *Coprosma robusta*) as treatments. Leaves taken from divaricates *Corokia cotoneaster* and *Coprosma propinqua* and non-divaricates *Corokia buddleioides* and *Coprosma robusta*. [significant results in bold].

| | Df | Sum of Sq | Mean Sq | F Value | Pr(F) |
|---|---|---|---|---|---|
| Light level | 1 | 1243529 | 1243529 | 2.04 | 0.172 |
| Water availability | 1 | 2506639 | 2506639 | 4.10 | 0.059 |
| **Genus** | 1 | 7238035 | 7238035 | 11.84 | **0.003** |
| **Habit** | 1 | 22273480 | 22273480 | 36.44 | **0.000** |
| Light level*Water availability | 1 | 454838 | 454838 | 0.74 | 0.400 |
| Light level*Genus | 1 | 685152 | 685152 | 1.12 | 0.305 |
| Water availability*Genus | 1 | 1255827 | 1255827 | 2.06 | 0.170 |
| Light level*Habit | 1 | 1183371 | 1183371 | 1.94 | 0.182 |
| Water availability*Habit | 1 | 51875 | 51875 | 0.09 | 0.774 |
| **Genus*Habit** | 1 | 12070377 | 12070377 | 19.75 | **0.000** |
| Light level*Water availability*Genus | 1 | 28877 | 28877 | 0.05 | 0.831 |
| Light level*Genus*Habit | 1 | 144882 | 144882 | 0.24 | 0.633 |
| Water availability*Genus*Habit | 1 | 185 | 185 | 0.00 | 0.986 |
| Residuals | 17 | 10390345 | 611197 | | |

Table A2.50 Analysis of variance table for the pigment ***Chlorophyll a in µmol per m$^{-2}$ leaf area***, samples taken in the glasshouse during late afternoon in June 2002. Light level (100% vs 75% of natural daylight), water availability (200 mls every 2 days and 50mls every 3 days) and genus (*Corokia cotoneaster, Coprosma propinqua, Corokia buddleioide* and *Coprosma robusta*) as treatments. Leaves taken from divaricates *Corokia cotoneaster* and *Coprosma propinqua* and non-divaricates *Corokia buddleioides* and *Coprosma robusta*. [significant results in bold].

| | Df | Sum of Sq | Mean Sq | F Value | Pr(F) |
|---|---|---|---|---|---|
| Light level | 1 | 40540599 | 40540599 | 1.90 | 0.186 |
| Water availability | 1 | 78838355 | 78838355 | 3.70 | 0.071 |
| **Genus** | 1 | 178267814 | 178267814 | 8.36 | **0.010** |
| **Habit** | 1 | 550895148 | 550895148 | 25.84 | **0.000** |
| Light level*Water availability | 1 | 7575501 | 7575501 | 0.36 | 0.559 |
| Light level*Genus | 1 | 21875745 | 21875745 | 1.03 | 0.325 |
| Water availability*Genus | 1 | 38792315 | 38792315 | 1.82 | 0.195 |
| Light level*Habit | 1 | 32486606 | 32486606 | 1.52 | 0.234 |
| Water availability*Habit | 1 | 4295379 | 4295379 | 0.20 | 0.659 |
| **Genus*Habit** | 1 | 309085769 | 309085769 | 14.50 | **0.001** |
| Light level*Water availability*Genus | 1 | 98241 | 98241 | 0.01 | 0.947 |
| Light level*Genus*Habit | 1 | 4790962 | 4790962 | 0.23 | 0.642 |
| Water availability*Genus*Habit | 1 | 767756 | 767756 | 0.04 | 0.852 |
| Residuals | 17 | 362468941 | 21321702 | | |

Table A2.51 Analysis of variance table for the pigment ***Chlorophyll b in µmol per m$^{-2}$ leaf area***, samples taken in the glasshouse during late afternoon in June 2002. Light level (100% vs 75% of natural daylight), water availability (200 mls every 2 days and 50mls every 3 days) and genus (*Corokia cotoneaster, Coprosma propinqua, Corokia buddleioide* and *Coprosma robusta*) as treatments. Leaves taken from divaricates *Corokia cotoneaster* and *Coprosma propinqua* and non-divaricates *Corokia buddleioides* and *Coprosma robusta*. [significant results in bold].

| | Df | Sum of Sq | Mean Sq | F Value | Pr(F) |
|---|---|---|---|---|---|
| Light level | 1 | 6254616 | 6254616 | 1.98 | 0.177 |
| Water availability | 1 | 13770135 | 13770135 | 4.36 | 0.052 |
| **Genus** | 1 | 26293883 | 26293883 | 8.33 | **0.010** |
| **Habit** | 1 | 90165876 | 90165876 | 28.58 | **0.000** |
| Light level*Water availability | 1 | 774188 | 774188 | 0.25 | 0.627 |
| Light level*Genus | 1 | 3085976 | 3085976 | 0.98 | 0.337 |
| Water availability*Genus | 1 | 7492267 | 7492267 | 2.38 | 0.142 |
| Light level*Habit | 1 | 4906346 | 4906346 | 1.56 | 0.229 |
| Water availability*Habit | 1 | 1748832 | 1748832 | 0.55 | 0.467 |
| **Genus*Habit** | 1 | 49477375 | 49477375 | 15.68 | **0.001** |
| Light level*Water availability*Genus | 1 | 4197 | 4197 | 0.00 | 0.971 |
| Light level*Genus*Habit | 1 | 512515 | 512515 | 0.16 | 0.692 |
| Water availability*Genus*Habit | 1 | 332532 | 332532 | 0.11 | 0.749 |
| Residuals | 17 | 53638061 | 3155180 | | |

Table A2.52 Analysis of variance table for the pigment ***Chlorophyll a+b in µmol per m$^{-2}$ leaf area***, samples taken in the glasshouse during late afternoon in June 2002. Light level (100% vs 75% of natural daylight), water availability (200 mls every 2 days and 50mls every 3 days) and genus (*Corokia cotoneaster, Coprosma propinqua, Corokia buddleioide* and *Coprosma robusta*) as treatments. Leaves taken from divaricates *Corokia cotoneaster* and *Coprosma propinqua* and non-divaricates *Corokia buddleioides* and *Coprosma robusta*. [significant results in bold].

| | Df | Sum of Sq | Mean Sq | F Value | Pr(F) |
|---|---|---|---|---|---|
| Light level | 1 | 78642721 | 78642721 | 1.97 | 0.178 |
| Water availability | 1 | 158505828 | 158505828 | 3.98 | 0.063 |
| **Genus** | 1 | 341490189 | 341490189 | 8.56 | **0.009** |
| **Habit** | 1 | 1086805093 | 1086805093 | 27.25 | **0.000** |
| Light level*Water availability | 1 | 13193186 | 13193186 | 0.33 | 0.573 |
| Light level*Genus | 1 | 41394375 | 41394375 | 1.04 | 0.323 |
| Water availability*Genus | 1 | 80381054 | 80381054 | 2.02 | 0.174 |
| Light level*Habit | 1 | 62642945 | 62642945 | 1.57 | 0.227 |
| Water availability*Habit | 1 | 11525780 | 11525780 | 0.29 | 0.598 |
| **Genus*Habit** | 1 | 605890882 | 605890882 | 15.19 | **0.001** |
| Light level*Water availability*Genus | 1 | 61828 | 61828 | 0.00 | 0.969 |
| Light level*Genus*Habit | 1 | 8437448 | 8437448 | 0.21 | 0.651 |
| Water availability*Genus*Habit | 1 | 2110839 | 2110839 | 0.05 | 0.821 |
| Residuals | 17 | 678086283 | 39887428 | | |

Table A2.53 Analysis of variance table for the pigment ***Chlorophyll a:b in µmol per m$^{-2}$ leaf area***, samples taken in the glasshouse during late afternoon in June 2002. Light level (100% vs 75% of natural daylight), water availability (200 mls every 2 days and 50mls every 3 days) and genus (*Corokia cotoneaster, Coprosma propinqua, Corokia buddleioide* and *Coprosma robusta*) as treatments. Leaves taken from divaricates *Corokia cotoneaster* and *Coprosma propinqua* and non-divaricates *Corokia buddleioides* and *Coprosma robusta*. [significant results in bold].

| | Df | Sum of Sq | Mean Sq | F Value | Pr(F) |
|---|---|---|---|---|---|
| Light level | 1 | 0.057978 | 0.0579779 | 0.75 | 0.400 |
| Water availability | 1 | 0.054547 | 0.0545472 | 0.70 | 0.414 |
| **Genus** | 1 | 0.910634 | 0.9106336 | 11.70 | **0.003** |
| Habit | 1 | 0.043805 | 0.0438046 | 0.56 | 0.463 |
| Light level*Water availability | 1 | 0.002783 | 0.0027833 | 0.03 | 0.852 |
| Light level*Genus | 1 | 0.001039 | 0.0010393 | 0.01 | 0.909 |
| Water availability*Genus | 1 | 0.082666 | 0.0826661 | 1.06 | 0.317 |
| Light level*Habit | 1 | 0.042210 | 0.0422096 | 0.54 | 0.472 |
| Water availability*Habit | 1 | 0.028911 | 0.0289114 | 0.37 | 0.550 |
| Genus*Habit | 1 | 0.025729 | 0.0257294 | 0.33 | 0.573 |
| Light level*Water availability*Genus | 1 | 0.005550 | 0.0055504 | 0.07 | 0.793 |
| Light level*Genus*Habit | 1 | 0.000346 | 0.0003457 | 0.00 | 0.948 |
| Water availability*Genus*Habit | 1 | 0.149794 | 0.1497937 | 1.93 | 0.183 |
| Residuals | 17 | 1.322730 | 0.0778077 | | |

Table A2.54 Analysis of variance table for the pigment ***ß-Carotene in µmol per m$^{-2}$ leaf area***, samples taken in the glasshouse during late afternoon in June 2002. Light level (100% vs 75% of natural daylight), water availability (200 mls every 2 days and 50mls every 3 days) and genus (*Corokia cotoneaster, Coprosma propinqua, Corokia buddleioide* and *Coprosma robusta*) as treatments. Leaves taken from divaricates *Corokia cotoneaster* and *Coprosma propinqua* and non-divaricates *Corokia buddleioides* and *Coprosma robusta*. [significant results in bold].

| | Df | Sum of Sq | Mean Sq | F Value | Pr(F) |
|---|---|---|---|---|---|
| Light level | 1 | 34222.8 | 34222.8 | 0.80 | 0.383 |
| Water availability | 1 | 27560.8 | 27560.8 | 0.65 | 0.433 |
| **Genus** | 1 | 274283.2 | 274283.2 | 6.42 | **0.021** |
| **Habit** | 1 | 671352.4 | 671352.4 | 15.72 | **0.001** |
| Light level*Water availability | 1 | 34871.9 | 34871.9 | 0.82 | 0.379 |
| Light level*Genus | 1 | 26398.4 | 26398.4 | 0.62 | 0.443 |
| Water availability*Genus | 1 | 12901.0 | 12901.0 | 0.30 | 0.590 |
| Light level*Habit | 1 | 36008.9 | 36008.9 | 0.84 | 0.371 |
| Water availability*Habit | 1 | 22695.0 | 22695.0 | 0.53 | 0.476 |
| **Genus*Habit** | 1 | 421334.6 | 421334.6 | 9.87 | **0.006** |
| Light level*Water availability*Genus | 1 | 8163.2 | 8163.2 | 0.19 | 0.667 |
| Light level*Genus*Habit | 1 | 10227.1 | 10227.1 | 0.24 | 0.631 |
| Water availability*Genus*Habit | 1 | 14068.1 | 14068.1 | 0.33 | 0.574 |
| Residuals | 17 | 725847.1 | 42696.9 | | |

Table A2.55 Analysis of variance table for ***vitamin E in µmol per m$^{-2}$ leaf area***, samples taken in the glasshouse during late afternoon in June 2002. Light level (100% vs 75% of natural daylight), water availability (200 mls every 2 days and 50mls every 3 days) and genus (*Corokia cotoneaster*, *Coprosma propinqua*, *Corokia buddleioide* and *Coprosma robusta*) as treatments. Leaves taken from divaricates *Corokia cotoneaster* and *Coprosma propinqua* and non-divaricates *Corokia buddleioides* and *Coprosma robusta*. [significant results in bold].

| | Df | Sum of Sq | Mean Sq | F Value | Pr(F) |
|---|---|---|---|---|---|
| **Light level** | 1 | 143 | 143 | 5.87 | **0.020** |
| **Water availability** | 1 | 146 | 146 | 6.01 | **0.018** |
| **Genus** | 1 | 1229 | 1229 | 50.60 | **0.000** |
| **Habit** | 1 | 3462 | 3462 | 142.58 | **0.000** |
| **Light level*Water availability** | 1 | 169 | 169 | 6.96 | **0.012** |
| Light level*Genus | 1 | 0 | 0 | 0.01 | 0.911 |
| Water availability*Genus | 1 | 96 | 96 | 3.95 | 0.053 |
| **Light level*Habit** | 1 | 117 | 117 | 4.82 | **0.034** |
| Water availability*Habit | 1 | 57 | 57 | 2.34 | 0.133 |
| **Genus*Habit** | 1 | 1833 | 1833 | 75.51 | **0.000** |
| Light level*Water availability*Genus | 1 | 45 | 45 | 1.84 | 0.182 |
| **Light level*Water availability*Habit** | 1 | 157 | 157 | 6.47 | **0.015** |
| Light level*Genus*Habit | 1 | 12 | 12 | 0.49 | 0.490 |
| Water availability*Genus*Habit | 1 | 12 | 12 | 0.49 | 0.490 |
| **Light level*Water availability*Genus* Habit** | 1 | 109 | 109 | 4.50 | **0.040** |
| Residuals | 43 | 1044 | 24 | | |

Table A2.56 Analysis of variance table for the pigment ***Violaxanthin in nmol per g fresh weight***, samples taken in the glasshouse during late afternoon in June 2002. Light level (100% vs 75% of natural daylight), water availability (200 mls every 2 days and 50mls every 3 days) and genus (*Corokia cotoneaster, Coprosma propinqua, Corokia buddleioide* and *Coprosma robusta*) as treatments. Leaves taken from divaricates *Corokia cotoneaster* and *Coprosma propinqua* and non-divaricates *Corokia buddleioides* and *Coprosma robusta*. [significant results in bold].

| | Df | Sum of Sq | Mean Sq | F Value | Pr(F) |
|---|---|---|---|---|---|
| Light level | 1 | 705.401 | 705.401 | 1.50 | 0.237 |
| Water availability | 1 | 499.192 | 499.192 | 1.06 | 0.317 |
| Genus | 1 | 266.234 | 266.234 | 0.57 | 0.462 |
| Habit | 1 | 284.847 | 284.847 | 0.60 | 0.447 |
| **Light level*Water availability** | 1 | 2681.832 | 2681.832 | 5.69 | **0.028** |
| Light level*Genus | 1 | 541.332 | 541.332 | 1.15 | 0.298 |
| Water availability*Genus | 1 | 817.729 | 817.729 | 1.73 | 0.204 |
| **Light level*Habit** | 1 | 2188.689 | 2188.689 | 4.64 | **0.045** |
| Water availability*Habit | 1 | 146.425 | 146.425 | 0.31 | 0.584 |
| **Genus*Habit** | 1 | 2795.550 | 2795.550 | 5.93 | **0.026** |
| Light level*Water availability*Genus | 1 | 922.195 | 922.195 | 1.96 | 0.179 |
| Light level*Genus*Habit | 1 | 151.771 | 151.771 | 0.32 | 0.577 |
| Water availability*Genus*Habit | 1 | 284.883 | 284.883 | 0.60 | 0.447 |
| Residuals | 18 | 8484.365 | 471.354 | | |

Table A2.57 Analysis of variance table for the pigment ***Antheraxanthin in nmol per g fresh weight***, samples taken in the glasshouse during late afternoon in June 2002. Light level (100% vs 75% of natural daylight), water availability (200 mls every 2 days and 50mls every 3 days) and genus (*Corokia cotoneaster, Coprosma propinqua, Corokia buddleioide* and *Coprosma robusta*) as treatments. Leaves taken from divaricates *Corokia cotoneaster* and *Coprosma propinqua* and non-divaricates *Corokia buddleioides* and *Coprosma robusta*. [significant results in bold].

| | Df | Sum of Sq | Mean Sq | F Value | Pr(F) |
|---|---|---|---|---|---|
| Light level | 1 | 16.8753 | 16.87529 | 0.32 | 0.576 |
| Water availability | 1 | 24.2054 | 24.20537 | 0.47 | 0.504 |
| Genus | 1 | 59.3162 | 59.31621 | 1.14 | 0.299 |
| Habit | 1 | 69.1654 | 69.16539 | 1.33 | 0.264 |
| Light level*Water availability | 1 | 8.2107 | 8.21071 | 0.16 | 0.696 |
| Light level*Genus | 1 | 12.9890 | 12.98901 | 0.25 | 0.623 |
| Water availability*Genus | 1 | 20.3129 | 20.31289 | 0.39 | 0.540 |
| Light level*Habit | 1 | 0.9389 | 0.93886 | 0.02 | 0.895 |
| Water availability*Habit | 1 | 12.5158 | 12.51583 | 0.24 | 0.630 |
| Genus*Habit | 1 | 63.2667 | 63.26665 | 1.21 | 0.284 |
| Light level*Water availability*Genus | 1 | 3.5311 | 3.53110 | 0.07 | 0.797 |
| Light level*Genus*Habit | 1 | 0.0223 | 0.02231 | 0.00 | 0.984 |
| Water availability*Genus*Habit | 1 | 0.8649 | 0.86489 | 0.02 | 0.899 |
| Residuals | 18 | 935.1839 | 51.95466 | | |

Table A2.58 Analysis of variance table for the pigment ***Zeaxanthin in nmol per g fresh weight***, samples taken in the glasshouse during late afternoon in June 2002. Light level (100% vs 75% of natural daylight), water availability (200 mls every 2 days and 50mls every 3 days) and genus (*Corokia cotoneaster, Coprosma propinqua, Corokia buddleioide* and *Coprosma robusta*) as treatments. Leaves taken from divaricates *Corokia cotoneaster* and *Coprosma propinqua* and non-divaricates *Corokia buddleioides* and *Coprosma robusta*. [significant results in bold].

| | Df | Sum of Sq | Mean Sq | F Value | Pr(F) |
|---|---|---|---|---|---|
| Light level | 1 | 437.991 | 437.9908 | 0.89 | 0.357 |
| Water availability | 1 | 395.010 | 395.0099 | 0.81 | 0.382 |
| Genus | 1 | 451.402 | 451.4024 | 0.92 | 0.350 |
| Habit | 1 | 411.502 | 411.5017 | 0.84 | 0.372 |
| Light level*Water availability | 1 | 71.659 | 71.6591 | 0.15 | 0.707 |
| Light level*Genus | 1 | 295.288 | 295.2877 | 0.60 | 0.448 |
| Water availability*Genus | 1 | 430.402 | 430.4015 | 0.88 | 0.362 |
| Light level*Habit | 1 | 120.954 | 120.9544 | 0.25 | 0.626 |
| Water availability*Habit | 1 | 265.292 | 265.2921 | 0.54 | 0.472 |
| Genus*Habit | 1 | 309.475 | 309.4755 | 0.63 | 0.438 |
| Light level*Water availability*Genus | 1 | 119.285 | 119.2854 | 0.240 | 0.628 |
| Light level*Genus*Habit | 1 | 57.516 | 57.5165 | 0.12 | 0.736 |
| Water availability*Genus*Habit | 1 | 302.227 | 302.2273 | 0.62 | 0.443 |
| Residuals | 18 | 8836.357 | 490.9087 | | |

Table A2.59 Analysis of variance table for the pigment ***Neoxanthin in nmol per g fresh weight***, samples taken in the glasshouse during late afternoon in June 2002. Light level (100% vs 75% of natural daylight), water availability (200 mls every 2 days and 50mls every 3 days) and genus (*Corokia cotoneaster, Coprosma propinqua, Corokia buddleioide* and *Coprosma robusta*) as treatments. Leaves taken from divaricates *Corokia cotoneaster* and *Coprosma propinqua* and non-divaricates *Corokia buddleioides* and *Coprosma robusta*. [significant results in bold].

| | Df | Sum of Sq | Mean Sq | F Value | Pr(F) |
|---|---|---|---|---|---|
| Light level | 1 | 162.571 | 162.5710 | 1.22 | 0.283 |
| Water availability | 1 | 179.053 | 179.0534 | 1.35 | 0.261 |
| Genus | 1 | 16.786 | 16.7863 | 0.13 | 0.726 |
| Habit | 1 | 60.984 | 60.9839 | 0.46 | 0.507 |
| Light level*Water availability | 1 | 256.698 | 256.6983 | 1.93 | 0.182 |
| Light level*Genus | 1 | 109.128 | 109.1277 | 0.82 | 0.377 |
| Water availability*Genus | 1 | 95.944 | 95.9435 | 0.72 | 0.407 |
| Light level*Habit | 1 | 0.007 | 0.0066 | 0.00 | 0.994 |
| Water availability*Habit | 1 | 0.848 | 0.8479 | 0.01 | 0.937 |
| Genus*Habit | 1 | 7.984 | 7.9838 | 0.06 | 0.809 |
| Light level*Water availability*Genus | 1 | 109.523 | 109.5232 | 0.82 | 0.376 |
| Light level*Genus*Habit | 1 | 2.270 | 2.2698 | 0.02 | 0.898 |
| Water availability*Genus*Habit | 1 | 7.679 | 7.6794 | 0.06 | 0.813 |
| Residuals | 18 | 2392.108 | 132.8949 | | |

Table A2.60 Analysis of variance table for the pigment ***Lutein in nmol per g fresh weight***, samples taken in the glasshouse during late afternoon in June 2002. Light level (100% vs 75% of natural daylight), water availability (200 mls every 2 days and 50mls every 3 days) and genus (*Corokia cotoneaster, Coprosma propinqua, Corokia buddleioide* and *Coprosma robusta*) as treatments. Leaves taken from divaricates *Corokia cotoneaster* and *Coprosma propinqua* and non-divaricates *Corokia buddleioides* and *Coprosma robusta*. [significant results in bold].

| | Df | Sum of Sq | Mean Sq | F Value | Pr(F) |
|---|---|---|---|---|---|
| Light level | 1 | 11798.2 | 11798.21 | 2.03 | 0.172 |
| Water availability | 1 | 3293.1 | 3293.14 | 0.57 | 0.462 |
| Genus | 1 | 1489.4 | 1489.38 | 0.26 | 0.619 |
| Habit | 1 | 3614.0 | 3613.97 | 0.62 | 0.441 |
| **Light level*Water availability** | 1 | 34929.3 | 34929.25 | 6.00 | **0.025** |
| Light level*Genus | 1 | 7707.3 | 7707.32 | 1.32 | 0.265 |
| Water availability*Genus | 1 | 10236.9 | 10236.87 | 1.76 | 0.202 |
| **Light level*Habit** | 1 | 39248.1 | 39248.09 | 6.74 | **0.018** |
| Water availability*Habit | 1 | 2185.3 | 2185.26 | 0.38 | 0.548 |
| **Genus*Habit** | 1 | 29871.1 | 29871.07 | 5.13 | **0.036** |
| Light level*Water availability*Genus | 1 | 14092.4 | 14092.39 | 2.42 | 0.137 |
| Light level*Genus*Habit | 1 | 822.5 | 822.55 | 0.14 | 0.712 |
| Water availability*Genus*Habit | 1 | 9633.9 | 9633.93 | 1.66 | 0.215 |
| Residuals | 18 | 104840.0 | 5824.44 | | |

Table A2.61 Analysis of variance table for the pigment ***Chlorophyll a in nmol per g fresh weight***, samples taken in the glasshouse during late afternoon in June 2002. Light level (100% vs 75% of natural daylight), water availability (200 mls every 2 days and 50mls every 3 days) and genus (*Corokia cotoneaster, Coprosma propinqua, Corokia buddleioide* and *Coprosma robusta*) as treatments. Leaves taken from divaricates *Corokia cotoneaster* and *Coprosma propinqua* and non-divaricates *Corokia buddleioides* and *Coprosma robusta*. [significant results in bold].

| | Df | Sum of Sq | Mean Sq | F Value | Pr(F) |
|---|---|---|---|---|---|
| Light level | 1 | 542699 | 542699 | 2.52 | 0.130 |
| Water availability | 1 | 93866 | 93866 | 0.43 | 0.517 |
| Genus | 1 | 2400 | 2400 | 0.01 | 0.917 |
| Habit | 1 | 213776 | 213776 | 0.99 | 0.332 |
| Light level*Water availability | 1 | 691955 | 691955 | 3.22 | 0.090 |
| Light level*Genus | 1 | 431536 | 431536 | 2.01 | 0.174 |
| Water availability*Genus | 1 | 477057 | 477057 | 2.22 | 0.154 |
| **Light level*Habit** | 1 | 1957938 | 1957938 | 9.11 | **0.007** |
| Water availability*Habit | 1 | 364016 | 364016 | 1.69 | 0.210 |
| **Genus*Habit** | 1 | 1400845 | 1400845 | 6.52 | **0.020** |
| Light level*Water availability*Genus | 1 | 237442 | 237442 | 1.10 | 0.307 |
| Light level*Genus*Habit | 1 | 959 | 959 | 0.00 | 0.948 |
| Water availability*Genus*Habit | 1 | 624564 | 624564 | 2.90 | 0.106 |
| Residuals | 18 | 3870773 | 215043 | | |

Table A2.62 Analysis of variance table for the pigment ***Chlorophyll b in nmol per g fresh weight***, samples taken in the glasshouse during late afternoon in June 2002. Light level (100% vs 75% of natural daylight), water availability (200 mls every 2 days and 50mls every 3 days) and genus (*Corokia cotoneaster, Coprosma propinqua, Corokia buddleioide* and *Coprosma robusta*) as treatments. Leaves taken from divaricates *Corokia cotoneaster* and *Coprosma propinqua* and non-divaricates *Corokia buddleioides* and *Coprosma robusta*. [significant results in bold].

| | Df | Sum of Sq | Mean Sq | F Value | Pr(F) |
|---|---|---|---|---|---|
| Light level | 1 | 92081.3 | 92081.3 | 2.82 | 0.110 |
| Water availability | 1 | 5299.5 | 5299.5 | 0.16 | 0.692 |
| Genus | 1 | 12078.7 | 12078.7 | 0.37 | 0.550 |
| Habit | 1 | 21378.9 | 21378.9 | 0.66 | 0.429 |
| Light level*Water availability | 1 | 101832.7 | 101832.7 | 3.12 | 0.094 |
| Light level*Genus | 1 | 52535.8 | 52535.8 | 1.61 | 0.221 |
| Water availability*Genus | 1 | 83212.0 | 83212.0 | 2.55 | 0.128 |
| **Light level*Habit** | 1 | 278650.6 | 278650.6 | 8.54 | **0.009** |
| Water availability*Habit | 1 | 35405.7 | 35405.7 | 1.09 | 0.311 |
| **Genus*Habit** | 1 | 189558.7 | 189558.7 | 5.81 | **0.027** |
| Light level*Water availability*Genus | 1 | 49740.9 | 49740.9 | 1.53 | 0.233 |
| Light level*Genus*Habit | 1 | 1.1 | 1.1 | 0.00 | 0.995 |
| Water availability*Genus*Habit | 1 | 86731.5 | 86731.5 | 2.66 | 0.120 |
| Residuals | 18 | 587110.9 | 32617.3 | | |

Table A2.63 Analysis of variance table for the pigment ***Chlorophyll a+b in nmol per g fresh weight***, samples taken in the glasshouse during late afternoon in June 2002. Light level (100% vs 75% of natural daylight), water availability (200 mls every 2 days and 50mls every 3 days) and genus (*Corokia cotoneaster, Coprosma propinqua, Corokia buddleioide* and *Coprosma robusta*) as treatments. Leaves taken from divaricates *Corokia cotoneaster* and *Coprosma propinqua* and non-divaricates *Corokia buddleioides* and *Coprosma robusta*. [significant results in bold].

| | Df | Sum of Sq | Mean Sq | F Value | Pr(F) |
|---|---|---|---|---|---|
| Light level | 1 | 1081871 | 1081871 | 2.63 | 0.123 |
| Water availability | 1 | 143773 | 143773 | 0.35 | 0.562 |
| Genus | 1 | 25247 | 25247 | 0.06 | 0.807 |
| Habit | 1 | 370363 | 370363 | 0.90 | 0.356 |
| Light level*Water availability | 1 | 1324688 | 1324688 | 3.22 | 0.090 |
| Light level*Genus | 1 | 785210 | 785210 | 1.91 | 0.184 |
| Water availability*Genus | 1 | 958750 | 958750 | 2.33 | 0.145 |
| **Light level*Habit** | 1 | 3713858 | 3713858 | 9.01 | **0.008** |
| Water availability*Habit | 1 | 626475 | 626475 | 1.52 | 0.233 |
| **Genus*Habit** | 1 | 2621020 | 2621020 | 6.36 | **0.021** |
| Light level*Water availability*Genus | 1 | 504535 | 504535 | 1.23 | 0.283 |
| Light level*Genus*Habit | 1 | 895 | 895 | 0.00 | 0.963 |
| Water availability*Genus*Habit | 1 | 1176782 | 1176782 | 2.86 | 0.108 |
| Residuals | 18 | 7415805 | 411989 | | |

Table A2.64 Analysis of variance table for the pigment ***Chlorophyll a:b in nmol per g fresh weight***, samples taken in the glasshouse during late afternoon in June 2002. Light level (100% vs 75% of natural daylight), water availability (200 mls every 2 days and 50mls every 3 days) and genus (*Corokia cotoneaster, Coprosma propinqua, Corokia buddleioide* and *Coprosma robusta*) as treatments. Leaves taken from divaricates *Corokia cotoneaster* and *Coprosma propinqua* and non-divaricates *Corokia buddleioides* and *Coprosma robusta*. [significant results in bold].

| | Df | Sum of Sq | Mean Sq | F Value | Pr(F) |
|---|---|---|---|---|---|
| Light level | 1 | 0.001679 | 0.0016788 | 0.01 | 0.922 |
| Water availability | 1 | 0.240011 | 0.2400108 | 1.41 | 0.251 |
| Genus | 1 | 0.530950 | 0.5309497 | 3.11 | 0.095 |
| Habit | 1 | 0.006675 | 0.0066754 | 0.04 | 0.845 |
| Light level*Water availability | 1 | 0.060083 | 0.0600826 | 0.35 | 0.560 |
| Light level*Genus | 1 | 0.035911 | 0.0359105 | 0.21 | 0.652 |
| Water availability*Genus | 1 | 0.000179 | 0.0001786 | 0.00 | 0.975 |
| Light level*Habit | 1 | 0.140799 | 0.1407988 | 0.83 | 0.376 |
| Water availability*Habit | 1 | 0.223376 | 0.2233762 | 1.31 | 0.267 |
| Genus*Habit | 1 | 0.003104 | 0.0031035 | 0.02 | 0.894 |
| Light level*Water availability*Genus | 1 | 0.135330 | 0.1353305 | 0.79 | 0.385 |
| Light level*Genus*Habit | 1 | 0.023745 | 0.0237454 | 0.14 | 0.713 |
| Water availability*Genus*Habit | 1 | 0.017629 | 0.0176294 | 0.10 | 0.752 |
| Residuals | 18 | 3.070108 | 0.1705616 | | |

Table A2.65 Analysis of variance table for the pigment ***ß-carotene in nmol per g fresh weight***, samples taken in the glasshouse during late afternoon in June 2002. Light level (100% vs 75% of natural daylight), water availability (200 mls every 2 days and 50mls every 3 days) and genus (*Corokia cotoneaster, Coprosma propinqua, Corokia buddleioide* and *Coprosma robusta*) as treatments. Leaves taken from divaricates *Corokia cotoneaster* and *Coprosma propinqua* and non-divaricates *Corokia buddleioides* and *Coprosma robusta*. [significant results in bold].

| | Df | Sum of Sq | Mean Sq | F Value | Pr(F) |
|---|---|---|---|---|---|
| Light level | 1 | 241.933 | 241.933 | 1.14 | 0.300 |
| Water availability | 1 | 3.155 | 3.155 | 0.02 | 0.904 |
| Genus | 1 | 360.076 | 360.076 | 1.70 | 0.209 |
| Habit | 1 | 322.830 | 322.830 | 1.52 | 0.233 |
| **Light level*Water availability** | 1 | 1077.435 | 1077.435 | 5.08 | **0.037** |
| Light level*Genus | 1 | 278.653 | 278.653 | 1.31 | 0.267 |
| Water availability*Genus | 1 | 167.050 | 167.050 | 0.79 | 0.386 |
| Light level*Habit | 1 | 888.111 | 888.111 | 4.19 | 0.056 |
| Water availability*Habit | 1 | 155.822 | 155.822 | 0.73 | 0.403 |
| Genus*Habit | 1 | 871.460 | 871.460 | 4.11 | 0.058 |
| Light level*Water availability*Genus | 1 | 400.778 | 400.778 | 1.89 | 0.186 |
| Light level*Genus*Habit | 1 | 0.102 | 0.102 | 0.00 | 0.983 |
| Water availability*Genus*Habit | 1 | 854.426 | 854.426 | 4.03 | 0.060 |
| Residuals | 18 | 3816.378 | 212.021 | | |

Table A2.66 Analysis of variance table for ***vitamin E in µmol per g fresh weight***, samples taken in the glasshouse during late afternoon in June 2002. Light level (100% vs 75% of natural daylight), water availability (200 mls every 2 days and 50mls every 3 days) and genus (*Corokia cotoneaster*, *Coprosma propinqua*, *Corokia buddleioide* and *Coprosma robusta*) as treatments. Leaves taken from divaricates *Corokia cotoneaster* and *Coprosma propinqua* and non-divaricates *Corokia buddleioides* and *Coprosma robusta*. [significant results in bold].

| | Df | Sum of Sq | Mean Sq | F Value | Pr(F) |
|---|---|---|---|---|---|
| Light level | 1 | 234 | 234 | 0.06 | 0.815 |
| **Water availability** | 1 | 21793 | 21793 | 5.16 | **0.028** |
| Genus | 1 | 3276 | 3276 | 0.78 | 0.383 |
| Habit | 1 | 15168 | 15168 | 3.59 | 0.065 |
| Light level*Water availability | 1 | 1739 | 1739 | 0.41 | 0.525 |
| Light level*Genus | 1 | 950 | 950 | 0.23 | 0.638 |
| Water availability*Genus | 1 | 9536 | 9536 | 2.26 | 0.140 |
| Light level*Habit | 1 | 5383 | 5383 | 1.28 | 0.265 |
| Water availability*Habit | 1 | 64 | 64 | 0.02 | 0.903 |
| Genus*Habit | 1 | 5917 | 5917 | 1.40 | 0.243 |
| Light level*Water availability*Genus | 1 | 2474 | 2474 | 0.59 | 0.448 |
| Light level*Water availability*Habit | 1 | 156 | 156 | 0.04 | 0.848 |
| Light level*Genus*Habit | 1 | 456 | 456 | 0.11 | 0.744 |
| Water availability*Genus*Habit | 1 | 13422 | 13422 | 3.18 | 0.082 |
| Light level*Water availability*Genus*Habit | 1 | 7016 | 7016 | 1.66 | 0.204 |
| Residuals | 43 | 181604 | 4223 | | |

## A3 Anova Tables of Glasshouse Data 2003

Table A3.1 Analysis of variance table for water potential in the glasshouse, (a) predawn, (b) noon and (c) evening measurements during January and predawn (d) December 2003. Light level (100% vs 75% of natural daylight), water availability (200 mls every 2.day and 50mls every 3.day) and habit (*Corokia cotoneaster, Corokia buddleioides* and *Corokia* hybrid) as treatments. Measurements recorded from shoots of *Corokia cotoneaster* and *Corokia* hybrid; leaves of *Corokia buddleioides*. [significant results in bold].

| a | Df | Sum of Sq | Mean Sq | F Value | Pr(F) |
|---|---|---|---|---|---|
| Light level | 1 | 0.09455 | 0.09455 | 0.30 | 0.5910 |
| **Water availability** | 1 | 13.09937 | 13.09937 | 40.89 | **0.0000** |
| Habit | 2 | 1.71015 | 0.85508 | 2.67 | 0.0858 |
| Light level*Water availability | 1 | 0.24831 | 0.24831 | 0.78 | 0.3857 |
| Light level*Habit | 2 | 0.14406 | 0.07203 | 0.22 | 0.800 |
| **Water availability*Habit** | 2 | 2.37042 | 1.18521 | 3.70 | **0.037** |
| Light level*Water availability*Habit | 2 | 0.28553 | 0.14276 | 0.45 | 0.645 |
| Residuals | 30 | 9.61167 | 0.32039 | | |

| b | Df | Sum of Sq | Mean Sq | F Value | Pr(F) |
|---|---|---|---|---|---|
| Light level | 1 | 0.010962 | 0.010962 | 0.10 | 0.756 |
| **Water availability** | 1 | 3.250680 | 3.250680 | 29.27 | **0.000** |
| **Habit** | 2 | 1.415164 | 0.707582 | 6.37 | **0.006** |
| Light level*Water availability | 1 | 0.102774 | 0.102774 | 0.93 | 0.346 |
| Light level*Habit | 2 | 0.336102 | 0.168051 | 1.51 | 0.240 |
| **Water availability*Habit** | 2 | 1.759296 | 0.879648 | 7.92 | **0.002** |
| Light level*Water availability*Habit | 2 | 0.233094 | 0.116547 | 1.05 | 0.366 |
| Residuals | 24 | 2.665358 | 0.111057 | | |

| c | Df | Sum of Sq | Mean Sq | F Value | Pr(F) |
|---|---|---|---|---|---|
| **Light level** | 1 | 1.64948 | 1.64948 | 28.26 | **0.000** |
| **Water availability** | 1 | 14.50516 | 14.50516 | 248.53 | **0.000** |
| **Habit** | 2 | 1.09897 | 0.54948 | 9.41 | **0.001** |
| **Light level*Water availability** | 1 | 0.84480 | 0.84480 | 14.47 | **0.001** |
| Light level*Habit | 2 | 0.09403 | 0.04701 | 0.81 | 0.457 |
| **Water availability*Habit** | 2 | 3.09344 | 1.54672 | 26.50 | **0.000** |
| Light level*Water availability*Habit | 2 | 0.12072 | 0.06036 | 1.03 | 0.369 |
| Residuals | 27 | 1.57583 | 0.05836 | | |

| d | Df | Sum of Sq | Mean Sq | F Value | Pr(F) |
|---|---|---|---|---|---|
| Light level | 1 | 0.019114 | 0.019114 | 0.58 | 0.451 |
| **Water availability** | 1 | 0.715106 | 0.715106 | 21.86 | **0.000** |
| **Habit** | 2 | 2.915456 | 1.457728 | 44.56 | **0.000** |
| **Light level*Water availability** | 1 | 0.205066 | 0.205066 | 6.27 | **0.018** |
| **Light level*Habit** | 2 | 0.254102 | 0.127051 | 3.88 | **0.032** |
| Water availability*Habit | 2 | 0.213783 | 0.106891 | 3.27 | 0.053 |
| **Light level*Water availability*Habit** | 2 | 0.574082 | 0.287041 | 8.77 | **0.001** |
| Residuals | 28 | 0.916042 | 0.032716 | | |

Table A3.2 Analysis of variance table for $F_v/F_m$ in the glasshouse, (a) predawn, (b) noon and (c) evening measurements during January and predawn (d) December 2003. Light level (100% vs 75% of natural daylight), water availability (200 mls every 2 days and 50mls every 3 days) and habit (*Corokia cotoneaster, Corokia buddleioides* and *Corokia* hybrid) as treatments. Measurements recorded from shoots of *Corokia cotoneaster* and *Corokia* hybrid; leaves of *Corokia buddleioides*. [significant results in bold].

| a | Df | Sum of Sq | Mean Sq | F Value | Pr(F) |
|---|---|---|---|---|---|
| Light level | 1 | 0.00159087 | 0.001590869 | 0.54 | 0.467 |
| Water availability | 1 | 0.00179303 | 0.001793030 | 0.61 | 0.440 |
| Habit | 2 | 0.00566583 | 0.002832914 | 0.97 | 0.391 |
| Light level*Water availability | 1 | 0.00130664 | 0.001306641 | 0.45 | 0.509 |
| Light level*Habit | 2 | 0.01591078 | 0.007955392 | 2.72 | 0.083 |
| Water availability*Habit | 2 | 0.00785577 | 0.003927883 | 1.34 | 0.277 |
| Light level*Water availability*Habit | 2 | 0.00644846 | 0.003224230 | 1.10 | 0.345 |
| Residuals | 29 | 0.08476150 | 0.002922810 | | |

| b | Df | Sum of Sq | Mean Sq | F Value | Pr(F) |
|---|---|---|---|---|---|
| **Light level** | 1 | 0.1092025 | 0.1092025 | 7.40 | **0.011** |
| **Water availability** | 1 | 0.0745046 | 0.0745046 | 5.05 | **0.033** |
| Habit | 2 | 0.0133972 | 0.0066986 | 0.45 | 0.640 |
| Light level*Water availability | 1 | 0.0103553 | 0.0103553 | 0.70 | 0.409 |
| Light level*Habit | 2 | 0.0165287 | 0.0082644 | 0.56 | 0.577 |
| Water availability*Habit | 2 | 0.0870366 | 0.0435183 | 2.95 | 0.069 |
| Light level*Water availability*Habit | 2 | 0.0293458 | 0.0146729 | 1.00 | 0.383 |
| Residuals | 28 | 0.4129667 | 0.0147488 | | |

| c | Df | Sum of Sq | Mean Sq | F Value | Pr(F) |
|---|---|---|---|---|---|
| **Light level** | 1 | 0.06036107 | 0.06036107 | 50.48 | **0.000** |
| Water availability | 1 | 0.00004858 | 0.00004858 | 0.04 | 0.842 |
| **Habit** | 2 | 0.09165923 | 0.04582962 | 38.33 | **0.000** |
| Light level*Water availability | 1 | 0.00037842 | 0.00037842 | 0.32 | 0.578 |
| **Light level*Habit** | 2 | 0.05037779 | 0.02518889 | 21.07 | **0.000** |
| **Water availability*Habit** | 2 | 0.01506626 | 0.00753313 | 6.30 | **0.005** |
| **Light level*Water availability*Habit** | 2 | 0.00861702 | 0.00430851 | 3.60 | **0.040** |
| Residuals | 29 | 0.03467450 | 0.00119567 | | |

| d | Df | Sum of Sq | Mean Sq | F Value | Pr(F) |
|---|---|---|---|---|---|
| **Light level** | 1 | 0.00513023 | 0.005130225 | 5.32 | **0.029** |
| Water availability | 1 | 0.00130698 | 0.001306976 | 1.36 | 0.254 |
| Habit | 2 | 0.00247727 | 0.001238633 | 1.29 | 0.292 |
| Light level*Water availability | 1 | 0.00178583 | 0.001785834 | 1.85 | 0.184 |
| Light level*Habit | 2 | 0.00397889 | 0.001989447 | 2.06 | 0.146 |
| Water availability*Habit | 2 | 0.00227383 | 0.001136916 | 1.18 | 0.322 |
| Light level*Water availability*Habit | 2 | 0.00033693 | 0.000168466 | 0.17 | 0.841 |
| Residuals | 28 | 0.02698642 | 0.000963801 | | |

Table A3.3 Analysis of variance table for the pigment ***Violaxanthin in mmol per mol total Chlorophyll***, samples taken in the glasshouse during late afternoon in December 2003. Light level (100% vs 75% of natural daylight), water availability (200 mls every 2 days and 50mls every 3 days) and habit (*Corokia cotoneaster*, *Corokia* hybrid and *Corokia buddleioides*) as treatments. [significant results in bold].

| | Df | Sum of Sq | Mean Sq | F Value | Pr(F) |
|---|---|---|---|---|---|
| Water availability | 1 | 1.1723 | 1.1723 | 0.08 | 0.787 |
| **Habit** | 2 | 414.8057 | 207.4028 | 13.59 | **0.001** |
| Water availability*Habit | 2 | 2.2887 | 1.1443 | 0.08 | 0.928 |
| Residuals | 11 | 167.8711 | 15.2610 | | |

Table A3.4 Analysis of variance table for the pigment ***Antheraxanthin in mmol per mol total Chlorophyll***, samples taken in the glasshouse during late afternoon in December 2003. Light level (100% vs 75% of natural daylight), water availability (200 mls every 2 days and 50mls every 3 days) and habit (*Corokia cotoneaster*, *Corokia* hybrid and *Corokia buddleioides*) as treatments. [significant results in bold].

| | Df | Sum of Sq | Mean Sq | F Value | Pr(F) |
|---|---|---|---|---|---|
| Water availability | 1 | 0.717269 | 0.7172687 | 1.44 | 0.256 |
| Habit | 2 | 0.648321 | 0.3241607 | 0.65 | 0.541 |
| Water availability*Habit | 2 | 0.025172 | 0.0125858 | 0.03 | 0.975 |
| Residuals | 11 | 5.489250 | 0.4990227 | | |

Table A3.5 Analysis of variance table for the pigment ***Zeaxanthin in mmol per mol total Chlorophyll***, samples taken in the glasshouse during late afternoon in December 2003. Light level (100% vs 75% of natural daylight), water availability (200 mls every 2 days and 50mls every 3 days) and habit (*Corokia cotoneaster*, *Corokia* hybrid and *Corokia buddleioides*) as treatments. [significant results in bold].

| | Df | Sum of Sq | Mean Sq | F Value | Pr(F) |
|---|---|---|---|---|---|
| Water availability | 1 | 50.3741 | 50.37407 | 2.98 | 0.112 |
| Habit | 2 | 24.8716 | 12.43581 | 0.74 | 0.501 |
| Water availability*Habit | 2 | 18.8050 | 9.40249 | 0.56 | 0.589 |
| Residuals | 11 | 185.8599 | 16.89635 | | |

Table A3.6 Analysis of variance table for pigments in the ***Deepoxidation state in mmol per mol sum Xanthophyll cycle pigments***, samples taken in the glasshouse during late afternoon in December 2003. Light level (100% vs 75% of natural daylight), water availability (200 mls every 2 days and 50mls every 3 days) and habit (*Corokia cotoneaster*, *Corokia* hybrid and *Corokia buddleioides*) as treatments. [significant results in bold].

| | Df | Sum of Sq | Mean Sq | F Value | Pr(F) |
|---|---|---|---|---|---|
| Water availability | 1 | 0.00895106 | 0.008951062 | 1.72 | 0.217 |
| Habit | 2 | 0.00072371 | 0.000361853 | 0.07 | 0.933 |
| Water availability*Habit | 2 | 0.00496102 | 0.002480508 | 0.48 | 0.633 |
| Residuals | 11 | 0.05725833 | 0.005205303 | | |

Table A3.7 Analysis of variance table for the pigment ***Neoxanthin in mmol per mol total Chlorophyll***, samples taken in the glasshouse during late afternoon in December 2003. Light level (100% vs 75% of natural daylight), water availability (200 mls every 2 days and 50mls every 3 days) and habit (*Corokia cotoneaster*, *Corokia* hybrid and *Corokia buddleioides*) as treatments. [significant results in bold].

| | Df | Sum of Sq | Mean Sq | F Value | Pr(F) |
|---|---|---|---|---|---|
| Water availability | 1 | 1.3875 | 1.38747 | 0.17 | 0.692 |
| **Habit** | 2 | 155.3622 | 77.68111 | 9.28 | **0.004** |
| Water availability*Habit | 2 | 45.6902 | 22.84508 | 2.73 | 0.109 |
| Residuals | 11 | 92.1318 | 8.37562 | | |

Table A3.8 Analysis of variance table for the pigment ***Lutein in mmol per mol total Chlorophyll***, samples taken in the glasshouse during late afternoon in December 2003. Light level (100% vs 75% of natural daylight), water availability (200 mls every 2 days and 50mls every 3 days) and habit (*Corokia cotoneaster, Corokia* hybrid and *Corokia buddleioides*) as treatments. [significant results in bold].

| | Df | Sum of Sq | Mean Sq | F Value | Pr(F) |
|---|---|---|---|---|---|
| Water availability | 1 | 100.7003 | 100.7003 | 1.49 | 0.247 |
| Habit | 2 | 468.3064 | 234.1532 | 3.47 | 0.068 |
| Water availability*Habit | 2 | 53.8378 | 26.9189 | 0.40 | 0.680 |
| Residuals | 11 | 742.3721 | 67.4884 | | |

Table A3.9 Analysis of variance table for the pigment ***ß-carotene in µmol per mol total Chlorophyll***, samples taken in the glasshouse during late afternoon in December 2003. Light level (100% vs 75% of natural daylight), water availability (200 mls every 2 days and 50mls every 3 days) and habit (*Corokia cotoneaster, Corokia* hybrid and *Corokia buddleioides*) as treatments. [significant results in bold].

| | Df | Sum of Sq | Mean Sq | F Value | Pr(F) |
|---|---|---|---|---|---|
| Water availability | 1 | 0.079 | 0.079 | 0.00056 | 0.9812949 |
| **Habit** | 2 | 1234.876 | 617.438 | 4.39370 | **0.0222871** |
| Water availability* Habit | 2 | 27.225 | 13.612 | 0.09687 | 0.9079925 |
| Residuals | 11 | 3794.257 | 140.528 | | |

Table A3.10 Analysis of variance table for the ***vitamin E in mol per mol total Chlorophyll***, samples taken in the glasshouse during late afternoon in December 2003. Light level (100% vs 75% of natural daylight), water availability (200 mls every 2 days and 50mls every 3 days) and habit (*Corokia cotoneaster, Corokia* hybrid and *Corokia buddleioides*) as treatments. [significant results in bold].

| | Df | Sum of Sq | Mean Sq | F Value | Pr(F) |
|---|---|---|---|---|---|
| **Light level** | 1 | 0.6783 | 0.6783 | 76.40 | **0.000** |
| Water availability | 1 | 0.0149 | 0.0149 | 1.68 | 0.206 |
| Habit | 2 | 0.0524 | 0.0262 | 2.95 | 0.069 |
| Light level*Water availability | 1 | 0.0203 | 0.0203 | 2.29 | 0.141 |
| **Light level*Habit** | 2 | 0.0745 | 0.0372 | 4.19 | **0.026** |
| **Water availability*Habit** | 2 | 0.0806 | 0.0403 | 4.54 | **0.020** |
| Light level*Water availability*Habit | 2 | 0.0335 | 0.0167 | 1.88 | 0.171 |
| Residuals | 28 | 0.2486 | 0.0089 | | |

Table A3.11 Analysis of variance table for the pigment ***Violaxanthin in µmol per m$^{-2}$ leaf area***, samples taken in the glasshouse during late afternoon in December 2003. Light level (100% vs 75% of natural daylight), water availability (200 mls every 2 days and 50mls every 3 days) and habit (*Corokia cotoneaster*, *Corokia* hybrid and *Corokia buddleioides*) as treatments. [significant results in bold].

| | Df | Sum of Sq | Mean Sq | F Value | Pr(F) |
|---|---|---|---|---|---|
| Water availability | 1 | 9.045 | 9.045 | 0.16 | 0.698 |
| **Habit** | 2 | 6278.594 | 3139.297 | 55.10 | **0.001** |
| Water availability*Habit | 2 | 1.340 | 0.670 | 0.01 | 0.988 |
| Residuals | 11 | 626.777 | 56.980 | | |

Table A3.12 Analysis of variance table for the pigment ***Antheraxanthin in µmol per m$^{-2}$ leaf area***, samples taken in the glasshouse during late afternoon in December 2003. Light level (100% vs 75% of natural daylight), water availability (200 mls every 2 days and 50mls every 3 days) and habit (*Corokia cotoneaster*, *Corokia* hybrid and *Corokia buddleioides*) as treatments. [significant results in bold].

| | Df | Sum of Sq | Mean Sq | F Value | Pr(F) |
|---|---|---|---|---|---|
| Water availability | 1 | 0.008366 | 0.008366 | 0.04 | 0.848 |
| **Habit** | 2 | 2.855947 | 1.427973 | 6.60 | **0.013** |
| Water availability*Habit | 2 | 0.041209 | 0.020605 | 0.10 | 0.910 |
| Residuals | 11 | 2.380867 | 0.216442 | | |

Table A3.13 Analysis of variance table for the pigment ***Zeaxanthin in µmol per m$^{-2}$ leaf area***, samples taken in the glasshouse during late afternoon in December 2003. Light level (100% vs 75% of natural daylight), water availability (200 mls every 2 days and 50mls every 3 days) and habit (*Corokia cotoneaster*, *Corokia* hybrid and *Corokia buddleioides*) as treatments. [significant results in bold].

| | Df | Sum of Sq | Mean Sq | F Value | Pr(F) |
|---|---|---|---|---|---|
| Water availability | 1 | 5.7675 | 5.7675 | 1.389 | 0.264 |
| **Habit** | 2 | 673.7616 | 336.8808 | 81.11 | **0.000** |
| Water availability*Habit | 2 | 1.8839 | 0.9420 | 0.23 | 0.801 |
| Residuals | 11 | 45.6899 | 4.1536 | | |

Table A3.14 Analysis of variance table for the pigment ***Neoxanthin in µmol per m$^{-2}$ leaf area***, samples taken in the glasshouse during late afternoon in December 2003. Light level (100% vs 75% of natural daylight), water availability (200 mls every 2 days and 50mls every 3 days) and habit (*Corokia cotoneaster*, *Corokia* hybrid and *Corokia buddleioides*) as treatments. [significant results in bold].

| | Df | Sum of Sq | Mean Sq | F Value | Pr(F) |
|---|---|---|---|---|---|
| Water availability | 1 | 26.324 | 26.324 | 0.42 | 0.530 |
| **Habit** | 2 | 6321.904 | 3160.952 | 50.57 | **0.000** |
| Water availability*Habit | 2 | 2.494 | 1.247 | 0.02 | 0.980 |
| Residuals | 11 | 687.637 | 62.512 | | |

Table A3.15 Analysis of variance table for the pigment ***Lutein in µmol per m$^{-2}$ leaf area***, samples taken in the glasshouse during late afternoon in December 2003. Light level (100% vs 75% of natural daylight), water availability (200 mls every 2 days and 50mls every 3 days) and habit (*Corokia cotoneaster*, *Corokia* hybrid and *Corokia buddleioides*) as treatments. [significant results in bold].

| | Df | Sum of Sq | Mean Sq | F Value | Pr(F) |
|---|---|---|---|---|---|
| Water availability | 1 | 243.08 | 243.08 | 0.48 | 0.503 |
| **Habit** | 2 | 65848.46 | 32924.23 | 65.03 | **0.000** |
| Water availability*Habit | 2 | 13.78 | 6.89 | 0.01 | 0.987 |
| Residuals | 11 | 5569.42 | 506.31 | | |

Table A3.16 Analysis of variance table for the pigment ***Chlorophyll a in µmol per m$^{-2}$ leaf area***, samples taken in the glasshouse during late afternoon in December 2003. Light level (100% vs 75% of natural daylight), water availability (200 mls every 2 days and 50mls every 3 days) and habit (*Corokia cotoneaster*, *Corokia* hybrid and *Corokia buddleioides*) as treatments. [significant results in bold].

| | Df | Sum of Sq | Mean Sq | F Value | Pr(F) |
|---|---|---|---|---|---|
| Water availability | 1 | 3994 | 3993.6 | 0.28 | 0.606 |
| **Habit** | 2 | 1416031 | 708015.5 | 49.88 | **0.000** |
| Water availability*Habit | 2 | 38 | 19.2 | 0.00 | 0.999 |
| Residuals | 11 | 156136 | 14194.2 | | |

Table A3.17 Analysis of variance table for the pigment ***Chlorophyll b in µmol per m$^{-2}$ leaf area***, samples taken in the glasshouse during late afternoon in December 2003. Light level (100% vs 75% of natural daylight), water availability (200 mls every 2 days and 50mls every 3 days) and habit (*Corokia cotoneaster*, *Corokia* hybrid and *Corokia buddleioides*) as treatments. [significant results in bold].

| | Df | Sum of Sq | Mean Sq | F Value | Pr(F) |
|---|---|---|---|---|---|
| Water availability | 1 | 470.7 | 470.70 | 0.30 | 0.595 |
| **Habit** | 2 | 154390.0 | 77194.98 | 49.22 | **0.000** |
| Water availability*Habit | 2 | 9.1 | 4.56 | 0.00 | 0.997 |
| Residuals | 11 | 17252.0 | 1568.37 | | |

Table A3.18 Analysis of variance table for the pigment ***Chlorophyll a+b in µmol per m$^{-2}$ leaf area***, samples taken in the glasshouse during late afternoon in December 2003. Light level (100% vs 75% of natural daylight), water availability (200 mls every 2 days and 50mls every 3 days) and habit (*Corokia cotoneaster*, *Corokia* hybrid and *Corokia buddleioides*) as treatments. [significant results in bold].

| | Df | Sum of Sq | Mean Sq | F Value | Pr(F) |
|---|---|---|---|---|---|
| Water availability | 1 | 7207 | 7207 | 0.29 | 0.603 |
| **Habit** | 2 | 2505579 | 1252789 | 49.80 | **0.000** |
| Water availability*Habit | 2 | 84 | 42 | 0.00 | 0.998 |
| Residuals | 11 | 276723 | 25157 | | |

Table A3.19 Analysis of variance table for the pigment ***Chlorophyll a:b in µmol per m$^{-2}$ leaf area***, samples taken in the glasshouse during late afternoon in December 2003. Light level (100% vs 75% of natural daylight), water availability (200 mls every 2 days and 50mls every 3 days) and habit (*Corokia cotoneaster*, *Corokia* hybrid and *Corokia buddleioides*) as treatments. [significant results in bold].

| | Df | Sum of Sq | Mean Sq | F Value | Pr(F) |
|---|---|---|---|---|---|
| Water availability | 1 | 0.0120471 | 0.0120471 | 0.92 | 0.359 |
| **Habit** | 2 | 0.5190970 | 0.2595485 | 19.76 | **0.000** |
| Water availability*Habit | 2 | 0.0204446 | 0.0102223 | 0.78 | 0.483 |
| Residuals | 11 | 0.1444583 | 0.0131326 | | |

Table A3.20 Analysis of variance table for the pigment ***ß-Carotene in µmol per m$^{-2}$ leaf area***, samples taken in the glasshouse during late afternoon in December 2003. Light level (100% vs 75% of natural daylight), water availability (200 mls every 2 days and 50mls every 3 days) and habit (*Corokia cotoneaster*, *Corokia* hybrid and *Corokia buddleioides*) as treatments. [significant results in bold].

| | Df | Sum of Sq | Mean Sq | F Value | Pr(F) |
|---|---|---|---|---|---|
| Water availability | 1 | 15.783 | 15.783 | 0.35 | 0.568 |
| **Habit** | 2 | 6527.318 | 3263.659 | 71.63 | **0.000** |
| Water availability*Habit | 2 | 0.115 | 0.057 | 0.00 | 0.999 |
| Residuals | 11 | 501.184 | 45.562 | | |

Table A3.21 Analysis of variance table for the ***vitamin E in µmol per m$^{-2}$ leaf area***, samples taken in the glasshouse during late afternoon in December 2003. Light level (100% vs 75% of natural daylight), water availability (200 mls every 2 days and 50mls every 3 days) and habit (*Corokia cotoneaster*, *Corokia* hybrid and *Corokia buddleioides*) as treatments. [significant results in bold].

| | Df | Sum of Sq | Mean Sq | F Value | Pr(F) |
|---|---|---|---|---|---|
| **Light level** | 1 | 3.98 | 3.98 | 10.47 | **0.003** |
| Water availability | 1 | 0.95 | 0.95 | 2.51 | 0.124 |
| **Habit** | 2 | 50.17 | 25.08 | 66.04 | **0.000** |
| Light level*Water availability | 1 | 0.71 | 0.71 | 1.86 | 0.183 |
| **Light level*Habit** | 2 | 7.19 | 3.60 | 9.47 | **0.001** |
| Water availability*Habit | 2 | 2.02 | 1.01 | 2.65 | 0.087 |
| Light level*Water availability*Habit | 2 | 1.81 | 0.91 | 2.39 | 0.109 |
| Residuals | 30 | 11.40 | 0.38 | | |

Table A3.22 Analysis of variance table for the pigment ***Violaxanthin in nmol per g fresh weight***, samples taken in the glasshouse during late afternoon in December 2003. Light level (100% vs 75% of natural daylight), water availability (200 mls every 2 days and 50mls every 3 days) and habit (*Corokia cotoneaster, Corokia* hybrid and *Corokia buddleioides*) as treatments. [significant results in bold].

| | Df | Sum of Sq | Mean Sq | F Value | Pr(F) |
|---|---|---|---|---|---|
| Water availability | 1 | 1.108 | 1.108 | 0.02 | 0.904 |
| **Habit** | 2 | 3193.212 | 1596.606 | 21.70 | **0.000** |
| Water availability*Habit | 2 | 126.614 | 63.307 | 0.86 | 0.446 |
| Residuals | 13 | 956.357 | 73.566 | | |

Table A3.23 Analysis of variance table for the pigment ***Antheraxanthin in nmol per g fresh weight***, samples taken in the glasshouse during late afternoon in December 2003. Light level (100% vs 75% of natural daylight), water availability (200 mls every 2 days and 50mls every 3 days) and habit (*Corokia cotoneaster, Corokia* hybrid and *Corokia buddleioides*) as treatments. [significant results in bold].

| | Df | Sum of Sq | Mean Sq | F Value | Pr(F) |
|---|---|---|---|---|---|
| Water availability | 1 | 0.113262 | 0.113262 | 0.15 | 0.701 |
| Habit | 2 | 2.112634 | 1.056317 | 1.43 | 0.274 |
| Water availability*Habit | 2 | 0.029497 | 0.014748 | 0.02 | 0.980 |
| Residuals | 13 | 9.583957 | 0.737227 | | |

Table A3.24 Analysis of variance table for the pigment ***Zeaxanthin in nmol per g fresh weight***, samples taken in the glasshouse during late afternoon in December 2003. Light level (100% vs 75% of natural daylight), water availability (200 mls every 2 days and 50mls every 3 days) and habit (*Corokia cotoneaster, Corokia* hybrid and *Corokia buddleioides*) as treatments. [significant results in bold].

| | Df | Sum of Sq | Mean Sq | F Value | Pr(F) |
|---|---|---|---|---|---|
| Water availability | 1 | 37.6557 | 37.6557 | 2.53 | 0.136 |
| **Habit** | 2 | 276.5981 | 138.2991 | 9.28 | **0.003** |
| Water availability*Habit | 2 | 33.7731 | 16.8865 | 1.13 | 0.352 |
| Residuals | 13 | 193.6730 | 14.8979 | | |

Table A3.25 Analysis of variance table for the pigment ***Neoxanthin in nmol per g fresh weight***, samples taken in the glasshouse during late afternoon in December 2003. Light level (100% vs 75% of natural daylight), water availability (200 mls every 2 days and 50mls every 3 days) and habit (*Corokia cotoneaster, Corokia* hybrid and *Corokia buddleioides*) as treatments. [significant results in bold].

| | Df | Sum of Sq | Mean Sq | F Value | Pr(F) |
|---|---|---|---|---|---|
| Water availability | 1 | 10.654 | 10.654 | 0.12 | 0.739 |
| **Habit** | 2 | 2223.935 | 1111.968 | 12.08 | **0.001** |
| Water availability*Habit | 2 | 93.634 | 46.817 | 0.51 | 0.613 |
| Residuals | 13 | 1196.800 | 92.062 | | |

Table A3.26 Analysis of variance table for the pigment ***Lutein in nmol per g fresh weight***, samples taken in the glasshouse during late afternoon in December 2003. Light level (100% vs 75% of natural daylight), water availability (200 mls every 2 days and 50mls every 3 days) and habit (*Corokia cotoneaster, Corokia* hybrid and *Corokia buddleioides*) as treatments. [significant results in bold].

| | Df | Sum of Sq | Mean Sq | F Value | Pr(F) |
|---|---|---|---|---|---|
| Water availability | 1 | 270.48 | 270.48 | 0.34 | 0.572 |
| **Habit** | 2 | 21447.01 | 10723.51 | 13.30 | **0.001** |
| Water availability*Habit | 2 | 1250.69 | 625.34 | 0.78 | 0.481 |
| Residuals | 13 | 10485.54 | 806.58 | | |

Table A3.27 Analysis of variance table for the pigment ***Chlorophyll a in nmol per g fresh weight***, samples taken in the glasshouse during late afternoon in December 2003. Light level (100% vs 75% of natural daylight), water availability (200 mls every 2 days and 50mls every 3 days) and habit (*Corokia cotoneaster, Corokia* hybrid and *Corokia buddleioides*) as treatments. [significant results in bold].

| | Df | Sum of Sq | Mean Sq | F Value | Pr(F) |
|---|---|---|---|---|---|
| Water availability | 1 | 1860.7 | 1860.7 | 0.08 | 0.785 |
| **Habit** | 2 | 419482.3 | 209741.2 | 8.74 | **0.004** |
| Water availability*Habit | 2 | 37586.3 | 18793.1 | 0.78 | 0.477 |
| Residuals | 13 | 311878.4 | 23990.6 | | |

Table A3.28 Analysis of variance table for the pigment ***Chlorophyll b in nmol per g fresh weight***, samples taken in the glasshouse during late afternoon in December 2003. Light level (100% vs 75% of natural daylight), water availability (200 mls every 2 days and 50mls every 3 days) and habit (*Corokia cotoneaster, Corokia* hybrid and *Corokia buddleioides*) as treatments. [significant results in bold].

| | Df | Sum of Sq | Mean Sq | F Value | Pr(F) |
|---|---|---|---|---|---|
| Water availability | 1 | 86.11 | 86.11 | 0.03 | 0.865 |
| **Habit** | 2 | 38152.79 | 19076.39 | 6.68 | **0.010** |
| Water availability*Habit | 2 | 3361.01 | 1680.50 | 0.59 | 0.569 |
| Residuals | 13 | 37139.72 | 2856.90 | | |

Table A3.29 Analysis of variance table for the pigment ***Chlorophyll a+b in nmol per g fresh weight***, samples taken in the glasshouse during late afternoon in December 2003. Light level (100% vs 75% of natural daylight), water availability (200 mls every 2 days and 50mls every 3 days) and habit (*Corokia cotoneaster, Corokia* hybrid and *Corokia buddleioides*) as treatments. [significant results in bold].

| | Df | Sum of Sq | Mean Sq | F Value | Pr(F) |
|---|---|---|---|---|---|
| Water availability | 1 | 2747.4 | 2747.4 | 0.06 | 0.805 |
| **Habit** | 2 | 705024.6 | 352512.3 | 8.16 | **0.005** |
| Water availability*Habit | 2 | 63288.4 | 31644.2 | 0.73 | 0.500 |
| Residuals | 13 | 561682.4 | 43206.3 | | |

Table A3.30 Analysis of variance table for the pigment ***Chlorophyll a:b in nmol per g fresh weight***, samples taken in the glasshouse during late afternoon in December 2003. Light level (100% vs 75% of natural daylight), water availability (200 mls every 2 days and 50mls every 3 days) and habit (*Corokia cotoneaster, Corokia* hybrid and *Corokia buddleioides*) as treatments. [significant results in bold].

| | Df | Sum of Sq | Mean Sq | F Value | Pr(F) |
|---|---|---|---|---|---|
| Water availability | 1 | 0.0231058 | 0.0231058 | 1.87 | 0.195 |
| **Habit** | 2 | 0.5307228 | 0.2653614 | 21.47 | **0.000** |
| Water availability*Habit | 2 | 0.0254902 | 0.0127451 | 1.03 | 0.384 |
| Residuals | 13 | 0.1607033 | 0.0123618 | | |

Table A3.31 Analysis of variance table for the pigment ***ß-carotene in nmol per g fresh weight***, samples taken in the glasshouse during late afternoon in December 2003. Light level (100% vs 75% of natural daylight), water availability (200 mls every 2 days and 50mls every 3 days) and habit (*Corokia cotoneaster, Corokia* hybrid and *Corokia buddleioides*) as treatments. [significant results in bold].

| | Df | Sum of Sq | Mean Sq | F Value | Pr(F) |
|---|---|---|---|---|---|
| Water availability | 1 | 63.597 | 63.597 | 0.87 | 0.368 |
| **Habit** | 2 | 3501.558 | 1750.779 | 23.90 | **0.000** |
| Water availability*Habit | 2 | 327.592 | 163.796 | 2.24 | 0.146 |
| Residuals | 13 | 952.321 | 73.255 | | |

Table A3.32 Analysis of variance table for the ***vitamin E in µmol per g fresh weight***, samples taken in the glasshouse during late afternoon in December 2003. Light level (100% vs 75% of natural daylight), water availability (200 mls every 2 days and 50mls every 3 days) and habit (*Corokia cotoneaster*, *Corokia* hybrid and *Corokia buddleioides*) as treatments. [significant results in bold].

| | Df | Sum of Sq | Mean Sq | F Value | Pr(F) |
|---|---|---|---|---|---|
| **Light level** | 1 | 82140 | 82140 | 8.85 | **0.006** |
| Water availability | 1 | 10155 | 10155 | 1.09 | 0.304 |
| Habit | 2 | 35935 | 17968 | 1.94 | 0.163 |
| Light level*Water availability | 1 | 3194 | 3194 | 0.34 | 0.562 |
| **Light level*Habit** | 2 | 99292 | 49646 | 5.35 | **0.011** |
| **Water availability*Habit** | 2 | 127664 | 63832 | 6.88 | **0.004** |
| Light level*Water availability*Habit | 2 | 40895 | 20448 | 2.20 | 0.129 |
| Residuals | 29 | 269153 | 9281 | | |

Table A3.33 Analysis of variance table for daytime respiration measurements in the glasshouse, taken in December 2003. Light level (100% vs 75% of natural daylight), water availability (200 mls every 2 days and 50mls every 3 days) and habit (*Corokia cotoneaster, Corokia buddleioides* and *Corokia* hybrid) as treatments [significant results in bold].

| | Df | Sum of Sq | Mean Sq | F Value | Pr(F) |
|---|---|---|---|---|---|
| Light level | 1 | 4.38440 | 4.38440 | 1.7326 | 0.198 |
| **Water availability** | 1 | 14.82654 | 14.82654 | 5.8591 | **0.022** |
| **Habit** | 2 | 75.41143 | 37.70571 | 14.900 | **0.000** |
| Light level*Water availability | 1 | 2.63123 | 2.63123 | 1.0398 | 0.316 |
| Light level*Habit | 2 | 0.91118 | 0.45559 | 0.1800 | 0.836 |
| Water availability*Habit | 2 | 11.91620 | 5.95810 | 2.3545 | 0.112 |
| Light level*Water availability*Habit | 2 | 1.48693 | 0.74347 | 0.2938 | 0.748 |
| Residuals | 30 | 75.91580 | 2.53053 | | |

Table A3.34 Analysis of variance table for measurements of quantum efficiency (QE) in the glasshouse, taken in December 2003. Light level (100% vs 75% of natural daylight), water availability (200 mls every 2 days and 50mls every 3 days) and habit (*Corokia cotoneaster, Corokia buddleioides* and *Corokia* hybrid) as treatments [significant results in bold].

| | Df | Sum of Sq | Mean Sq | F Value | Pr(F) |
|---|---|---|---|---|---|
| Light level | 1 | 0.000179884 | 0.000179884 | 0.615 | 0.439 |
| Water availability | 1 | 0.001038388 | 0.001038388 | 3.550 | 0.070 |
| **Habit** | 2 | 0.002421448 | 0.001210724 | 4.139 | **0.026** |
| Light level*Water availability | 1 | 0.000072745 | 0.000072745 | 0.249 | 0.622 |
| Light level*Habit | 2 | 0.000476372 | 0.000238186 | 0.814 | 0.453 |
| Water availability*Habit | 2 | 0.000706918 | 0.000353459 | 1.208 | 0.313 |
| Light level*Water availability*Habit | 2 | 0.000016034 | 0.000008017 | 0.027 | 0.973 |
| Residuals | 29 | 0.008483333 | 0.000292529 | | |

Table A3.35 Analysis of variance table for measurements of the maximum photosynthetic rate (Amax) in the glasshouse, taken in December 2003. Light level (100% vs 75% of natural daylight), water availability (200 mls every 2 days and 50mls every 3 days) and habit (*Corokia cotoneaster, Corokia buddleioides* and *Corokia* hybrid) as treatments [significant results in bold].

| | Df | Sum of Sq | Mean Sq | F Value | Pr(F) |
|---|---|---|---|---|---|
| Light level | 1 | 11.3341 | 11.3341 | 1.3385 | 0.256 |
| Water availability | 1 | 2.9341 | 2.9341 | 0.3465 | 0.561 |
| **Habit** | 2 | 354.9180 | 177.4590 | 20.958 | **0.000** |
| **Light level*Water availability** | 1 | 36.9760 | 36.9760 | 4.3668 | **0.045** |
| Light level*Habit | 2 | 37.3567 | 18.6784 | 2.2059 | 0.128 |
| Water availability*Habit | 2 | 45.5578 | 22.7789 | 2.6902 | 0.084 |
| Light level*Water availability*Habit | 2 | 30.7273 | 15.3636 | 1.8144 | 0.180 |
| Residuals | 30 | 254.0251 | 8.4675 | | |

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