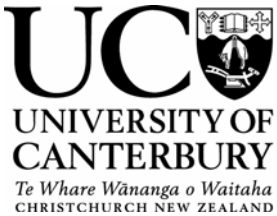
REPRESENTING NUTRITION OF *PINUS RADIATA* IN PHYSIOLOGICAL HYBRID PRODUCTIVITY MODELS

A thesis
submitted in partial fulfilment
of the requirements for the degree of

Doctor of Philosophy

By

Horacio E. Bown



University of Canterbury
New Zealand
2007

Greenhouse University of Canterbury, March 2005

Pinus radiata trees were cultivated in silica sand with a factorial combination of nitrogen and phosphorus supply to assess internal nutrient cycling and carbon allocation patterns

Tekapo, September 2005

Highly-stocked (40 000 stems ha^{-1}) control and fertilized mini-plots of *Pinus radiata* were measured to assess patterns of carbon allocation in five sites in the South Island of New Zealand

ABSTRACT

Hybrid physiological models are being increasingly used to assess productivity, carbon sequestration, water and nutrient use and environmental impacts of management decisions. Users include forest managers, politicians, environmental agencies and scientists. However a wider use of these models has been prevented as a result of an incomplete understanding of the mechanisms regulating carbon allocation, nutrient availability in soils and nutrient uptake by trees. On-going innovation in clonal forestry, genetic improvement and vegetation management techniques is also poorly represented in hybrid models.

This thesis examines means to represent nutrition and genotype-nutrition interactions in productivity physiological hybrid models. Nutrient limitations and growth differences between genotypes were hypothesized to operate through key physiological processes: photosynthesis, carbon allocation and nutrient internal cycling. In order to accomplish the aims of the study both greenhouse and field experimentation were carried out.

In a first experiment, responses of photosynthesis (A) to intercellular CO_2 concentration (C_i) were measured in a fast- and a slow-growing clone of *Pinus radiata* D. Don cultivated in a greenhouse in a factorial combination of nitrogen and phosphorus supply, and analyzed using the biochemical model of leaf photosynthesis described by Farquhar *et al.* (1980). There were significant positive linear relationships between the parameters, V_{cmax}, J_{max}, T_p and both foliar nitrogen (N_a) and phosphorus (P_a) concentration on an area basis. The study showed that the effects of nitrogen and phosphorus supply on photosynthesis were statistically independent and that the photosynthetic behaviour of the two clones was equivalent.

In a similar study, gas exchange and chlorophyll fluorescence were simultaneously measured to determine internal transfer conductance (g_m) based on the "constant J method". Transfer conductance may pose significant limitations to photosynthesis which may be differentially affected by nutrition and genotype in *Pinus radiata*. Values of g_m were similar to those of stomatal conductance (g_s) and their ratio (g_m / g_s) was not influenced by nutrient supply or clone being on average (± 1 SE) 1.22 ± 0.04. Relative mesophyll limitations (L_M, 16%) to photosynthesis were marginally greater than those imposed by stomata (L_S, 13%), and together smaller than the relative limitations posed to photosynthesis by biochemical processes (L_B, 71%). The CO_2 concentration in the intercellular air spaces (C_i) was (± 1 SE) 53 ± 3 μmol mol^{-1}

lower than in the atmosphere (C_a) while CO_2 concentration in the chloroplasts (C_c) was (± 1 SE) 48 ± 2 μmol mol^{-1} less than C_i. Values of L_S, L_M and L_B and CO_2 diffusion gradients posed by g_s (C_a-C_i) and g_m (C_i-C_c) did not change with nutrient supply or clone.

In a third experiment, one-year old *Pinus radiata* cuttings from four genotypes were cultivated in silica sand with a factorial combination of nitrogen (N_0=1.43 and N_1=7.14 mM) and phosphorus (P_0=0.084 and P_1=0.420 mM) supply for 24 months. N supply was enriched with ^{15}N to 2.5‰ (labelled N) during the first year, then plants transferred to clean sand and cultivated for another year with ^{15}N at levels close to natural abundance (0.3664899 atom percent ^{15}N, $\delta^{15}N$ 0.5115 ‰) provided by the source of N in nutrient solution applied during the second year. Recovery of labelled and unlabelled N was used to estimate N remobilization. N remobilization scaled with plant growth, N content and N and P supply. In relative terms, 65% of all stored N was remobilized in the high-nutrient supply regime compared to 42-48% at lower N and P addition rates. Most N remobilization occurred during spring-summer (77%), coincidently with the largest proportion of needle development (80%), indicating that N remobilization was driven by sink-strength. Foliage was by far the main source for internal cycling while roots were the main sink (40%). Clones exhibited differences in N remobilization capacity, but these differences were completely explained by the size of the N pool before remobilization took place, indicating that N remobilization performance was similar among clones.

In a fourth study, four clones were cultivated in silica sand with a factorial combination of nitrogen and phosphorus supply for ten months, and patterns of carbon allocation examined using a carbon balance approach. Gross-primary productivity (GPP) scaled mainly with nitrogen but also with phosphorus supply. The fraction of GPP (GPP = ANPP + APR + TBCA) allocated to above-ground components (ANPP) increased with N and P supply at the expense of total-below ground C allocation (TBCA) with no apparent effect on the fraction of GPP partitioned to above-ground plant respiration (APR). Carbon use efficiency (NPP:GPP) scaled with nutrient supply, being 0.42 in the low-nutrient supply regime compared to 0.51 in the high-nutrient supply regime, suggesting that in poor fertility environments a larger proportion of the C budget is respired compared to the net productivity. Fast-growing clones allocated about 2-4% more carbon to above-ground components (ANPP) at the expense of carbon allocated below-ground (TBCA) with no effect on carbon respired above-ground (APR), indicating that

faster-growing genotypes allocate more carbon to leaf area which may compound and increase overall GPP over time.

The field component of this thesis was conducted in a subset of locations where ENSIS (formerly New Zealand Forest Research Institute) had established trials to test the influence of species, soil disturbance and plant nutrition on sustainability indicators. Plots were small in size (3 m × 3 m) with trees spaced at 0.5 m × 0.5 m (40 000 trees ha^{-1}) with nine measurement trees surrounded by a two-row buffer. All sites were planted in winter 2001 and harvested in spring 2005. The aim of this pilot study was to examine patterns of carbon allocation during the fourth year after planting in control and fertilized mini-plots of *Pinus radiata* in five sites with contrasting climate and soil conditions in the South Island of New Zealand. The study showed that the fraction of gross-primary productivity allocated belowground increased as the soil C:N ratio increased. However, these results should be interpreted with caution due to the unusual nature of the trial and the reduced number of sites studied.

Two existing physiological models were selected for the discussion in this thesis (3-PG, Landsberg and Waring 1997; canopy net carbon exchange model, Whitehead *et al*. 2002). Potential improvements for the nutritional component of 3-PG comprise: accounting for reductions in carbon use efficiency (NPP:GPP) in poor-fertility environments, adding a preliminary fertility modifier (F_N, 0-1) driven by soil C : N ratio and soil N, adding a preliminary relationship between carbon allocation to roots and the soil C : N ratio and representing faster-growing genotypes by increasing their leaf area but not their photosynthetic performance. The canopy net carbon exchange model (NCE) combines the coupled model of leaf photosynthesis - stomatal conductance described by Leuning (1995) with canopy structure and a water balance model to scale carbon assimilation from leaves to canopies. Potential improvements to account for nutrient deficiencies in the leaf model by Leuning (1995), comprise using nutrient ratios to discriminate nitrogen ($N_a/P_a < 23$ mol mol^{-1}) from phosphorus deficiencies ($N_a/P_a > 23$ mol mol^{-1}), adding relationships between photosynthetic model parameters V_{cmax} and J_{max} to P_a, and correcting the estimation of photosynthetic parameters V_{cmax} and J_{max} by accounting for transfer conductance (g_m). The canopy net carbon exchange model may be also modified to account for carbon-use efficiency, carbon allocation to roots and genotype in a similar form to that proposed for 3-PG.

The results previously outlined provide a preliminary framework to represent tree and soil nutrition in physiological hybrid productivity models.

ACKNOWLEDGMENTS

This study is by far the most stimulating and challenging project of my career. The project was born by the initiative of Dr. Euan Mason (NZ School of Forestry) and Dr. Brian Richardson (ENSIS, former NZ Forest Research Institute), who suggested that means to represent nutrition in physiological hybrid models should be addressed. The project was a cooperative undertaking between ENSIS and the University of Canterbury, and I am indebted to all those people from both institutions, that supported and encourage me throughout this study.

I would like to specially thank my supervisors Dr. Euan Mason, Dr. Peter Clinton, Dr. Michael Watt and Dr. Brian Richardson for their encouragement, empathy and outstanding supervision. Dr. Michael Watt was part of the ENSIS team reporting on the Site Quality Project when I started my Ph.D., and I was fortunate to benefit from his experience and previous work in the project, in order to define a suitable set of sites, to identify key hypotheses and for taking the time for teaching and discussing the best methods to address the research questions for this thesis. Furthermore, Dr. Michael Watt exceeded his responsibilities at ENSIS and provided his personal time to support this project in the fourteen trips (four days each) to measure the site quality plots and at harvest in days that were routinely 12 hours long and extending up to 18 hours. Mike also encouraged and spent considerable time in the greenhouse experiments particularly at times of overlapping activities, and helped immensely with the structuring, and editing of this thesis (in particular Chapter *Two*, *Four*, *Five* and *Six*), and also provided considerable assistance in translating these chapters to viable papers.

Dr. Euan Mason as university supervisor always was ready to help with financial and administrative matters and provided invaluable encouragement and support throughout this huge undertaking. As a modeller, he introduced me to the fascinating world of hybrid modelling and hard world of statistics, provided sound advice and constructive criticism, and helped immensely with the structuring and editing of this thesis (in particular Chapters *One*, *Six*, *Seven* and *Eight*). As an ENSIS supervisor, Dr. Peter Clinton, helped me to keep funds flowing and plan ahead. As a scientist, Peter helped me to understand soil and plant nutrition and to keep the big picture in mind. He helped me immensely in the design of the ammonium and nitrate experiment (Appendix B), in the design of the nutrient storage and remobilization experiment, in the monthly sampling of soil exchangeable ions, and with the structuring and editing of this

thesis particularly of Chapters *Four*, *Six* and *Eight*. So thanks Guys! I would not have done it without your expertise, enthusiasm and valuable comments. In addition, special thanks to Dr. David Whitehead and Dr. Roger Sands for their kind and generous advice at different stages of the study. I would like to also thank the referees for this thesis, Dr. John Adams (University of Canterbury) and Dr. Robin Rose (Oregon State University), who validated the scientific rigour of this study and their constructive criticism improved the final version of the document.

Funding was provided during 2003 and 2004 by ENSIS and the University of Canterbury and from 2005 onwards by Education New Zealand. I feel truly indebted to all these institutions for funding and trusting me to carry out this project. I feel exceedingly grateful also to the University of Chile, and to my colleagues at the Faculty of Forestry, first for their friendship and also for their support and encouragement to work towards a Ph.D degree.

Last but not least, I would like to thank my beloved wife Laura for her endless patience and support, and daughter Mariana for her understanding and for sprinkling with joy my life everyday. Thanks Girls!

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF SYMBOLS

Symbol	Description	Units
A	rate of net photosynthesis	μmol m^{-2} s^{-1}
C_a	CO_2 concentration in the air outside the leaf	μmol mol^{-1}
C_i	CO_2 concentration in intercellular air spaces	μmol mol^{-1}
C_c	CO_2 concentration in the chloroplast	μmol mol^{-1}
Γ^*	chloroplastic CO_2 compensation point	μmol mol^{-1}
C_i^*	intercellular CO_2 compensation point in the absence of day respiration	μmol mol^{-1}
R_d	rate of mitochondrial respiration in the light.	μmol m^{-2} s^{-1}
A_{sat}	light-saturated rate of photosynthesis at ambient C_a	μmol m^{-2} s^{-1}
E_N	instantaneous photosynthetic nitrogen use efficiency	μmol mol^{-1} s^{-1}
E_P	instantaneous photosynthetic phosphorus use efficiency	μmol mol^{-1} s^{-1}
L_S	relative stomatal (plus boundary layer) limitation to photosynthesis	%
L_M	relative mesophyll limitations to photosynthesis	%
L_B	relative biochemical limitations to photosynthesis	%
$1 - L_S$	Non-stomatal limitations to photosynthesis	%
g_s	stomatal (plus boundary layer) conductance to CO_2 diffusion	mmol m^{-2} s^{-1}
g_m	Internal transfer conductance to CO_2 diffusion	mmol m^{-2} s^{-1} bar^{-1}
V_{cmax}	*in vivo* maximum rate of ribulose-1,5-*bis*phosphate (RuBP) carboxylase-oxygenase (Rubisco) carboxylation	μmol CO_2 m^{-2} s^{-1}
J_{max}	electron transport driving regeneration of RuBP	μmol electrons m^{-2} s^{-1}
T_p	rate of triose phosphate export	μmol triose-P m^{-2} s^{-1}
Φ_{PSII}	Photochemical efficiency of photosystem II	no units
N_a, P_a	foliage nitrogen and phosphorus concentration on an area basis	mmol m^{-2}
N_m, P_m	foliage nitrogen and phosphorus concentration on a mass basis	μmol g^{-1}
N_a/P_a	foliage nitrogen to phosphorus concentration ratio	mol mol^{-1}
M	Leaf area to mass ratio	m^2 kg^{-1}
VPD	Leaf-to-air vapour pressure deficit	kPa

LIST OF SYMBOLS

Symbol	Description	Units
e	Natural log base (2.71828)	no units
π	Pi (3.1416)	no units
ln	Natural log	no units
r^2	Coefficient of determination	no units
SE	Standard Error	variable units
SD	Standard Deviation	variable units
P_G, GPP	gross-primary productivity	kg C m^{-2} $year^{-1}$
P_N, NPP	net-primary productivity	kg C m^{-2} $year^{-1}$
NEP	net-ecosystem productivity	kg C m^{-2} $year^{-1}$
ANPP	Above-ground net primary productivity	kg C m^{-2} $year^{-1}$
APR	Above-ground plant respiration	kg C m^{-2} $year^{-1}$
TBCA	Total below-ground carbon allocation	kg C m^{-2} $year^{-1}$
CUE	Carbon use efficiency	kg kg^{-1}
F_A	Aboveground litterfall	kg C m^{-2} $year^{-1}$
F_W	Tree mortality	kg C m^{-2} $year^{-1}$
C_C	Foliage carbon content	kg C m^{-2} $year^{-1}$
C_W	carbon content of aboveground wood	kg C m^{-2} $year^{-1}$
L_{RC}	Leaf construction respiration	kg C m^{-2} $year^{-1}$
L_{RM}	Leaf maintenance respiration	kg C m^{-2} $year^{-1}$
W_R	Wood construction and maintenance respiration	kg C m^{-2} $year^{-1}$
F_S	Soil respiration	kg C m^{-2} $year^{-1}$
F_E	Carbon lost from the soil by leaching or erosion	kg C m^{-2} $year^{-1}$
C_S	Carbon content of the mineral soil	kg C m^{-2} $year^{-1}$
C_R	Carbon content of root biomass	kg C m^{-2} $year^{-1}$
C_L	Carbon content of the litter layer	kg C m^{-2} $year^{-1}$
R_a	Autotrophic respiration	kg C m^{-2} $year^{-1}$
R_h	Heterotrophic respiration	kg C m^{-2} $year^{-1}$
Q_{10}	Proportional increase in respiration rate with a 10 °C increase in temperature	no units
LAI	Leaf area index	m^2 m^{-2}
θ	Volumetric water content	m^3 m^{-3}

CHAPTER ONE
GENERAL INTRODUCTION

INTRODUCTION

Background

Pinus radiata is the most outstanding and widely planted forest species in New Zealand and the Southern Hemisphere (Lewis and Ferguson 1993). It represents 89% of the 1.8 million ha of plantation forestry area in New Zealand, and its predominance is explained by higher productivity (> 20 m^3 ha^{-1} yr^{-1}), greater adaptability to soil and environmental conditions, better response to tree breeding and silviculture, and better range in end-uses than most other forest species (Turner and Lambert 1986, Cown 1997). Such outstanding characteristics contribute to the success of the New Zealand forest industry that provides 1.1% of world timber consumption from only 0.05% of the global forest cover (N.Z.F.O.A. 2007).

Pinus radiata, once considered a low-quality timber, is now intensively tended and managed for a wide range of uses (MacLaren 1993). Additionally, tree breeding programmes have contributed to improve stem growth and form by up to 23 %, among other traits, and have led to the development of a widely used system of genetic improvement ratings (Vincent and Dunstan 1989). Clonal forestry, being practised at a moderate commercial scale in New Zealand, is envisaged to play an increasing role in the improvement of *Pinus radiata* for timber production and quality (Cown 1997, Sorensson *et al*. 1997, Sorensson and Shelbourne 2005).

Increasing productivity of existing plantations at the global scale in order to fulfil current and future needs of wood for industrial and fuel consumption would require more intensive forest management and tree breeding strategies (Nambiar 1984, Turner and Lambert 1986). However unless nutrient and water requirements are optimized, the effects of intensive silviculture and tree breeding will not be realized (Webber 1978, Nambiar 1984, Turner and Lambert 1986, Raison and Myers 1992, Madgwick 1994). Because plantation forestry was historically relegated to land with low agricultural potential (Boomsma and Hunter 1990, Hunter and Smith 1996), fertilization has been an effective management tool permitting the New Zealand forestry sector to produce fast-growing radiata pine plantations in nutrient deficient areas (Mead and Gadgil 1978, Mead 2005*a*).

Major nutrient deficiencies noted in New Zealand comprise nitrogen, phosphorus, magnesium and boron, and localized deficiencies of potassium, manganese, copper, zinc and

molybdenum have also been recorded (Will 1985, MacLaren 1993, Hunter *et al*. 1991, Mead 2005*b*). Severe nitrogen deficiencies are widespread in coastal sands, dredge tailings and generally where soils contain no or little organic matter or after topsoil removal such as skid sites and landings (Will 1978, 1985). Less severe nitrogen deficiencies with good fertilization responses have been noted in gley-podzols (pakihi soils) in Westland, undrained peats in Southland (Will 1978), eroded Moutere Gravel soils in Nelson (Mead and Gadgil 1978), podzolized sands and clays in North Auckland (Will 1978, 1985), and alluvial soils in Canterbury (Hunter *et al*. 1991). Marginal nitrogen deficiencies have been observed in other soils such as the central North Island pumice plateau where fertilization responses in growth have also been substantial (Will 1978, 1985).

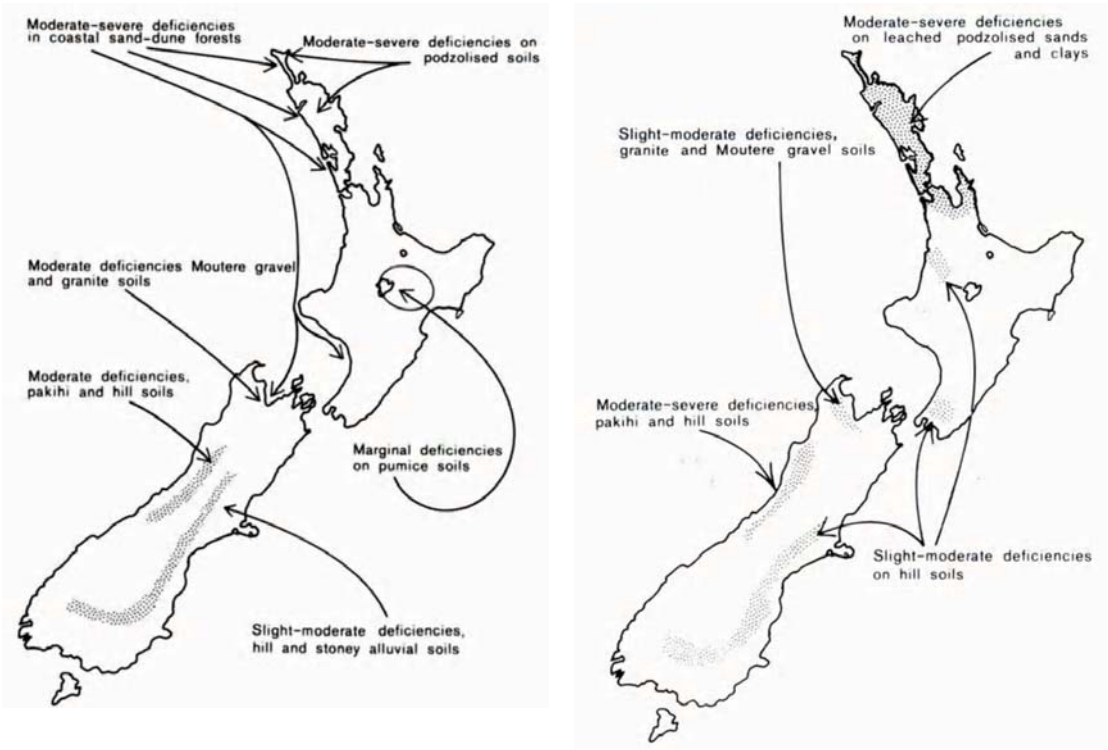


Figure 1.1. Major areas of nitrogen (left) and phosphorus (right) deficiencies in *Pinus radiata* forests in New Zealand. Source: Will (1985) p. 13,18.

Most New Zealand soils and their parental materials are low in phosphorus, and hence sustained agricultural and forest productivity are dependent on phosphate fertilizers (Will 1985). Moderate to severe phosphorus deficiencies have been observed in podzolized sands (e.g. Waipoua State Forest, Hunter and Graham 1983) and clay soils (e.g. Riverhead State Forest, Will 1965, Hunter and Graham 1982, 1983) in North Auckland and Northland, in the pakihi

soils in Westland, leached granite soils (e.g. Kaiteriteri hills) and Moutere gravels in Nelson and rhyolitic and andesitic parent materials in the Coromandel Peninsula (Will 1978, 1985, Hunter and Graham 1983). Moderate phosphorus deficiencies have been noted in coastal Canterbury, whereas in most areas in north-east and inland Canterbury phosphorus levels are satisfactory (Hunter *et al*. 1991). Satisfactory levels of phosphorus are found in most soils where *Pinus radiata* grows on the pumice plateau region (Hunter *et al*. 1991).

Nutrient deficiencies are usually diagnosed by visual symptoms but more commonly by foliage analysis (Will 1985, MacLaren 1993, Madgwick 1994). Severe nitrogen and phosphorus deficiencies have been associated with foliage nutrient concentrations of less than 1.2 % and 0.12 % respectively (Will 1985, Turner and Lambert 1986). However the interpretation of foliage analysis, being clear-cut under severe nutrient deficiencies, remains largely uncertain at marginal levels as a predictive tool, probably because nutrients are highly dynamic both in the soil and within the tree (Turner and Lambert 1986, Landsberg and Gower 1997). Soil testing despite being widely used in agriculture has proved of limited use for identifying nutrient deficiencies in forestry (Will 1985, MacLaren 1993, Madgwick 1994). One noticeable exception has been the use of Bray extractable phosphorus (< 9 ppm) as indicative that phosphate fertilizer will be probably required soon after planting *Pinus radiata* in New Zealand (Will 1985).

Most nutritional problems in *Pinus radiata* plantations in New Zealand are now routinely solved using commercial fertilizers (Turner and Lambert 1986). Other methods of fertilization include the use of legumes to supply nitrogen, and the use of municipal, industrial and farm-waste water or sludge (biosolids) to supply water and nutrients (Mead 2005*b*). In young trees, deficiencies are usually corrected by applying fertilizers in a slot beside each tree, while in large trees aerial systems are usually preferred (Will 1985, MacLaren 1993, Mead 2005*b*).

Genetically improved trees on the basis of differential nutritional requirements may also potentially contribute to alleviate forest nutritional problems (Turner and Lambert 1986). Several studies have reported differences in nutrient use efficiency among *Pinus radiata* genotypes (Burdon 1976, Forrest and Ovington 1971, Knight 1978) but selection criteria for genetic improvement has not yet been defined (Turner and Lambert 1986). Additionally there is some controversy about whether some families that respond well to poor fertility sites will be less responsive under more fertile conditions, and this question is also extended to the interaction between nutrients and water (Turner and Lambert 1986). However a recent study by Carson *et al*. (2004) showed that genotype × fertility interactions in *Pinus radiata* are seldom

significant, suggesting that selecting genotypes for better growth performance in poor fertility sites would not be substantially better than selecting for growth on all sites irrespective of nutrient availability.

Responses to fertilization in nutrient deficient sites have been often large, particularly with nitrogen and phosphorus (Nambiar 1984, Hunter *et al*. 1986). For instance, Mead and Gadgil (1978) showed mid-rotation growth responses to nitrogen fertilization that often exceed 8 m^3 ha^{-1} $year^{-1}$ on fertile soils in the central North Island, while shorter-lived larger growth responses of up to 17 m^3 ha^{-1} $year^{-1}$ have been observed in less fertile sites in the Nelson region. Woollons *et al*. (1988) reported growth responses in basal area at mid-rotation of up to 6.2 m^2 ha^{-1} a few years after fertilization, and this response was enhanced and compounded over time in three out of four fertilization trials in New Zealand and Australia. The authors suggested that enduring responses to fertilization will be found in most sites in Australasia, but that in marginal sites periodic applications of fertilizers may be required to sustain early growth responses.

The widespread use of fertilizers has been undoubtedly encouraged by the massive growth responses reported in a large number of research trials located since the 1950s in different soil, environmental and management conditions in New Zealand. However this large body of knowledge has been difficult to synthesize due to differences in methods and reporting practices (Madgwick 1994). Nambiar (1984) argues that practical solutions to many nutritional problems are known but that much of that knowledge is empirical, and that further innovation depends on a better understanding of the mechanisms underlying plant responses to shortages in site resources. The complexity of trees and tree-environment interactions has encouraged the development of multi-disciplinary research which has lead to the development of physiological models as means to explore such systems (Sands and Mulligan 1990, Benson *et al*. 1992).

Tree growth models

Mensurational forest models describe historical patterns of growth in order to forecast future development of stands (Proe *et al*. 1994). These models are appropriate when future stand and environmental conditions remain similar to those of the past. However, intensive silviculture, genetically improved material and global environmental change make present conditions arguably different to those of the past and likely to be different to those in the future (Kimmins *et al*. 1990, Johnsen *et al*. 2001). Mason and Milne (1999) illustrated this point when observing that regional growth and yield models failed to predict the effects of weed control,

fertilization and soil cultivation when extrapolated from short-term experiments to later in the rotation in *Pinus radiata* in the Canterbury Region in New Zealand.

Some research has been carried out to incorporate effects of fertilization in mensurational growth and yield models of *Pinus radiata* in New Zealand. An elegant approach was used by Lowell (1986) to incorporate the effect of fertilization in a stand-level growth and yield model developed by Garcia (1984) for *Pinus radiata* in Golden Downs in Nelson, New Zealand. The author determined that among top height, stocking and basal area, only the latter required modification within the growth and yield model, being incorporated through three multiplicative components: the time elapsed since fertilization, fertilization rates and the state of the stand at the time of fertilization. The model was robust in the conditions for which it was constructed, explaining 86% of the variance in the basal area response to fertilization. Yet the applicability of such an approach to different soil and environmental conditions remained limited.

Physiological forest models, on the other hand, are based on a (more) mechanistic understanding of physiological processes, and may predict productivity over a wider range of environmental conditions (Landsberg and Gower 1997, Johnsen *et al*. 2001, Landsberg 2003) such as rising temperature and CO_2 concentration in the atmosphere. These models play a major role in assessing global environmental change, carbon balance, primary production, and water and nutrient use-efficiency (Jarvis 1995). However the general application of physiological models to forest management has been restricted by the lack of precise data, complex calibration required to operate these models, and incomplete understanding of key physiological processes (Mäkelä *et al*. 2000, Johnsen *et al*. 2001).

Despite that mensurational growth and yield models may provide more accurate growth and yield predictions, physiological models play a key role in understanding the growth potential and limiting factors of a site, and the biological consequences of management decisions, and may help to find gaps where new research could be developed (Kimmins *et al.* 1990). For instance, McMurtrie *et al.* (1990*a*, 1990*b*) described the use of a physiological model (BIOMASS) to describe root-zone water balance and annual canopy photosynthesis in fertilization × thinning and fertilization × irrigation experiments of *Pinus radiata* in Woodhill (New Zealand) and Canberra (Australia), respectively. The application of the model showed gaps of knowledge on the effects of nutrient limitations on photosynthesis, carbon allocation, plant respiration and nutrient cycling of forest stands.

Hybrid models combine the predictive power and robustness of mensurational growth and yield models with the flexibility of physiological models, providing enhanced biological

realism yet requiring less parameters than physiological models (Kimmins *et al*. 1990, Mäkelä *et al*. 2000, Landsberg 2003). For instance, significant improvements in a mensurational yield model were achieved when replacing time for potentially intercepted radiation, and that model used to interpret a complex vegetation management experiment of Douglas-fir subject to varying levels of competition (Mason *et al.* 2007). Another novel application is given by Valentine and Mäkelä (2005) who fitted a hybrid model to standard forest inventory variables. The model was formulated using a carbon balance approach based on pipe model theory and fitted using both physiological and morphological parameters. This illustrates ample possibilities to combine the strengths of both mensurational and physiological approaches.

Hybrid models are at the stage at which they can be used to predict growth and yield and explore the effects of management decisions on forest stands (Landsberg 2003). The hybrid approach, as compared to mensurational and physiological ones, may better assist particular needs of a broader range of decision-makers from forest managers to politicians and scientists alike. However, further research in carbon allocation processes, nutrient availability in soils and nutrient uptake and cycling within the plant are seen as major challenges for future model development (Raison and Myers 1992, Waring *et al*. 1998, Johnsen *et al*. 2001, Landsberg 2003). Additionally, from a management and environmental perspective, the impact of genetically improved material and vegetation management techniques should be assessed (Boomsma and Hunter 1990, Raison and Myers 1992, Landsberg 2003).

Framework for modelling tree nutrition

Plant growth depends on carbon assimilation and distribution (Landsberg and Gower 1997) and therefore accounting for the carbon balance becomes the natural method to represent plant growth in physiological and hybrid models (Mäkelä 2003, Valentine and Mäkelä 2005). The carbon balance of a forest ecosystem is the net result of CO_2 assimilation by the fundamental process of photosynthesis and plant respiration (Figure 1.2), and strongly affected by site resource availability such as nutrients, water and light (Landsberg 1986). Plant respiration consists of both construction respiration that is proportional to plant growth and maintenance respiration proportional to each live biomass component (Makela 1986, Makela 2003).

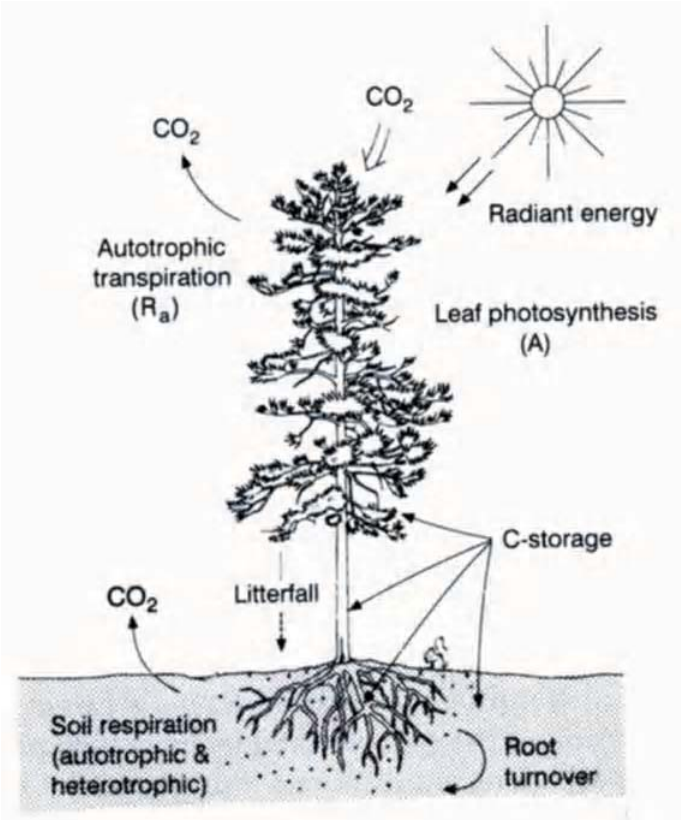


Figure 1.2. Simplified representation of the major components of the carbon balance of forest ecosystems. Leaf photosynthesis transforms CO_2 from the atmosphere into carbohydrates that are either stored in leaves, stems and roots or CO_2 respired by the tree (autotrophic respiration, R_a) or the soil microbial population (heterotrophic respiration, R_h). Litterfall and root turnover provides carbon to the soil that can be either respired ($R_a + R_h$) or stored. Net primary productivity (NPP) is the difference between total net carbon assimilated through photosynthesis (gross primary productivity, GPP) and autotrophic respiration (R_a). Net ecosystem productivity (NEP) is the net CO_2 flux to or from a forest ecosystem, and equal to total carbon assimilated (GPP) minus auto- (R_a) and heterotrophic respiration (R_h). Source: Landsberg and Gower (1997), p. 126.

The total net carbon assimilated through photosynthesis over a given time interval is known as gross-primary productivity (GPP), while net primary productivity (NPP) represents overall carbon assimilated minus autotrophic respiration (GPP-R_a) (Waring *et al.* 1998). Carbon assimilated through photosynthesis is allocated among tree components defining their ability for growth and survival (Landsberg and Gower 1997). Net ecosystem production (NEP) is the net flux of CO_2 from forest ecosystems and is calculated as the total net carbon assimilated through photosynthesis (GPP) minus autotrophic (R_a) and heterotrophic (R_h) respiration. Soil microbial respiration (heterotrophic) is caused by the decomposition of the litter and soil organic matter. Organic matter is added to the soil through litterfall and root and mycorrhizal turnover. NEP is the variable of primary concern for ecologists because it measures the net change in carbon content at the ecosystem level comprising vegetation, detritus and soil (Landsberg and Gower 1997).

Our ability to estimate carbon assimilated through photosynthesis has greatly improved in recent years with the advent of new experimental and modelling techniques (Waring *et al* 1998). Eddy correlation techniques currently allow precise measurements of CO_2 ecosystem exchange over complete growing seasons permitting testing and validation of many radiation interception and canopy photosynthesis models that provide reliable long-term estimates of GPP (Waring *et al*. 1998). However predicting forest productivity would usually require NPP, which has proven difficult to calculate because of the uncertainties associated with estimating below-ground carbon allocation and autotrophic respiration (Waring *et al*. 1998). Principles for studying plant growth and maintenance respiration were well established in the 1970s (Penning de Vries 1972, 1975, Amthor 2000), but theoretical and experimental improvements are continuously being made (e.g. Ryan *et al*. 1996, Stockfors and Linder 1998), yet the task ahead seems formidable particularly for the below-ground components (Waring *et al*. 1998).

Considering the processes involved in the carbon balance of forest ecosystems, the understanding of photosynthesis far outweighs the comprehension of respiration and carbon allocation (Landsberg and Gower 1997). Williams and Eamus (1997) emphasized that advances in instrumentation (i.e. portable infra-red gas analyzers) have allowed detailed studies on the relationship between assimilation, transpiration, stomatal conductance and environmental factors, but that the ecophysiology of roots and associated fungi remains largely in the 19th century. Advances in the study of belowground processes have been slow mainly because of sampling and measurement problems and assumptions difficult to hold besetting all current methods utilized to estimate below-ground carbon fluxes and particularly fine-root production and turnover (Landsberg and Gower 1997, Waring *et al*. 1998).

Trees allocate a greater proportion of carbon to roots in poor fertility environments as shown by many studies (e.g. Chapin 1980, Albaugh *et al*. 1998, Zerihun and Montagu 2004). In early modelling studies, allocation coefficients were assumed to be constant providing qualitative realistic trends of whole-stand growth (McMurtrie and Wolf 1983). Since the causal explanation of carbon allocation is largely unknown, Landsberg (1986) proposed the use of allometric equations to constrain carbon allocation, in a way that after growth and turnover, the allometric proportions would be maintained. Causal explanations of carbon allocation have been given through the transport-resistance model (Thornley, 1969, 1972, 1991), the nitrogen-productivity concept (Ågren and Ingestad 1987), and pipe-model theory (Valentine 1985, Mäkelä 1986). Yet the validity of these models as predicting tools remains largely untested.

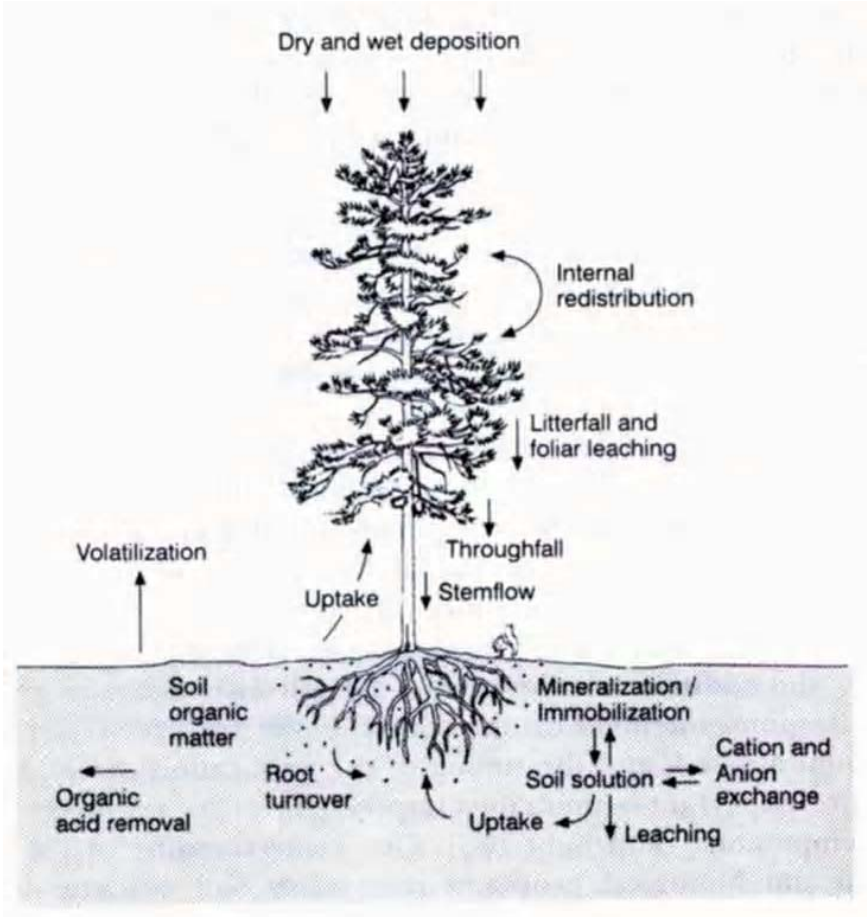


Figure 1.3. Simplified representation of the major components of the nutrient cycle of forest ecosystems. Soil weathering provides most phosphorus while microbially-based mineralization provides most nitrogen taken up by plants. Source: Landsberg and Gower (1997), p. 186.

The integration of the carbon (Figure 1.2) and the nutrient cycles (Figure 1.3) provides a sound framework for testing hypotheses on the influence of plant and soil nutrition on the carbon balance of forest ecosystems. While the weathering of soils and parent materials is the main source for phosphorus, nitrogen becomes available to plants almost exclusively by the action of the soil microbial population which either fixes atmospheric nitrogen into the soil (nitrogen fixation) or breaks-down the soil organic matter into inorganic forms of nitrogen that are taken up by the plant (Landsberg and Gower 1997). Soil mineralization provides the main source of nitrogen in a form that can be readily used by plants, explaining the often observed good correlation between forest productivity and nitrogen mineralization in temperate forests (Reich *et al*. 1997, Landsberg and Gower 1997). Litterfall and root turnover are the main sources of organic matter, and hence mineralization, to forest soils (Landsberg and Gower 1997).

Most plants acquire a large proportion of some nutrients through symbiotic relations with mycorrhizae (Read 1991). Mycorrhizae are thought to uptake nutrients at a lower carbon cost than roots as a result of their greater surface to volume ratio (Aerts and Chapin 2000). However, factors controlling the distribution and function of mycorrhizal fungi in the field are poorly understood (Sylvia and Jarstfer 1997) and conditions under which the carbon cost of mycorrhizae outweigh the plant benefits are unknown (Read 1991). Soderstrom (1991) postulated that plants may invest as much as 20% of assimilated carbon into mycorrhizae, and Hobbie (2006) showed that carbon allocation to ectomycorrhizae ranged from 1% to 21% of net primary production in culture studies, being highest under low nutrient availability conditions. Respiration costs of mycorrhizae remain, however, largely unknown (Landsberg and Gower 1997).

Nutrient remobilization from aging foliage and other tissues has been shown to be an essential and significant source of some nutrients for growth (Fife and Nambiar 1982, Aerts and Chapin 2000). Nutrient remobilization may account for 50-60% of nitrogen and phosphorus demand required to drive new growth in *Pinus radiata* (Turner and Lambert 1986). Nutrient remobilization is seen today as a plant mechanism to supply nutrients to new growth rather than an exclusive mechanism of adaptation to low fertility environments (Nambiar and Fife 1987, Millard and Proe 1993).

Nutrient remobilization does not occur evenly throughout the growing season but follows a cyclic pattern (Knight 1978, Fife and Nambiar 1982, 1984, Nambiar and Fife 1991). Sheriff *et al*. (1986), working with *Pinus radiata* in South Australia's Mediterranean climate, showed that the content of N and P in needles fluctuated in a cycle, showing distinct phases of accumulation (spring to early summer), remobilization (summer) and replenishment (autumn-winter). Similarly, Bloom *et al*. (1985) argue that nutrients may accumulate when they are abundantly supplied and consumed in subsequent growth when these resources are externally limited acting as a buffering factor offsetting the asynchrony of resources. Yet controversy still exists as to whether remobilization efficiency is larger in poor or rich fertility environments, and also whether genotypes may exhibit differences in remobilization performance.

The corollary to this review is that further advances in the representation of nutrition in physiological growth models requires experimentation and sound hypothesis testing in the processes that control the carbon balance of forest ecosystems i.e. photosynthesis, respiration and carbon allocation, and how these processes are affected by soil and plant nutrition.

AIMS AND SCOPE OF THE STUDY

Objectives

The aim of this thesis was to assess means to represent nutrition and genotype-fertility interactions in tree productivity hybrid models and specifically,

1. To assess stomatal, mesophyll and biochemical limitations to photosynthesis as posed by nutrient deficiencies
2. To estimate internal nutrient remobilization responses to nutrient deficiencies
3. To compare growth and carbon allocation patterns of *Pinus radiata* across fertility gradients
4. To assess key physiological processes that may explain differences in genotypic growth performance in relation to nutrition
5. To discuss means by which the physiological processes previously outlined (objectives 1-3) may be integrated in physiological or hybrid models to represent nutrition and genotype-fertility interactions.

The study targets *Pinus radiata*, as the most outstanding conifer species in plantation forestry in the Southern Hemisphere (Lewis and Ferguson 1993), and nitrogen and phosphorus as the most important nutrient deficiencies limiting plantation forestry in New Zealand (Watt *et al*. 2005) and in most terrestrial ecosystems (Aerts and Chapin 2000).

Hypotheses

The influence of nutrition on the carbon balance was hypothesized to operate through key physiological processes i.e. photosynthesis, carbon allocation and nutrient remobilization. The hypothetical model was developed conceptually as described in Figure 1.4. Improved nutrition is hypothesized to increase both leaf area and rates of photosynthesis per unit leaf area, which overall increases canopy photosynthesis (GPP). Improved nutrition may affect carbon allocation to net primary production (NPP), auto- (R_a) and heterotrophic (R_h) respiration, and NPP allocation to different plant components. Improved nutrition may also affect nutrient remobilization which in turn may increase leaf area and photosynthetic rates.

Genotype was hypothesized to be expressed through the same physiological processes as nutrition. Faster-growing genotypes may allocate more carbon to photosynthetic and structural organs at the expense of below-ground components, may exhibit higher photosynthetic rates that may increase leaf area and together overall assimilation, and may exhibit higher nutrient remobilization efficiency than slower-growing genotypes. Hence, the integration of all these processes may help to explain differences in genotypic growth performance.

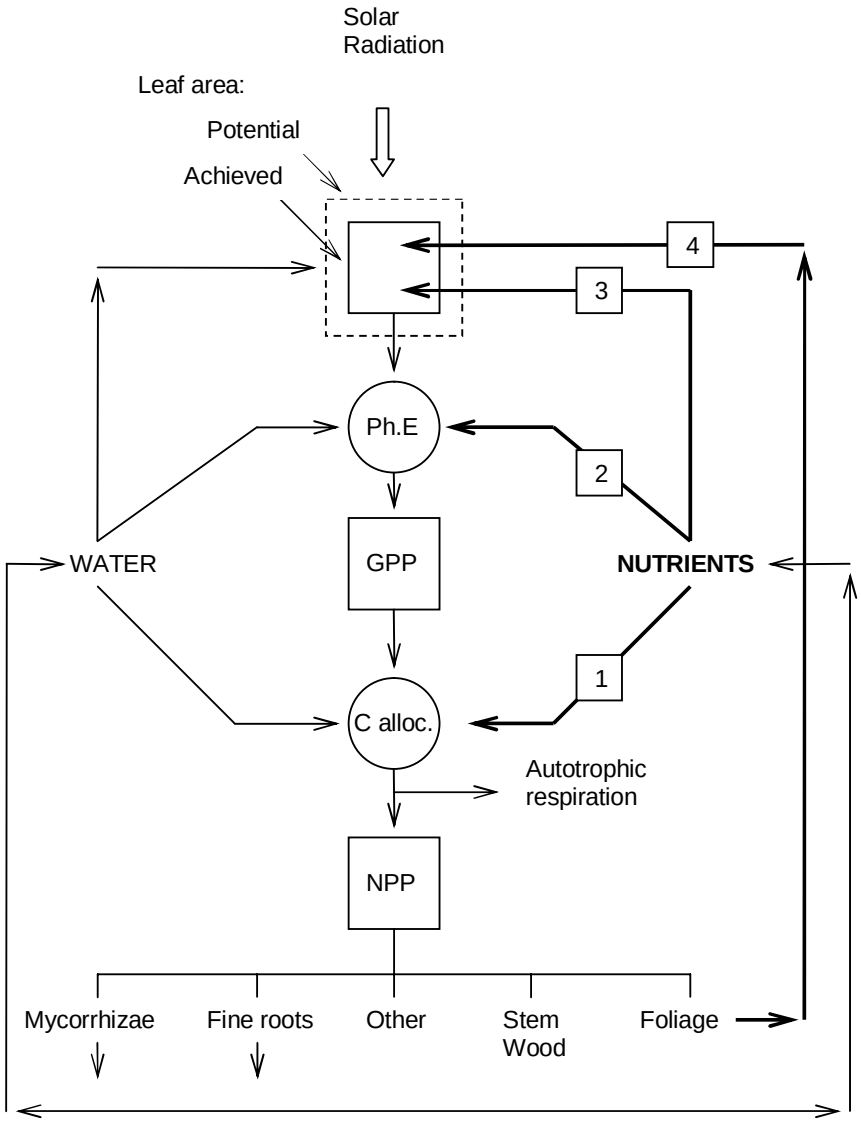


Figure 1.4. Hypothetical model to explain the influence of nutrition and genotype on productivity of *Pinus radiata*. Bold arrows and numbers within boxes represent main influences of nutrition on: 1, carbon allocation; 2, photosynthetic efficiency (Ph.E.); 3, nutrient remobilization and 4, leaf area development. Genotype is hypothesized to operate through the same pathways as nutrition. Net primary productivity (NPP) is gross-primary productivity (GPP) minus autotrophic respiration. Mycorrhizal symbiosis is seen as a source of nutrients and a sink for carbon. Redrawn and modified from the description of FORCYTE by Kimmins *et al*. (1990).

Synopsis of experiments

Two experiments were carried out in the greenhouse: one to assess internal nitrogen cycling and the second to assess patterns of carbon allocation. Both were carried out with one-year old *Pinus radiata* cuttings from four genotypes (two in common) cultivated in silica sand with a factorial combination of nitrogen (N_0=1.43 and N_1=7.14 mM) and phosphorus (P_0=0.084 and P_1=0.420 mM) supply. Clones were selected within a set of 400 genotypes planted in the Purokohukohu Experimental Basin (Beets *et al*. 2004), based on growth data and the known relationship that fast-growing genotypes exhibit lower nutrient concentrations but higher nutrient content than slow-growing genotypes (Carson *et al*. 2004). In the internal nitrogen cycling experiment, N supply was enriched with ^{15}N to 2.5‰ (labelled N) during the first year, then plants transferred to clean sand and cultivated for another year with ^{15}N at natural abundance. In the carbon allocation experiment, plants were cultivated for ten months and carbon allocation above- and below-ground determined based on a carbon balance approach (Giardina *et al*. 2003).

Plant material from the internal nitrogen cycling experiment was used to assess stomatal, mesophyll and biochemical limitations to photosynthesis (Objective 1). Recovery of labelled nitrogen was used to assess nitrogen remobilization responses under different nitrogen and phosphorus supply regimes (Objective 2). Whole-carbon budgets were used to compare plant patterns of carbon allocation across different nutrient supply regimes (Objective 3). Genotypes were compared in terms of photosynthesis, internal nitrogen cycling and carbon allocation patterns using plant material from both greenhouse experiments (Objective 4).

Field experimentation was carried out in a subset of locations where ENSIS (formerly New Zealand Forest Research Institute) had established trials to test the influence of species (*Cupressus lusitanica* or *Pinus radiata*), soil disturbance (disturbed or undisturbed) and plant nutrition (fertilized or control) on sustainability indicators (Watt *et al*. 2005). A carbon balance method was used to estimate patterns of carbon allocation in control and fertilized mini-plots of *Pinus radiata* located in five sites with contrasting soil and climatic conditions in the South Island of New Zealand. Examination of patterns of carbon allocation in the greenhouse and the field were complementary (Objective 3).

Findings from both the greenhouse and the field, were applied to one hybrid and one physiological model (i.e. Landsberg and Waring 1997, Whitehead *et al*. 2002), in order to represent nutrition and genotype in hybrid models (Objective 5).

Thesis structure

The hypothesis for the study was that understanding the effects of nutrient limitations on key physiological processes (i.e. photosynthesis, carbon allocation and nutrient remobilization) may allow us to better represent plant nutrition in physiological and hybrid models. These physiological processes are addressed in Chapters 2-6, and integrated in physiological and hybrid models in Chapter 7 (Figure 1.5). As greenhouse experiments comprised several clones, genetic controls to productivity are addressed in chapters 2-5 in relation to photosynthesis (Chapter 2 and 3), nutrient remobilization (Chapter 4) and carbon allocation (Chapter 5), and also integrated in Chapter 7 as a means to represent genotype-fertility interactions on productivity physiological and hybrid models.

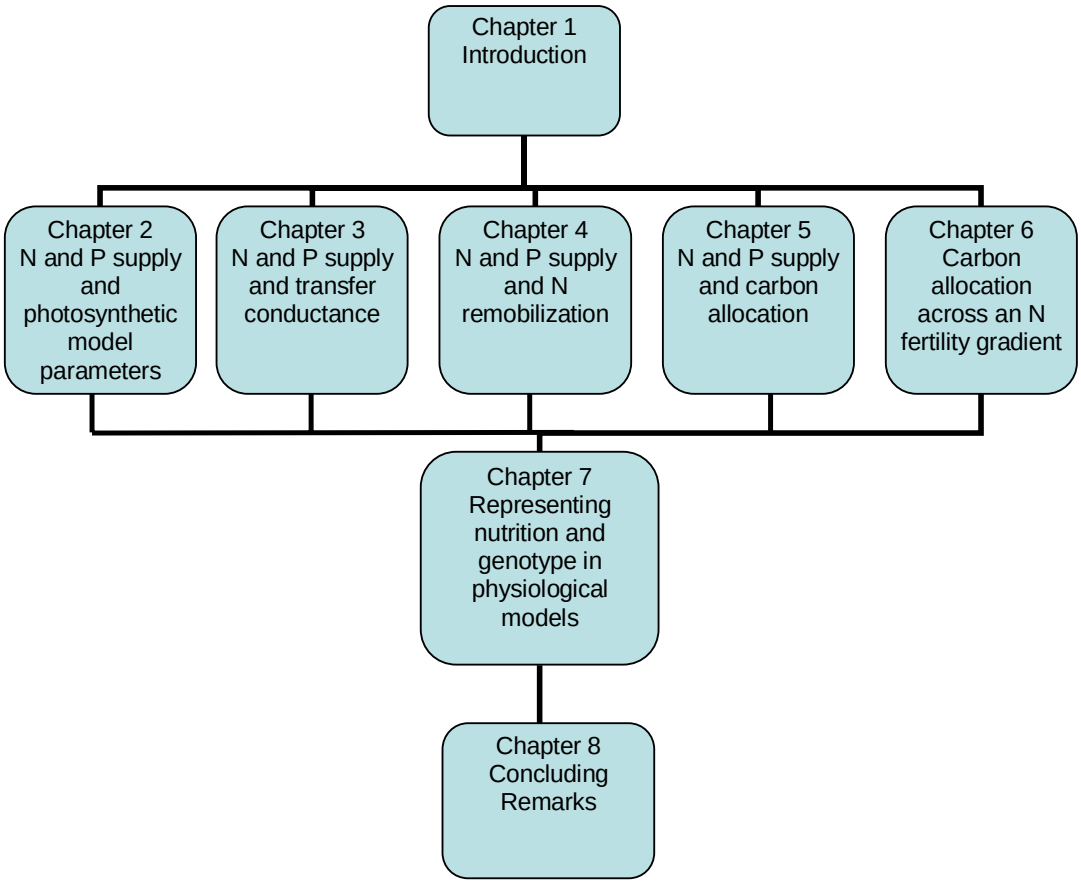


Figure 1.5 Diagrammatic representation of chapters in this thesis

The aim of Chapter *Two* was to determine the combined influence of nitrogen and phosphorus supply on photosynthetic model parameters, and to determine whether genotypes with contrasting growth may differ in photosynthetic performance. The biochemical model of leaf photosynthesis by Farquhar *et al*. (1980) is widely used in ecophysiological research, and its parameters V_{cmax} and J_{max} have been shown to scale linearly with foliar nitrogen concentration (Field and Mooney 1986, Evans 1989, Walcroft *et al*. 1997). However, only a few studies have characterized the relationship between these parameters and foliar phosphorus in pines (Conroy *et al*. 1986, Reich and Schoettle 1988, Loustau *et al*. 1999), and studies on the interactive influence of nitrogen and phosphorus supply on these photosynthetic parameters have apparently not been conducted.

The CO_2 concentration in the chloroplasts, once assumed to be equal to that in the intercellular air spaces, is now known to be lower and measured by a term called transfer conductance (Harley *et al*. 1992, Loreto *et al*. 1992, von Caemmerer 2000, Warren *et al*. 2003*b*). Transfer conductance has been measured mostly in angiosperms but only in a few conifers (e.g. Warren *et al*. 2003*b*, De Lucia *et al*. 2003, Warren 2006), and most studies have focused on comparisons across plant functional groups (e.g. Loreto *et al*. 1992) rather than gradients in environmental stresses (e.g. Warren *et al*. 2004, Grassi and Magnani 2005). Nitrogen and phosphorus are the most likely nutrients limiting primary productivity (Aerts and Chapin 2000, Hall *et al*. 2005) and therefore reductions in transfer conductance with nutrient deficiencies might be expected. The aim of Chapter *Three* was to examine simultaneous influences of nitrogen and phosphorus supply on transfer conductance, and to test whether two genotypes with contrasting growth patterns may exhibit differences in transfer conductance to combined nitrogen and phosphorus limitations.

Foliage nutrient concentrations in conifers in Mediterranean climates are known to follow a seasonal pattern (Fife and Nambiar 1982, 1984, Nambiar and Fife 1991) with distinct phases of accumulation (spring to early summer), remobilization (summer) and replenishment (autumn-winter) (Sheriff *et al*. 1986), and this phenomena has been attributed to nutrient remobilization from old tissues to new tissues to support new growth (Nambiar and Fife 1991). Nutrient remobilization in conifers accounts for 50% or more of nutrient demand (Sheriff *et al*. 1986), and is recognized of prime significance in the nutrient economy of plants (Aerts and Chapin 2000). Chapter *Four* examined the influence of nitrogen and phosphorus supply on nitrogen storage and remobilization capacity of four clones.

Whole-carbon budgets are a relatively recent development which has been greatly assisted by the development of portable gas exchange systems, and methods to estimate carbon

allocation belowground (Raich and Nadelhoffer 1989, Giardina and Ryan 2002). This tool has permitted us to better understand environmental controls on productivity, and to determine patterns of carbon allocation in a so far reduced range of forest ecosystems (e.g. Giardina *et al*. 2002, Hamilton *et al*. 2002, Giardina *et al*. 2003, Stape *et al*. 2004*b*). Chapter *Five* examines patterns of carbon allocation in *Pinus radiata* clones cultivated in a greenhouse with a factorial combination of nitrogen and phosphorus supply using a carbon balance approach. Similarly Chapter *Six* examines patterns of carbon allocation in control and fertilized mini-plots of *Pinus radiata* in five sites with different soil and environmental conditions in the South Island of New Zealand.

Chapter *Seven* discusses ways to advance our ability to model nutrition from a mechanistic perspective based on results from Chapters *Two* to *Six*. One hybrid and one physiological model (i.e. Landsberg and Waring 1997, Whitehead *et al*. 2002) are used as a framework for the discussion. These models were selected because they represent different mechanistic approaches and had been used successfully to model the outcome of physiological processes in *Pinus radiata* plantations in New Zealand.

Chapter *Eight*, concluding remarks, summarizes key findings of this thesis, with emphasis on physiological and hybrid models and future directions in which representing plant nutrition may be directed. This thesis comprises eight chapters which except for the first and the last two, stand alone as one or several original papers available for submission to a journal. Chapter *Two* has been published (*Tree Physiology* 27:335-344, 2007). Some preliminary results and potential journal articles which complement the results of the thesis are also presented in Appendices A-D.

CHAPTER TWO

PARTITIONING CONCURRENT INFLUENCES OF NITROGEN AND PHOSPHORUS SUPPLY ON PHOTOSYNTHETIC MODEL PARAMETERS OF *PINUS RADIATA*

Summary Responses of photosynthesis (A) to intercellular CO_2 concentration (C_i) were measured in a fast- and a slow-growing clone of *Pinus radiata* D. Don cultivated in a greenhouse with a factorial combination of nitrogen and phosphorus supply. Stomatal limitations scaled with nitrogen and phosphorus supply as a fixed proportion of the light-saturated photosynthesis rate (18.5%) independent of clone. Photosynthetic rates at ambient CO_2 were mainly in the portion of the CO_2 response rate that is limited by maximal carboxylation rate (V_{cmax}) at low- nitrogen supply and at the transition between V_{cmax} and J_{max} at high-nitrogen supply. Nutrient limitations to photosynthesis were partitioned based on the ratio of foliage nitrogen to phosphorus expressed on a leaf area basis (N_a / P_a), by minimizing the mean square error of segmented linear models relating photosynthetic parameters (V_{cmax}, J_{max}, T_p) to foliar nitrogen and phosphorus concentrations. A value of N_a / P_a equal to 23 (mole basis) was identified as the threshold separating nitrogen (N_a / $P_a \leq 23$) from phosphorus (N_a / $P_a > 23$) limitations independent of clones. On an area basis, there were significant positive linear relationships between the parameters, V_{cmax}, J_{max}, T_p and N_a and P_a, but only the relationships between T_p and N_a and P_a differed significantly between clones. These findings suggest that, in genotypes with contrasting growth, the responses of V_{cmax} and J_{max} to nutrient limitation are equivalent. The relationships between the parameters V_{cmax}, J_{max}, T_p and foliage nutrient concentration on a mass basis were unaffected by clone, because the slow-growing clone had a significantly greater leaf area to mass ratio than the fast-growing clone. These results may be useful in discriminating nitrogen-limited from phosphorus-limited photosynthesis.

Keywords: electron transport, genotype, nutrient limitation, nutrient ratio, Rubisco carboxylation, stomatal limitation, triose phosphate.

INTRODUCTION

Recent physiological models of forest production include biochemical descriptions of CO_2 assimilation to represent the carbon balance of leaves, canopies and ecosystems (Wullschleger 1993), because CO_2 plays a major role in global environmental change, carbon balance, net primary production and water- and nutrient-use efficiency (Jarvis 1995). Despite the existence of several models of carbon assimilation, the biochemical model originally proposed by Farquhar *et al*. (1980), and later improved (von Caemmerer and Farquhar 1981, Sharkey 1985, Harley and Sharkey 1991), is widely used in ecophysiological research for describing CO_2 exchange processes (e.g. McMurtrie *et al*. 1992, Baldocchi and Harley 1995, Whitehead *et al*. 2004*a*). For instance, this model has been used to explain the mechanisms of photosynthetic acclimation to elevated atmospheric CO_2 concentration (Kellomäki and Wang 1996, Hogan *et al.* 1996, Turnbull *et al*. 1998, Murray *et al*. 2000, Griffin *et al*. 2000), the influence of global warming on plant carbon budgets (Turnbull *et al*. 2002), and for identifying factors limiting photosynthesis and estimating net carbon uptake in shrubland and forests in New Zealand (Whitehead *et al*. 2002, Whitehead *et al*. 2004*b*, Whitehead and Walcroft 2005).

Leaf photosynthetic rates are influenced by plant nutrition, radiation, leaf age, and water stress (Farquhar *et al*. 1980, von Caemmerer and Farquhar 1981, Leuning 1995). Among these factors, nutrition seems to be the one currently represented with less confidence in physiological models (Landsberg *et al*. 1991). Nitrogen and phosphorus are the nutrients most frequently limiting primary producers in terrestrial (Aerts and Chapin 2000) and aquatic ecosystems (Hall *et al*. 2005). These nutrients provide an interesting contrast because they differ in their mobility in the soil, their metabolic function in the plant and their partitioning within the leaf (Conroy 1992).

In the C_3 photosynthesis model described by Farquhar *et al*. (1980), CO_2 assimilation is limited by the maximal rate of ribulose-1,5-*bis*phosphate (RuBP) carboxylase-oxygenase (Rubisco) carboxylation (V_{cmax}), by the maximal electron transport rate driving regeneration of RuBP (J_{max}), and in some cases, particularly at high irradiance and high CO_2 concentrations, by the rate of triose phosphate export (T_p) (von Caemmerer and Farquhar 1981, Sharkey 1985, Harley and Sharkey 1991, von Caemmerer 2000). Values of V_{cmax} and J_{max} have been shown to scale linearly with foliar nitrogen concentration (Field and Mooney 1986, Evans 1989, Walcroft *et al*. 1997). However, few studies have characterized the relationships between V_{cmax} and J_{max} and foliar phosphorus in pines (Conroy *et al*. 1986, Reich and Schoettle 1988, Loustau *et al*.

1999), and investigations on the interactive influence of nitrogen and phosphorus on these photosynthetic parameters in pines have apparently not been conducted.

Pinus radiata D. Don is the most widely planted conifer species in New Zealand and in the southern hemisphere (Lewis and Ferguson 1993). Therefore understanding how limitations are imposed on the carbon budget is relevant for assessing sustainability, and for estimating carbon uptake by this species. Recently, Watt *et al*. (2005) identified key productivity drivers across 35 sites covering gradients of environmental and edaphic conditions for New Zealand plantation forests. Temperature and rainfall were identified as the main environmental drivers, and total soil nitrogen and total soil phosphorus were identified as the key edaphic determinants of forest productivity. Phosphorus is expected to be even more important in the future because higher foliar phosphorus would be required to realize the maximum growth potential of pines at elevated CO_2 concentrations (Conroy *et al*. 1990).

The aim of the study was to examine concurrent influences of nitrogen and phosphorus supply on photosynthetic parameters of the Farquhar *et al*. (1980) model, and to determine if individual nutrient influences on photosynthesis can be partitioned in *Pinus radiata*. A third objective of the study was to test the extent to which two genotypes with contrasting growth patterns may exhibit differences in photosynthetic response to combined nitrogen and phosphorus limitation.

MATERIALS AND METHODS

Plant material

Plant material was selected from a greenhouse experiment laid out in a factorial design with two clones, two nitrogen supply regimes (N_0=1.43 and N_1=7.14 mM) and two phosphorus supply regimes (P_0=0.084 and P_1=0.420 mM). A fast- and a slow- growing clone (Clones A and B, respectively) were selected within a set of 400 genotypes planted in the Purokohukohu Experimental Basin (Beets *et al*. 2004).

One-year old *Pinus radiata* cuttings from Clones A and B were raised under standard ENSIS (formerly New Zealand Forest Research Institute) nursery conditions and transplanted to 4.25-dm^3 pots containing silica sand. Nutrient treatments were randomly allocated to the plants and applied for nine months from winter 2004 until photosynthesis measurements were taken in early autumn 2005. Plants received 0.5 dm^3 of nutrient solution per week and were watered daily. Nutrients other than N and P were provided at 1.023 mol m^{-3} K, 0.250 mol m^{-3} Ca, 0.411

mol m^{-3} Mg, 0.281 mol m^{-3} S, 12.532 mmol m^{-3} Fe, 0.445 mmol m^{-3} Zn, 0.473 mmol m^{-3} Cu, 7.281 mmol m^{-3} Mn, 0.073 mmol m^{-3} Mo, 18.502 mmol m^{-3} B, 0.946 mmol m^{-3} Cl and 0.145 mmol m^{-3} Na following Ingestad (1979). Plants were grown in a thermostatically controlled greenhouse where temperature fluctuated between 16 and 30 °C during the day, and between 8 and 18 ºC during the night depending on weather conditions. Roots of all plants were artificially inoculated with spores of *Rhizopogon rubescens* Tul. and confirmed as mycorrhizal either by visual inspection of roots or by the presence of fruiting bodies.

Gas exchange measurements

Photosynthesis was measured in 7-10 plants per treatment per clone. Plants were moved from the greenhouse to a growth cabinet the day before measurements were made. The growth cabinet provided a 12-h photoperiod at 720 μmol m^{-2} s^{-1}, with a day / night temperature of 20 / 18°C and a relative humidity of 80%. All gas exchange measurements were made in the growth cabinet during daylight hours in early autumn from March 8 to 23, 2005.

The response of net assimilation to internal CO_2 concentration (A / C_i curves) was measured with a portable photosynthesis system (Li-6400, Li-Cor, Lincoln, NE). For each plant, three fascicles were arranged in a 6-cm^2 cuvette avoiding shading between needles. Temperature in the cuvette was maintained at 20 ºC while leaf-to-air vapor pressure deficit (VPD) ranged from 1 to 1.5 kPa. External CO_2 concentration (C_a) was supplied with a CO_2 mixer across the series 360, 300, 200, 150, 125, 100, 75, 50, 360, 450, 600, 800, 1000, 1200, 1500 μmol mol^{-1}, with saturating irradiance, Q (400-700 nm), maintained at 1500 μmol photons m^{-2} s^{-1}. The CO_2 response curve for each plant was generally completed in one hour. Measurements were recorded after values of A, C_i and g_s were stable (coefficient of variation ≤ 2%), which typically took between 2 to 4 min (cf. Long and Bernacchi 2003).

A/Q curves were used to check that illumination from one side of the cuvette was sufficient to saturate photosynthesis over the entire needle area for both clones in all treatments. An index of light saturation (L) was determined as the ratio of A_{sat} measured at Q equal to 1500 and 2000 μmol photons m^{-2} s^{-1}. Main and interactive effects of nutrient treatment and clone on L were not significant ($F_{7,67} = 0.78$, $P = 0.61$). Mean values (± standard deviation) of L were 97.6% ± 2.7%, suggesting that saturating photosynthesis was effectively achieved and that the gas exchange measurements were not confounded by differences in the leaf area to mass ratio across treatments or clones.

Stomatal and non-stomatal limitations to photosynthesis

The leaf photosynthesis model described by Farquhar *et al*. (1980), and later improved (von Caemmerer and Farquhar 1981, Sharkey 1985, Harley and Sharkey 1991), was used to examine the limitations that a factorial combination of nitrogen and phosphorus supply impose on carbon assimilation. These equations were fitted to the A/C_i curves by non-linear least squares regression (SigmaPlot, Version 7.1, SPSS, Chicago, IL). Values of V_{cmax} and R_d were estimated from the lower part of the A/C_i curve (C_i < 220 μmol mol^{-1}), and these values were then used to estimate J_{max} and T_p over the whole range of measured C_i. The model fitted the data well (r^2 > 0.96) with little apparent bias (data not shown). Michaelis-Menten constants of Rubisco for CO_2 and O_2, K_c and K_o, and CO_2 compensation point in the absence of mitochondrial respiration, Γ^*, used in the fitting were 302 μmol mol^{-1}, 256 mmol mol^{-1} and 34.6 mol mol^{-1}, respectively, as published by Leuning (1995).

The A to C_i response was also used to separate stomatal from non-stomatal limitations, using the method of Farquhar and Sharkey (1982) as $L_S = (1 - A/A_0)$ where A is the photosynthetic rate at atmospheric CO_2 concentration (C_a = 360 μmol mol^{-1}) and A_0 is the photosynthetic rate that would occur if stomatal conductance (g_s) to CO_2 were infinite (C_i = 360 μmol mol^{-1}). For this calculation, mesophyll conductance (g_m) was considered to be infinitely large. Relative non-stomatal limitations to photosynthesis were estimated as 1 - L_S.

Genotypes were compared in terms of stomatal and non-stomatal limitations to photosynthesis, light-saturated photosynthetic rate at ambient C_a (A_{sat}) and instantaneous photosynthetic nutrient-use efficiencies which were calculated as the ratio of A_{sat} to either N_a (nitrogen-use efficiency; E_N) or P_a (phosphorus-use efficiency; E_P).

Foliage surface area and nutrient concentrations

Following the completion of A/C_i curves, foliage samples were carefully removed from the cuvette and cut to match the leaf area exposed to gas exchange. Total surface area of needles was determined based on water volume displacement as described by Johnson (1984). All measurements and analysis are reported on a total leaf area basis. To convert gas exchange measurements to a projected leaf area basis, values should be multiplied by π (Grace 1987). Foliage samples were dried at 70 °C to constant mass and dry mass recorded. For foliar chemical analysis, leaf samples were finely ground and acid-digested by a Kjeldahl method (Blakemore *et al*. 1987). Nitrogen and phosphorus in the digests were determined

colorimetrically by the Landcare Research Laboratories, Palmerston North, New Zealand. Nitrogen and phosphorus concentrations were expressed on a total leaf area (N_a, P_a) and a mass basis (N_m, P_m).

Growth and fascicle size measurements

Plant diameter and height growth are reported for the nine months of the experiment. Estimates of leaf area were determined as the product of leaf mass and the leaf area to mass ratio. Foliage mass at month 9 was estimated from the following equations of foliage dry mass, W, (g) against plant diameter, D, (mm) developed at month 11; Clone A, $W = 0.0387\ D^{2.7218}$, $r^2 = 0.94$, $P < 0.001$; and Clone B, $W = 0.0863\ D^{2.4091}$, $r^2 = 0.92$, $P < 0.001$. Reported measurements of fascicle diameter, length and leaf area were determined at month 11.

Data analysis

All subsequent analyses were made at the plant level with SAS software (1996; SAS Institute, Cary, NC). Variables were tested for normality and homogeneity of variance and transformations were made as necessary to meet the underlying statistical assumptions of the models used. The main and interactive effects of nitrogen and phosphorus supply and genotype on photosynthetic model parameters were examined by analysis of variance. Tukey's least significant difference test was used to distinguish among individual means where applicable with a confidence level of $P \leq 0.05$. Differences in slopes and intercepts between clones in the linear relationships between photosynthetic model parameters and foliage nutrient concentration were tested for significance by analysis of covariance.

With the N_a / P_a ratio as a threshold value, the population of observations were partitioned as either nitrogen deficient ($N_a / P_a \leq$ threshold) or phosphorus deficient ($N_a / P_a >$ threshold). Segmented linear models of V_{cmax}, J_{max} and T_p were fitted to foliage nitrogen and phosphorus concentrations, based on threshold values of N_a / P_a ranging from 15 to 27 mol mol^{-1}. From this range the threshold was empirically determined as the value which minimized the model mean square error. To ensure the final threshold value was realistic, the range over which the threshold value was determined (15 to 27 mol mol^{-1}) was constrained to values of N_a / P_a over which neither nitrogen nor phosphorus was severely deficient. These bounds were determined as the upper and lower 95% confidence limits, respectively, for N_a / P_a within the nitrogen-deficient treatment, N_0P_1, (mean and SD = 9.4 ± 2.8) and the phosphorus-deficient

treatment, N_1P_0, (mean and SD = 50.4 ± 12.1), respectively. Both populations were normally distributed (Shapiro-Wilkinson test, $P > 0.08$).

To determine whether A at ambient CO_2 was in the V_{cmax}, J_{max} or T_p limited portion of the CO_2 response, values of C_i at ambient CO_2 were compared with values of C_i at the intersections between the V_{cmax}/J_{max} and J_{max}/T_p portions of the CO_2 response curve. Based on that categorical classification, Fisher's exact test was performed to determine whether nutrient treatment and clone had an influence on the main biochemical limitation at ambient CO_2 concentration.

RESULTS

Treatment influences on growth

Plant growth in diameter, height and leaf area was strongly influenced by the main effects of nutrient treatment ($F_{3,60}$ = 24 - 47, $P < 0.001$) and clone ($F_{1,60} > 7$ - 28, $P < 0.01$) but not by their interaction ($F_{3,60} < 0.18$ - 1.84, $P > 0.15$) during the 9-month experiment (Table 2.1). All three growth variables scaled positively with nutrient supply and were greater in clone A than clone B (Table 2.1). Fascicle diameter, length and leaf area were also strongly influenced by the main effects of nutrient treatment ($F_{3,60}$ = 16 - 34, $P < 0.001$) and clone ($F_{1,60}$ = 8 - 43, $P < 0.007$) but not by their interaction ($F_{3,60}$ = 0.22 - 0.52, $P > 0.67$). Fascicle diameter, length and leaf area scaled positively with nutrient supply and were greater in clone A than clone B (Table 2.1). The leaf area to mass ratio (M) was significantly higher in clone B than clone A ($F_{1,60}$ = 80, $P < 0.001$) and also sensitive to the interaction between clone and treatment ($F_{3,60}$ = 6, $P < 0.01$).

Stomatal and non-stomatal limitation to photosynthesis

Stomatal limitations, L_S, were relatively small (18.5%) compared with non-stomatal limitations (81.5%) and this proportion was not influenced by main or interactive effects of nutrient treatment and clone ($F_{7,60}$ = 2.02, P = 0.07) (Table 2.1). In absolute terms stomatal conductance (g_s) significantly increased with nutrient supply ($F_{3,60}$ = 3.08, P = 0.03) from about 38 to about 51 mmol m^{-2} s^{-1} in the low-nutrient compared with the high-nutrient supply treatment independent of clone ($F_{1,60}$ = 11.13, P = 0.29) (Table 2.1).

Table 2.1. Plant, foliage and photosynthetic characteristics of *Pinus radiata* growing in a factorial combination of nitrogen and phosphorus supply. Values are presented as means (± 1 SE) of plant growth in basal diameter, height and leaf area over the duration of the experiment (9 months), fascicle diameter, length, and leaf area per fascicle, leaf area to mass ratio (M), foliage nitrogen (N_a) and phosphorus (P_a) concentrations on an area basis and their ratio (N_a/P_a), stomatal conductance to CO_2 diffusion (g_s), relative stomatal limitation to photosynthesis (L_S), maximal rate of rubisco carboxylation (V_{cmax}), maximal rate of electron transport driving regeneration of RuBP (J_{max}) and rate of triose phosphate export (T_p); n = 7-10. Significance of main effects of clones (C) and nutrient treatments (T) or the interaction between clones and treatments (C × T) are shown as: ns, non significant; *, significant at $P < 0.05$; **, significant at $P < 0.01$; ***, significant at $P < 0.001$). Separation of means was determined by a Tukey test where applicable. Different letters within treatments or clones indicate that means were significantly different at $P < 0.05$. Values of M and P_a are separated by clone (labeled at the far right of the row) as the interaction C × T was significant. The P-range between clones within the same treatment is reported in brackets for M and P_a.

Parameter	Treatments				Clones		ANOVA statistics		
	N_0P_0	N_0P_1	N_1P_0	N_1P_1	A	B	C	T	C x T
Diameter Growth (mm)	5.01 ± 0.23 a	5.59 ± 0.22 a	6.23 ± 0.20 b	8.42 ± 0.39 c	6.99 ± 0.31 a	5.72 ± 0.22 b	***	***	ns
Height Growth (mm)	299.9 ± 21.9 a	369.6 ± 18.8 a	358.1 ± 25.1 a	537.3 ± 19.7 b	421.5 ± 22.5 a	361.3 ± 19.0 b	***	***	ns
Leaf area growth (m^2)	0.28 ± 0.02 a	0.33 ± 0.02 ab	0.37 ± 0.02 b	0.63 ± 0.04 c	0.45 ± 0.03 a	0.35 ± 0.02 b	***	***	ns
Fascicle diameter (mm)	1.17 ± 0.03 a	1.23 ± 0.03 ab	1.34 ± 0.05 b	1.46 ± 0.04 c	1.41 ± 0.03 a	1.2 ± 0.03 b	***	***	ns
Fascicle Length (mm)	88.8 ± 1.9 a	89.6 ± 2.5 ab	103.0 ± 3.5 b	123.9 ± 3.8 c	105.6 ± 3.4 a	96.8 ± 2.9 b	**	***	ns
Fascicle area (cm^2)	6.70 ± 0.3 a	6.95 ± 0.32 a	8.76 ± 0.49 b	11.61 ± 0.53 c	9.31 ± 0.47 a	7.70 ± 0.38 b	***	***	ns
M (m^2 kg^{-1})	A 15.57 ± 0.21 a (***)	16.04 ± 0.51 a (***)	16.49 ± 0.61 a (ns)	15.83 ± 0.49 a (***)			***	ns	**
	B 20.60 ± 0.57 a	20.15 ± 0.89 a	17.32 ± 0.41 b	19.72 ± 0.55 a					
N_a (mmol m^{-2})	28.00 ± 1.14 a	28.66 ± 1.43 a	51.38 ± 2.64 c	39.29 ± 1.33 b	36.34 ± 1.89 a	36.96 ± 2.16 a	ns	***	ns
P_a (mmol m^{-2})	A 1.34 ± 0.08 b (ns)	3.88 ± 0.29 d (***)	0.97 ± 0.07 a (ns)	1.90 ± 0.06 c (ns)			ns	***	**
	B 1.30 ± 0.09 a	2.80 ± 0.27 c	1.11 ± 0.07 a	1.99 ± 0.11 b					
N_a / P_a (mol mol^{-1})	21.57 ± 0.97 b	9.37 ± 0.67 a	50.4 ± 2.94 c	20.43 ± 0.81 b	24.99 ± 2.99 a	25.52 ± 2.81 a	ns	***	ns
L_S (%)	19.24 ± 2.39 a	18.42 ± 1.54 a	16.15 ± 1.78 a	20.22 ± 2.79 a	20.5 ± 1.76 a	16.79 ± 1.25 a	ns	ns	ns
g_s (mmol m^{-2} s^{-1})	37.9 ± 3.1 a	39.9 ± 3.2 a	41.1 ± 3.2 a	50.6 ± 3.2 b	40.0 ± 3.0 a	44.8 ± 2.6 a	ns	*	ns
V_{cmax} (µmol CO_2 m^{-2} s^{-1})	15.05 ± 1.11 a	14.9 ± 0.96 a	17.25 ± 0.97 a	21.33 ± 1.07 b	17.56 ± 0.95 a	16.6 ± 0.74 a	ns	***	ns
J_{max} (µmol electrons m^{-2} s^{-1})	24.65 ± 1.31 a	24.9 ± 1.19 a	26.39 ± 1.59 a	33.22 ± 1.34 b	26.33 ± 1.16 a	27.86 ± 1.07 a	ns	***	ns
T_p (µmol triose-P m^{-2} s^{-1})	1.51 ± 0.11 a	1.53 ± 0.11 a	1.76 ± 0.11 a	2.2 ± 0.11 b	1.61 ± 0.07 a	1.90 ± 0.11 b	**	***	ns

Photosynthetic parameters $V_{cmax,}$ J_{max} and T_p were strongly influenced by nutrient treatment ($F_{3,60}$ = 8.5-10.2, $P < 0.001$) but only T_p was significantly influenced by clone ($F_{1,60}$ = 11.6, $P < 0.01$) (Table 2.1). Interactive effects of nutrient treatment and clone on $V_{cmax,}$ J_{max} and T_p were not significant ($F_{3,60}$ = 1.24-1.34, $P > 0.27$). Values of $V_{cmax,}$ J_{max} and T_p were on average 42%, 35% and 46% greater in the high-nutrient (N_1P_1) compared with the low-nutrient supply treatment (N_0P_0) (Table 2.1). Photosynthetic rates at ambient CO_2 concentration were mainly in the V_{cmax}-limited portion of the CO_2 response at low-nitrogen supply and at the transition between V_{cmax} and J_{max} at high-nitrogen supply (Fisher's exact test, P = 0.02) independent of clone (Fisher's exact test, P = 0.58).

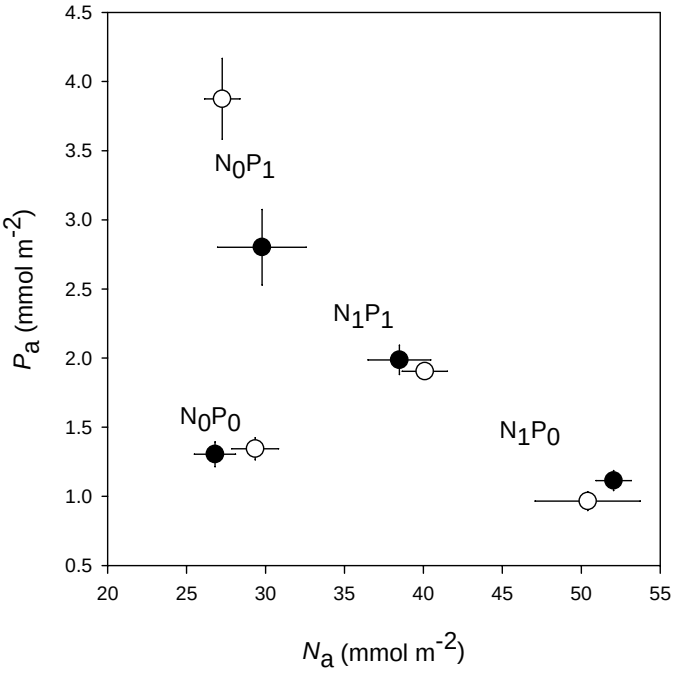


Figure 2.1. Comparison of foliage nitrogen and phosphorus concentrations on an area basis across nutrient treatments and clones. Nutrient treatments comprised two nitrogen supply regimes (N_0=1.43 and N_1=7.14 mM) and two phosphorus supply regimes (P_0=0.084 and P_1=0.420 mM). Values are presented as means (± 1 SE) for each treatment and clone. Symbols: ○ = Clone A; and ● = Clone B.

Photosynthetic parameters and foliage nutrient concentration

Foliar nitrogen and phosphorus concentrations conformed to the nutrient treatments (Table 2.1, Figure 2.1) and were significantly affected by them ($F_{3,60}$ = 37-106, $P < 0.001$). At the tree level, observed N_a ranged almost fourfold from 18 to 75 mmol m^{-2} while P_a ranged eightfold from 0.7 to 5.6 mmol m^{-2}. The N_a / P_a ratio ranged 15-fold from 4.4 to 66.2 (mole basis). Values of N_a / P_a did not differ significantly between the N_0P_0 and N_1P_1 treatment with

an average (± 1 SE) of 21 ± 0.6. However, N_a / P_a values of plants in N_0P_1 and N_1P_0 treatments differed significantly, being on average 9.4 ± 0.7 and 50.4 ± 2.9, respectively (Table 2.1).

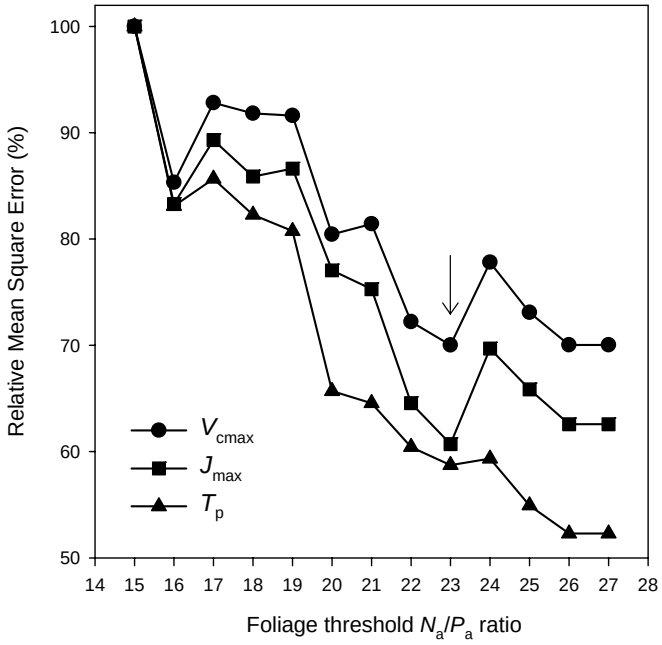


Figure 2.2. Relative model mean square error (%) of maximal rate of Rubisco carboxylation, V_{cmax}; maximal rate of electron transport driving regeneration of RuBP, J_{max}; and rate of triose phosphate export, T_p; to foliage nutrient concentrations when using different thresholds of N_a / P_a to discriminate nitrogen ($N_a / P_a \leq$ threshold) from phosphorus ($N_a / P_a >$ threshold) deficiencies.

Segmented linear models relating photosynthetic parameters to foliar nitrogen and phosphorus concentrations were fitted for different threshold values of N_a / P_a ranging from 15 to 27. A value of N_a / P_a equal to 23 (mole basis) was identified as the threshold that minimized the model mean square error for V_{cmax} and J_{max} for both clones (Figure 2.2). No clear optimum was found for T_p. This analysis assumes that there is not nutrient co-limitation, that nutrient concentration is a good surrogate for nutrient supply under the experimental conditions, and that threshold values can be empirically determined. Nutrient co-limitation was further tested by fitting linear equations of photosynthetic parameters (V_{cmax}, J_{max}, T_p) to N_a under phosphorus deficient conditions ($N_a/P_a > 23$) and to P_a under nitrogen deficient conditions ($N_a/P_a \leq 23$). Correlations were in all cases nonsignificant ($P > 0.32$, $r^2 < 0.025$), indicating that the effects of nitrogen and phosphorus supply on photosynthesis were statistically independent and that the photosynthetic behavior of the two clones was effectively similar.

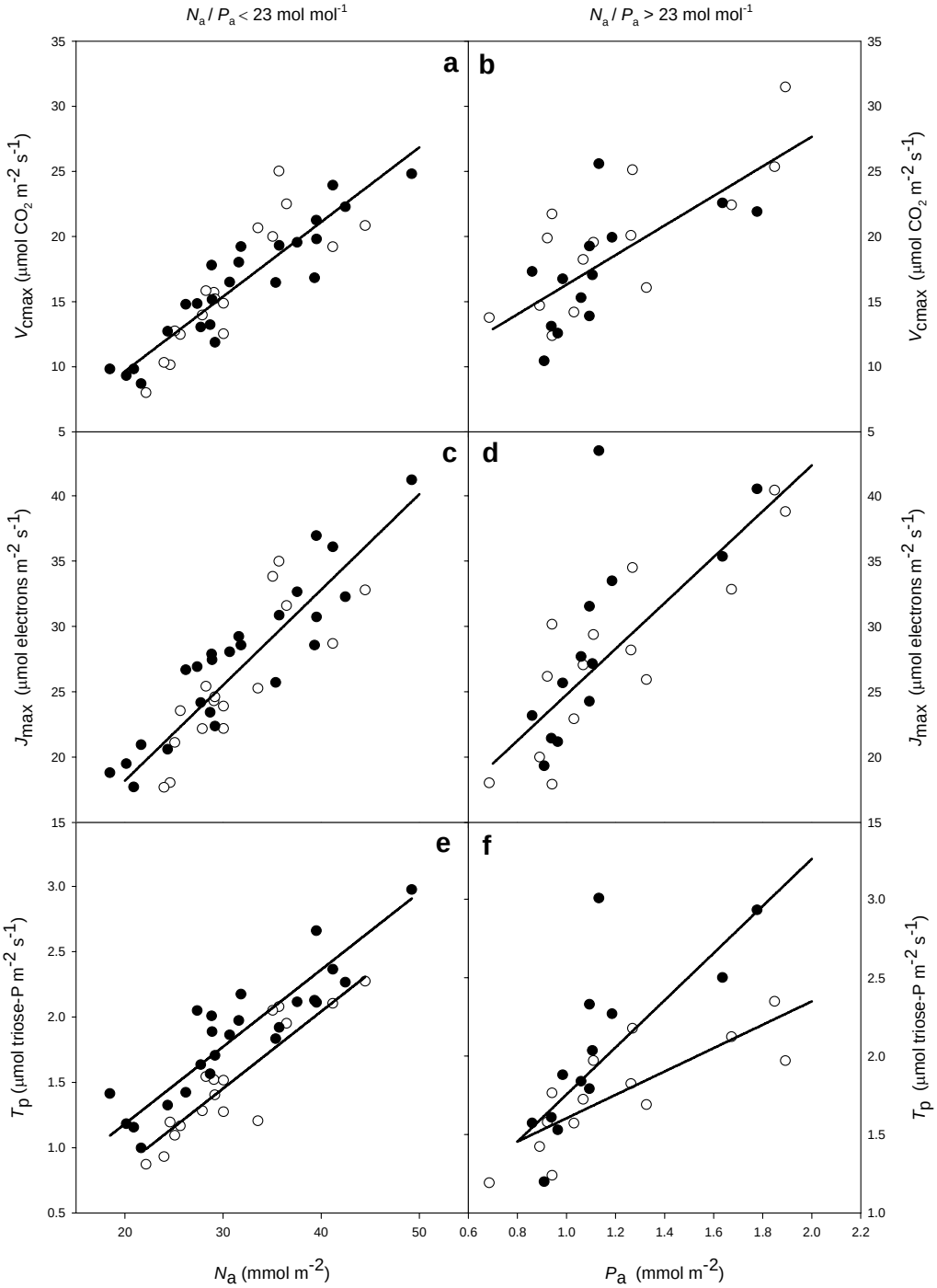


Figure 2.3. Relationship between (**a, b)** maximal rate of rubisco carboxylation, V_{cmax}, (**c, d)** maximal rate of electron transport driving regeneration of RuBP, J_{max}, and (**e, f)** rate of triose phosphate export, T_p, and foliage nitrogen and phosphorus concentration on an area basis (N_a, P_a) for Clones A (○) and B (●). On the left side, measurements are nitrogen limited (N_a / $P_a \leq 23$), and on the right side, measurements are phosphorus limited (N_a / $P_a > 23$). Different lines were fitted to clones where the relationship varied significantly. Under conditions of nitrogen limitation: $V_{cmax} = 0.573\ N_a - 1.806$, $r^2 = 0.79$, $P < 0.001$; $J_{max} = 0.731\ N_a + 3.574$, $r^2 = 0.78$, $P < 0.001$; $T_p = 0.059\ N_a - 0.3170$, $r^2 = 0.82$, $P < 0.001$ (Clone A); $T_p = 0.059\ N_a + 0.0028$, $r^2 = 0.80$, $P < 0.001$ (Clone B). Under conditions of phosphorus limitation: $V_{cmax} = 11.363\ P_a + 4.933$, $r^2 = 0.59$, $P < 0.001$; $J_{max} = 17.559\ P_a + 7.210$, $r^2 = 0.75$, $P < 0.001$; $T_p = 0.745\ P_a + 0.859$, $r^2 = 0.64$, $P < 0.001$ (Clone A); $T_p = 1.502\ P_a + 0.253$, $r^2 = 0.79$, $P < 0.001$ (Clone B).

Values of V_{cmax}, J_{max} and T_p exhibited highly significant positive linear relationships with both N_a ($F_{1,37}$ = 130-149, $P < 0.001$) and P_a ($F_{1,23}$ = 23-60, $P < 0.001$). Slopes and intercepts of the relationship between V_{cmax}, as well as J_{max}, and either N_a or P_a did not differ significantly between clones (Figure 2.3 a-d). In contrast, the relationships between T_p and N_a and P_a were significantly influenced by clone (Figure 2.3 e and 2.3f).

Influence of genotype on photosynthesis

Analysis of covariance revealed no significant clonal influences ($P > 0.05$) on either slopes or intercepts of the relationship between photosynthetic parameters (V_{cmax}, J_{max} and T_p) and either N_m or P_m (mass basis). Thus, under nitrogen limitations ($N_a / P_a = N_m / P_m \leq 23$): V_{cmax} = 25.8446 N_m + 1.2779, $r^2 = 0.69$, $P < 0.001$; J_{max} = 36.3443 N_m + 5.6874, $r^2 = 0.78$, $P < 0.001$ and T_p = 3.0858 N_m – 0.0364, $r^2 = 0.80$, $P < 0.001$. Under phosphorus limitations ($N_a / P_a = N_m / P_m > 23$): V_{cmax} = 0.5005 P_m + 8.1310, $r^2 = 0.38$, $P < 0.001$; J_{max} = 0.9101 P_m + 9.3846, $r^2 = 0.66$, $P < 0.001$ and T_p = 0.0549 P_m + 0.7381, $r^2 = 0.61$, $P < 0.001$. Differences were completely removed when values were expressed on a mass basis because the slow-growing clone had a generally significantly greater leaf area to mass ratio than the fast-growing clone.

Supporting evidence was obtained by comparing the rate of photosynthesis at saturating irradiance and ambient CO_2 (A_{sat}), photosynthetic nitrogen- (E_N) and phosphorus-use efficiency (E_P) across nutrient treatments and clones (Figure 2.4). Values of A_{sat} scaled positively with nutrient treatment ($F_{3,60}$ = 10.62, $P < 0.001$) being 41% greater in the high-nutrient supply treatment (4.48 ± 0.17 μmol m^{-2} s^{-1}) compared with the low nutrient supply treatment (3.18 ± 0.15 μmol m^{-2} s^{-1}) (Figure 2.4a); however these values did not differ significantly between clones ($F_{1,60}$ = 0.84, $P = 0.36$). Similarly, values of E_N and E_P were strongly influenced by nutrient treatment ($F_{3,60}$ = 34-65, $P < 0.001$) but were independent of clone ($F_{1,60}$ = 0.06-1.71, P = 0.20-0.81) (Figure 2.4b and 2.4c).

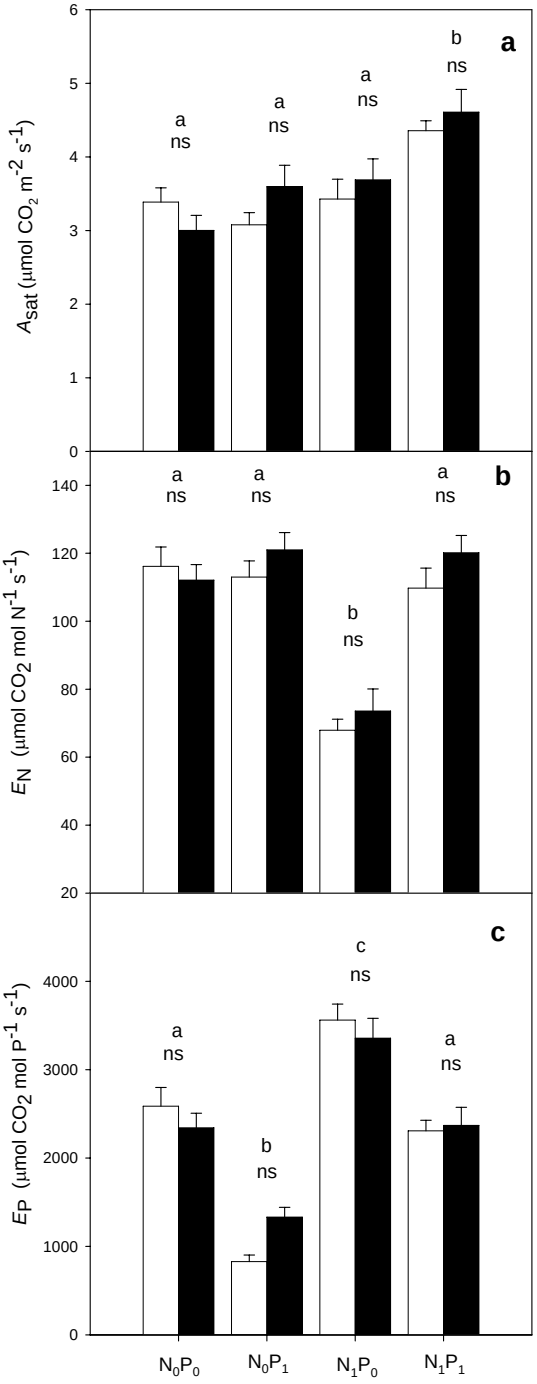


Figure 2.4. Influence of a factorial combination of nitrogen and phosphorus supply and clone on (a) the rate of photosynthesis at saturating irradiance and ambient CO_2 (A_{sat}), (b) photosynthetic nitrogen-use efficiency (E_N) and (c) photosynthetic phosphorus-use efficiency (E_P). Treatments comprised a combination of two nitrogen supply regimes (N_0=1.43 and N_1=7.14 mM) and two phosphorus supply regimes (P_0=0.084 and P_1=0.420 mM). Values are presented as means (± 1 SE) for each treatment and clone. Different letters indicate significant differences among treatments at $P < 0.05$. Differences between clones were non significant (ns). Open bars represent Clone A and closed bars represent Clone B. Interactive effects between nutrient treatment and clone were not significant ($P > 0.05$).

DISCUSSION

Nutrition affected photosynthesis mainly through biochemical limitations. Although g_s scaled positively with increasing nutrition, and photosynthetic model parameters (i.e., g_s increased as V_{cmax} and J_{max} increased), the relative stomatal limitation (L_S) was small (18.5%) and no increase in L_S with increasing nutrition was observed. Similar results were found by Loustau *et al*. (1999) across a phosphorus gradient and Grassi *et al*. (2002) across a nitrogen gradient in seedlings of *Pinus pinaster* and *Eucalyptus grandis*, respectively.

The mean values of V_{cmax} and J_{max} reported in this study for plants of *Pinus radiata* with abundant nutrient supply were 21 and 33 μmol m^{-2} s^{-1}, respectively, at a leaf temperature of 20ºC. These values fall within the range (mean ± standard deviation) of V_{cmax} and J_{max} (25 ± 12 and 40 ± 32 μmol m^{-2} s^{-1}, respectively) reported for conifers by Wullschleger (1993). Also, values of T_p from this study fall within the range reported by Lewis *et al*. (1994) for *Pinus taeda* across a gradient in phosphorus supply (0.5-2.5 μmol m^{-2} s^{-1}).

Photosynthetic rates are known to be closely related to foliar nitrogen concentrations (Field and Mooney 1986, Walcroft *et al*. 1997, Grassi *et al*. 2002, Ripullone *et al*. 2003). This is explained by the high proportion of total nitrogen partitioned to the carboxylating enzyme Rubisco (Sage and Pearcy 1987, Evans 1989, Warren and Adams 2002, Takashima *et al*. 2004) and also by the strong coupling effect among capacities (von Caemmerer and Farquhar 1981, Sharkey 1985). Strong linear relationships between photosynthetic parameters and foliar nitrogen (r^2 = 0.78-0.82) were found in this study, that closely matched those reported by Walcroft *et al*. (1997) for *Pinus radiata* under similar experimental conditions (V_{cmax} = 0.573 N_a – 1.806 cf. V_{cmax} = 0.520 N_a - 3.784; and J_{max} = 0.742 N_a + 2.668 cf. J_{max} = 0.731 N_a + 3.574, respectively).

Less is known about the processes limiting photosynthesis induced by phosphorus deficiencies. Photosynthetic rates in pines scale with foliar phosphorus because of limitations to carboxylation activity (Loustau *et al*. 1999), electron transport (Conroy *et al*. 1986) and triose phosphate export (Lewis *et al*. 1994). These findings are consistent with results found in this study showing strong linear relationships between photosynthetic parameters and foliar phosphorus (r^2 = 0.59-0.79). Mooney and Gulmon (1982) suggested that the amount of Rubisco in leaves is modulated to match the amount of the most limiting environmental resource, and Warren and Adams (2002) reported that foliage phosphorus was positively related to Rubisco concentration in *Pinus pinaster*. In this study, values of V_{cmax} scaled positively with P_a suggesting that either the activity or concentration of Rubisco, or both, are controlled by phosphorus supply. Additionally, J_{max} and T_p scaled positively with P_a suggesting that these capacities are regulated to match V_{cmax}.

Limitations in T_p occurred either as an abrupt change in shape or a decline in CO_2 assimilation at high intercellular CO_2 concentrations. Lewis *et al*. (1994) reported T_p limitations for *Pinus taeda*, whereas Walcroft *et al*. (1997) did not for *Pinus radiata*. This suggests that T_p limitations can occur at different times for reasons not completely understood. Azcón-Bieto (1983) observed drastic reductions in the rate of CO_2 assimilation at high C_i values, after exposing wheat plants to high irradiance (600-700 μmol m^{-2} s^{-1}) for 5 h. These reductions correlated well with carbohydrate accumulation, suggesting end-product inhibition of photosynthesis. Similarly, plants in this study, which were kept in a growth cabinet at an irradiance of 720 μmol m^{-2} s^{-1} for several hours before and during the gas exchange measurements, showed T_p limitations. Another possible reason for the observed T_p limitations may be that plants were large relative to the 4.25-dm^3 pots, and therefore they may have developed a sink limitation and accumulated carbohydrates. It might be also argued that the reason why some authors report T_p limitations whereas others do not is associated with inherent difficulties in identifying T_p limitations in A / C_i curves.

To determine whether gas exchange was nitrogen or phosphorus limited, photosynthetic measurements were partitioned based on N_a / P_a ratios. Nutrient ratios have been extensively used to address optimum nutrition and explain particular nutrient limitations (e.g. Ingestad 1971, 1979, Ingestad and Lund 1986). Aerts and Chapin (2000) suggested that the ratio of foliage nitrogen to phosphorus is more important than tissue concentrations of either nutrient in determining nutrient limitations. Knecht and Göransonn (2004) argue that the optimum ratio of nitrogen to phosphorus in terrestrial plants is similar for a wide range of species and about 10 on a mass basis (i.e., 23 on a molal basis). Several authors (Reich and Schoettle 1988, Marschner 1995, Aerts and Chapin, 2000) suggest that deviations should therefore lead to nitrogen ($N_a / P_a \leq 23$) or phosphorus ($N_a / P_a > 23$) deficiencies. In this study, the threshold value of N_a / P_a that minimized model mean square error of V_{cmax} and J_{max} to both N_a and P_a was 23, suggesting that the optimum stoichiometry ratios for growth and photosynthesis may be similar. Using this partitioning approach, significant relationships between V_{cmax}, J_{max} and T_p to N_a and P_a were found, showing that the effects of nitrogen and phosphorus supply on photosynthesis were statistically independent (assuming that foliage nutrient concentration is a good surrogate for nutrient supply under these experimental conditions). Given this independence, separating the data by stoichiometry ratios may provide a useful means of modelling nutrient-limited photosynthesis at the scale of leaves, plants and ecosystems.

Values of V_{cmax}, J_{max} and T_p were strongly correlated with foliage nutrient concentration on an area basis, with only T_p being influenced by genotype, suggesting that, in genotypes with contrasting growth performance, the response of V_{cmax} and J_{max} to nutrient limitation are

equivalent. The similarity in photosynthetic nutrient-use efficiency between clones provides further confirmation of this equivalence. Contrary to what was expected, the slow-growing clone had a higher T_p than the fast-growing clone. Because the fast-growing clone was larger than the slow-growing clone relative to the 4.25-dm^3 pots, it is possible that lower T_p in the fast-growing clone was attributable to development of greater sink limitations and the accumulation of carbohydrates. However, it seems feasible that significant differences in the leaf area to mass ratios between genotypes accounted for the differences in T_p, because the genotype effect was completely removed when values of V_{cmax}, J_{max} and T_p were correlated with foliage nutrient concentration on a mass basis. This result appears to support the global convergence in plant functioning theory by Wright *et al.* (2004) who argue that relationships between photosynthesis rates, foliage nutrient concentrations on a mass basis and leaf area to mass ratio are universal across plant functional groups. The results in this study suggest that there is no relationship between growth performance of a genotype and its photosynthetic nutrient-use efficiency or photosynthetic performance. Therefore, genotypic differences in plant growth may be explained, at least partially, by the additional amount of light intercepted by the larger amount and size of needles of the fast-growing clone.

In this study, V_{cmax}, J_{max} and T_p were calculated based on responses of A to C_i rather than CO_2 concentration in the chloroplast, C_c, with the implicit assumption that mesophyll conductance, g_m, was infinite. However, g_m has been shown to be finite causing C_c to be lower than C_i and thus leading to underestimates of V_{cmax}, J_{max} and T_p (Harley *et al.* 1992, Loreto *et al.* 1992, von Caemmerer 2000, Long and Bernacchi 2003). Warren *et al.* (2003*b*) found that g_m limited photosynthesis by 20% compared with a 30% limitation imposed by stomatal processes in 50-year-old *Pseudotsuga mensiezii* trees. De Lucia *et al.* (2003) found that values of g_m were similar in magnitude to the values of stomatal conductance (g_s) in *Pinus radiata* seedlings grown in nursery conditions, suggesting that limitations imposed by the mesophyll and stomata on CO_2 diffusion might be equivalent. An assumption of similarity in the values of g_s and g_m for this study suggest that biochemical limitations predominate (about 60%) over g_s (about 20%) and g_m (about 20%) limitations.

In conclusion, this chapter examined the effects of nitrogen and phosphorus supply on the behavior of the photosynthetic parameters of the Farquhar *et al.* (1980) model in two contrasting clones of *Pinus radiata*. The model showed that the effects of nitrogen and phosphorus supply on photosynthesis are statistically independent and that the photosynthetic behavior of the two clones was effectively similar.

CHAPTER THREE

INFLUENCE OF NITROGEN AND PHOSPHORUS SUPPLY ON TRANSFER CONDUCTANCE LIMITATIONS TO PHOTOSYNTHESIS IN *PINUS RADIATA*

Summary Transfer conductance (g_m) may pose significant limitations to photosynthesis which may be differentially affected by nutrition and genotype in *Pinus radiata*. Simultaneous measurements of gas exchange and chlorophyll fluorescence were made to determine g_m, using the constant *J* method (Harley *et al.* 1992), in a fast- and a slow-growing clone of *Pinus radiata* D. Don cultivated in a greenhouse with a factorial combination of nitrogen and phosphorus supply. Values of g_m scaled with nutrient supply, and approximately with the rate of photosynthesis at saturating irradiance and ambient CO_2 ($g_m = 0.020\ A_{sat}$, $r^2 = 0.25$, $P < 0.001$) and with stomatal conductance ($g_m = 1.16\ g_s$, $r^2 = 0.14$, $P < 0.001$). Values of g_m were greater than those of stomatal conductance (g_s) and their ratio (g_m / g_s) was not influenced by nutrient supply or clone being on average (± 1 SE) 1.22 ± 0.06. Relative mesophyll limitations (L_M, 16%) to photosynthesis were generally greater than those imposed by stomata (L_S, 13%), and collectively smaller than the relative limitations to photosynthesis posed by biochemical processes (L_B, 71%). The CO_2 concentration in the intercellular air spaces (C_i) was (± 1 SE) 53 ± 3 μmol mol^{-1} lower than in the atmosphere (C_a) while CO_2 concentration in the chloroplasts (C_c) was (± 1 SE) 48 ± 2 μmol mol^{-1} less than C_i. Values of L_S, L_M and L_B and CO_2 diffusion gradients posed by g_s (C_a-C_i) and g_m (C_i-C_c) did not significantly differ with nutrient supply or clone. Fascicle size scaled with nutrient supply and was larger in the fast- compared with the slow-growing clone, but did not influence g_m or the relative limitation posed by g_m. Values of maximal carboxylation rate (V_{cmax}) and maximal electron transport rate (J_{max}) calculated on a C_c basis were on average 15.4 % and 3.1 % greater than those on a C_i basis, which translated into different slopes of the J_{max} / V_{cmax} relationship (C_c basis: $J_{max} = 2.11\ V_{cmax}$, $r^2 = 0.88$, $P < 0.001$; C_i basis: $J_{max} = 2.43\ V_{cmax}$, $r^2 = 0.86$, $P < 0.001$). These results may be useful in correcting estimates of V_{cmax} and J_{max} in the biochemical description of carbon assimilation of *Pinus radiata* in many currently used ecosystem physiological models.

Keywords: chloroplastic CO_2 concentration, electron transport, genotype, mesophyll conductance, nutrient limitation, Rubisco carboxylation, stomatal limitation.

INTRODUCTION

The C_3 model of leaf photosynthesis originally proposed by Farquhar *et al*. (1980) is widely used in ecophysiological research, being particularly relevant at a time of great concern on global environmental change impacting the carbon cycle in the biosphere. In this model, CO_2 assimilation is mostly limited by the maximal rate of ribulose-1,5-*bis*phosphate (RuBP) carboxylase-oxygenase (Rubisco) carboxylation (V_{cmax}) and by the maximal electron transport rate driving regeneration of RuBP (J_{max}) (Farquhar *et al*. 1980, von Caemmerer and Farquhar 1981, von Caemmerer 2000). These parameters are fitted to the *in vivo* response of net assimilation to internal CO_2 concentration (A / C_i curves), and commonly used in process-based models to scale carbon assimilation from leaves to canopies.

Several diffusive conductances limit the transfer of CO_2 along a concentration gradient from the atmosphere to the sites of carboxylation (von Caemmerer and Evans 1991, Harley *et al*. 1992, De Lucia *et al*. 2003). Boundary layer and stomatal resistances restrict the diffusion of CO_2 from the ambient air to the intercellular air spaces of the leaf mesophyll, whereas internal resistances limit the diffusion of CO_2 in a liquid-phase through the plasma membrane, cytoplasm and chloroplast envelope to reach the sites of Rubisco carboxylation (von Caemmerer 2000). As a result of these air-phase and liquid-phase resistances, CO_2 concentration in the intercellular air spaces (C_i) is less than in ambient air (C_a), but greater than in the chloroplasts (C_c). The internal transfer resistance was once considered sufficiently small that it could be neglected (Farquhar *et al*. 1980). However, mesophyll conductance is now known to be finite imposing similar limitations to photosynthesis as those imposed by stomata (Harley *et al*. 1992, Loreto *et al*. 1992, von Caemmerer 2000, Warren *et al*. 2003*b*).

The exact mechanisms that control the diffusion of CO_2 from the intercellular air spaces to the sites of Rubisco carboxylation in the chloroplasts are not clearly understood. However, there is emerging evidence that g_m is enzyme regulated rather than a single physical diffusional process (Bernacchi *et al*. 2001). Some authors have suggested that carbonic anhidrase (Gillon and Yakir 2000) and aquaporins may be proteins regulating the transfer of CO_2 from the intercellular air spaces to the sites of Rubisco carboxylation (Terashima and Ono 2002, Tyerman *et al*. 2002). Transfer conductance has been found to be correlated with photosynthetic capacity (von Caemmerer 2000), stomatal conductance (Loreto *et al*. 1992), chloroplast surface area exposed to intercellular air spaces (von Caemmerer and Evans 1991, Hanba *et al*. 2002), and leaf nitrogen (von Caemmerer and Evans 1991), among others.

Transfer conductance has been measured mostly in angiosperm tree species but only in a few conifers (e.g. Warren *et al*. 2003*b*, De Lucia *et al*. 2003, Warren 2006). Also, most studies have focused on comparing g_m across species and plant functional groups (e.g. Loreto *et al*. 1992) but only a few have focused on the influence of environmental stresses on transfer conductance in conifers (e.g. Warren *et al*. 2004, Grassi and Magnani 2005). Because nitrogen and phosphorus are the elements which most limit primary producers in terrestrial (Aerts and Chapin 2000) and aquatic ecosystems (Hall *et al*. 2005), it is of interest to know how these nutrients influence g_m.

Increasing productivity of existing plantations at the global scale in order to fulfil current and future needs of wood for industrial and fuel consumption would require more intensive forest management and tree breeding strategies (Nambiar 1984, Turner and Lambert 1986). However unless nutrient and water requirements are optimized, the effects of intensive silviculture and tree breeding will not be realized (Webber 1978, Nambiar 1984, Turner and Lambert 1986, Raison and Myers 1992, Madgwick 1994). Therefore, understanding how nutrient availability affects photosynthesis, in general, and transfer conductance, in particular, is relevant to assess sustainability and to estimate carbon sequestration by this species. This seems relevant at a time when plantation forestry is expected to play a key role to meet New Zealand environmental commitments and particularly those subscribed to under the Kyoto Protocol (NZ Ministry for the Environment 2006). Clonal forestry, being practised at a moderate commercial scale in New Zealand, is expected to play an increasing role in the improvement of *Pinus radiata* for timber production and quality (Cown 1997, Sorensson et. al 1997, Sorensson and Shelbourne 2005). It therefore seems relevant to test whether physiological processes such as those described by transfer conductance are amenable to be used or discarded as selection criteria.

The aim of this study was to examine how variation in nitrogen and phosphorus supply influenced g_m in two clones with contrasting growth and leaf anatomy, and to compare the photosynthetic parameters of the Farquhar *et al*. (1980) model when estimated on an intercellular- and chloroplastic CO_2 concentration basis. A third objective for the study was to develop adjustments to account for g_m in the photosynthetic parameters of the Farquhar *et al*. (1980) model for *Pinus radiata*.

MATERIALS AND METHODS

Plant material

A greenhouse experiment was laid out in a factorial design with two clones, two nitrogen supply regimes (N_0=1.43 and N_1=7.14 mM) and two phosphorus supply regimes (P_0=0.084 and P_1=0.420 mM). A fast- and a slow- growing clone (Clones A and B, respectively) were selected within a set of 400 genotypes planted in the Purokohukohu Experimental Basin (Beets *et al*. 2004). These clones were the same as those used in the previous chapter.

One-year old *Pinus radiata* cuttings from Clones A and B were raised under standard ENSIS (formerly New Zealand Forest Research Institute) nursery conditions and cultivated in 4.25- and 42-dm^3 pots containing silica sand during the first and second year of growth, respectively. Nutrient treatments were randomly allocated to the plants and applied for 24 months ending in the winter 2006. Nutrient solution was supplied to plants in increasing amounts (0.5-1.0 dm^3) and with increasing frequency from once to twice a week to account for increasing demands with plant size. Plants received the same amount at each daily watering in excess to that required by the largest plants. Nutrients other than N and P were provided at 1.023 mol m^{-3} K, 0.250 mol m^{-3} Ca, 0.411 mol m^{-3} Mg, 0.281 mol m^{-3} S, 12.532 mmol m^{-3} Fe, 0.445 mmol m^{-3} Zn, 0.473 mmol m^{-3} Cu, 7.281 mmol m^{-3} Mn, 0.073 mmol m^{-3} Mo, 18.502 mmol m^{-3} B, 0.946 mmol m^{-3} Cl and 0.145 mmol m^{-3} Na following Ingestad (1979). Plants were grown in a thermostatically controlled greenhouse where temperature fluctuated between 6 and 34°C (18 ± 4 °C) during the day, and between 1 and 23 ºC (15 ± 4 °C) during the night depending on weather conditions. Roots of all plants were artificially inoculated with spores of *Rhizopogon rubescens* Tul. and confirmed as mycorrhizal either by visual inspection of roots or by the presence of fruiting bodies.

Gas exchange measurements

Simultaneous gas exchange and chlorophyll fluorescence measurements were made in 6 plants per treatment per clone. Plants were moved from the greenhouse to a thermostatically controlled room (20 °C) the day before measurements were undertaken. All measurements were made with a portable photosynthesis system (LI-6400, Li-Cor, Lincoln, NE) equipped with an integrated chlorophyll fluorescence and gas exchange chamber (LI-6400-40), during daylight hours in late summer from January 16 to February 8, 2006.

For each plant, three fascicles were placed inside a 2 cm^2 cuvette avoiding shading between needles. Temperature in the cuvette was maintained at 20 ºC while leaf-to-air vapour pressure deficit (VPD) was maintained, with the exception of three plants, below 1 kPa. Plants were left to equilibrate for 10 min at 360 µmol mol^{-1} CO_2 concentration and saturating irradiance (1500 µmol m^{-2} s^{-1}), before measuring the response of net assimilation (A) to intercellular CO_2 concentration (C_i). External CO_2 concentration (C_a) was supplied with a CO_2 mixer across the series 360, 300, 200, 150, 125, 100, 75, 50, 360, 450, 600, 800, 1000, 1200, 1500 µmol mol^{-1}, with saturating irradiance, Q (400-700 nm), maintained at 1500 µmol photons m^{-2} s^{-1}. Measurements were recorded after values of A, C_i and g_s were stable (coefficient of variation ≤ 2%), but with a minimum waiting time of 4 min at each step within the series. The CO_2 response curve for each plant was generally completed in two hours.

Mesophyll conductance calculations

Mesophyll conductance (g_m) was estimated based on the "constant J" method (Harley *et al*. 1992, Loreto *et al*. 1992). This method is applied in the RuBP-regeneration limited portion of the A/C_i curve where rates of electron transport become constant. Within this region, further increases in photosynthesis with increasing C_i are due to suppression of photorespiration as the rate of carboxylation progressively substitutes the rate of oxygenation. Thus, photosynthetic rates are a function of C_c and the relative CO_2/O_2 specificity of Rubisco, normally described by the chloroplastic CO_2 compensation point (Γ^*). The relationship of J with A may be described as a function of C_i (Harley *et al.* 1992) using,

$$J = (A + R_d)\,\frac{4\left[\left(C_i - A/g_m\right) + 2\Gamma^*\right]}{\left(C_i - A/g_m\right) - \Gamma^*} \qquad (3.1)$$

where J is the rate of electron transport, A is net photosynthesis, Γ^* is the chloroplastic CO_2 concentration at which the rate of carboxylation equals the rate of photorespiratory CO_2 release (von Caemmerer 2000) and R_d is the rate of mitochondrial respiration in the light. The constant J method is relatively insensitive to errors in R_d but highly sensitive to errors in Γ^* (Harley *et al*. 1992), and therefore these parameters were estimated for each plant using the Laisk method (von Caemmerer 2000). Briefly, the A / C_i response was measured at three different irradiances (in most cases: Q = 50, 100 and 300 µmol m^{-2} s^{-1}) across the following series of values of C_a : 200, 150, 100, 80, 60, 40 and 0 µmol mol^{-1}. Linear relationships between A and C_i were fitted and intersections between fitted lines averaged to yield a point which when projected

orthogonally to the *A* axis was taken as the rate of day respiration (R_d), and to the C_i axis, the intercellular CO_2 compensation point in the absence of day respiration (C_i^*) (Figure 3.1).

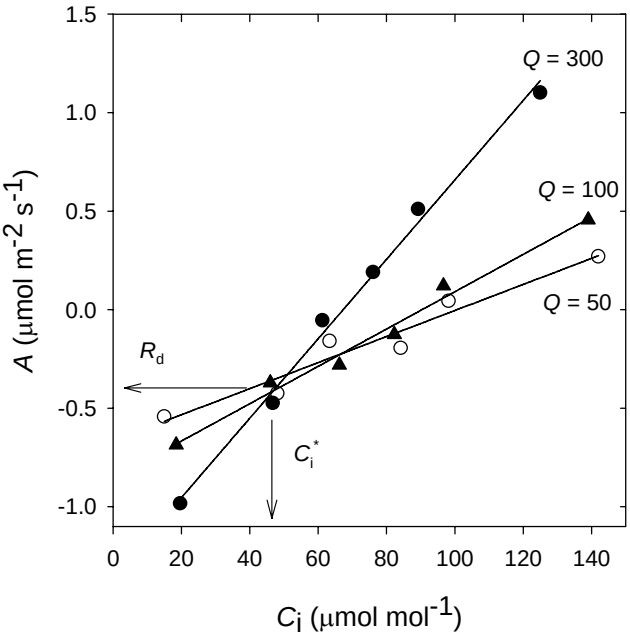


Figure 3.1. The response of net photosynthesis (*A*) to intercellular CO_2 concentration (C_i) at three different irradiances (Q = 300, 100 and 50 μmol m^{-2} s^{-1}, 400-700 nm) for a representative foliage sample. Linear relationships between *A* and C_i were fitted for each Q level ($r^2 > 0.87$, $P < 0.001$, in this case) and their intersections averaged to yield a point which when projected to the *A* axis was taken as the rate of day respiration (R_d) and when projected to the C_i axis was taken as the intercellular CO_2 compensation concentration in the absence of day respiration (C_i^*). The mitochondrial CO_2 compensation concentration (Γ^*) was calculated as $\Gamma^* = C_i^* + R_d / g_m$ (von Caemmerer 2000), where g_m is mesophyll conductance to CO_2 transfer. Method originally proposed by Laisk, A. (von Caemmerer 2000) to determine C_i^* and R_d. This figure is equivalent to the one presented by De Lucia *et al*. (2003) but with data drawn from this study.

The chloroplastic CO_2 compensation point (Γ^*) is given as a function of C_i^*, R_d and g_m (von Caemmerer 2000, Peisker and Apel 2001) by,

$$\Gamma^* = C_i^* + R_d / g_m \qquad (3.2)$$

Equation (3.2) was replaced into (3.1) and *J* values were then calculated for three or more photosynthetic rates measured above the values of C_i yielding constant rates of electron transport, using different values of g_m as proposed by Warren (2006). Then, optimal g_m was resolved iteratively using the solver add-in of Microsoft Excel (Figure 3.2), as the value that best explained (minimum *J* variance) changes in photosynthesis with changes in intercellular CO_2 concentration (Harley *et al*. 1992).

Photochemical efficiency of photosystem II (Φ_{PSII}) was estimated based on chlorophyll fluorescence measurements as (F_m' - F) / F_m', where F and F_m' are the steady and maximal

fluorescence in the light-adapted sample respectively (Schreiber *et al*. 1994). Genty *et al*. (1989) showed that values of Φ_{PSII} are directly proportional to the rate of electron transport through photosystem II, and therefore can be used to determine the portion of the A/C_i curve where the rate of electron transport was constant.

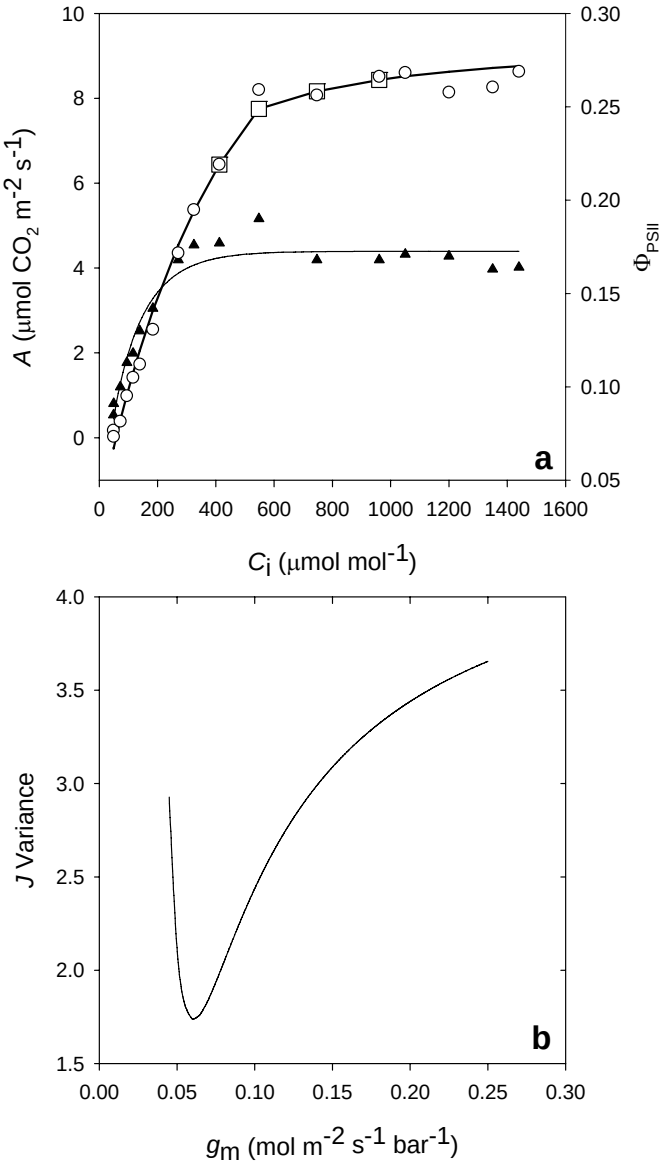


Figure 3.2. Graphic description of the constant *J* method to determine transfer conductance. **a.** The rate of net photosynthesis (*A;* open circles) and photochemical efficiency of photosystem II (Φ_{PSII}; solid triangles) as a function of the intercellular CO_2 concentration (C_i) for a representative foliage sample. Solid lines represent a least-squares fit to the A/C_i and Φ_{PSII}/C_i response. Open squares are interpolated values used to estimate mesophyll conductance (g_m). These values are within the portion of the A/C_i response where Φ_{PSII} indicated that electron transport rate was constant. **b.** The variance of estimated electron transport rates, *J*, for different values of g_m. Values of *J* were estimated for each of the four *A* values indicated as open squares in Figure 3.2a using the equation given by Harley *et al*. (1992). The g_m that minimized the variance of *J* estimates for this foliage sample was 0.061 mol m^{-2} s^{-1} bar^{-1}. This figure is equivalent to the ones presented by Harley *et al*. (1992) and De Lucia *et al*. (2003) but with data drawn from this study.

Stomatal, mesophyll and biochemical limitations to photosynthesis

The leaf photosynthesis model described by Farquhar *et al*. (1980) was fitted to the A/C_i and A/C_c curves by non-linear least squares regression (SigmaPlot, Version 7.1, SPSS, Chicago, IL). Values of V_{cmax} and R_d were estimated from the lower part of the A/C_i or A/C_c curve (C_i < 220 μmol mol^{-1}), and these values were then used to estimate J_{max} over the entire range of measured C_i or C_c. The model fitted the data precisely ($r^2 > 0.93$) with little apparent bias (data not shown). Michaelis-Menten constants of Rubisco for CO_2 and O_2, K_c and K_o, and CO_2 compensation point in the absence of mitochondrial respiration, Γ^*, used in the fitting (20 °C) were 231.20 μmol mol^{-1}, 213.94 mmol mol^{-1} and 32.80 mol mol^{-1}, respectively, as published by Bernacchi *et al*. (2001).

The A to C_i response, g_s and g_m, were used to separate stomatal, mesophyll and biochemical limitations to photosynthesis. These calculations were based on estimates of potential photosynthesis rates assuming these conductances were either infinite or as measured (Warren 2006). Relative stomatal limitations were calculated using the method of Farquhar and Sharkey (1982) as $L_S = 1 - A_{measured\text{-}gs} / A_{infinite\text{-}gs}$ where $A_{measured\text{-}gs}$ and $A_{infinite\text{-}gs}$ are estimated A values assuming actual g_s and infinite g_s, respectively. For this calculation, g_m was considered to be infinitely large. Following Bernacchi *et al*. (2002), relative limitation to photosynthesis imposed by g_m was calculated as: $L_M = 1 - A_{measured\text{-}gm} / A_{infinite\text{-}gm}$ where $A_{measured\text{-}gm}$ and $A_{infinite\text{-}gm}$ are estimated A values assuming actual g_m and infinite g_m, respectively. For this calculation, actual g_s values were used. Biochemical (L_B) limitations were calculated as $1 - L_S - L_M$. The CO_2 concentration in the chloroplasts, C_c, was calculated based on measured values of A, C_i and g_m as: $C_c = C_i - (A/g_m)$.

Foliage surface area and nutrient concentrations

Following the completion of A/C_i curves, foliage samples were carefully removed from the cuvette and cut to match the leaf area exposed to gas exchange and chlorophyll fluorescence measurements. Leaf area of needles (s) was calculated based on fascicle diameter (d), length (l) and the number of needles per fascicle (n) as: $s = (\pi d + nd)\ l$ (Turnbull *et al*. 1998). All measurements and analysis are reported on a total leaf area basis. Foliage samples were dried at 70 °C to constant mass and dry mass recorded. For foliar chemical analysis, leaf samples were finely ground and acid-digested by a Kjeldahl method (Blakemore *et al*. 1987). Nitrogen and phosphorus in the digests were determined colorimetrically by the Landcare Research

Laboratories, Palmerston North, New Zealand. Nitrogen and phosphorus concentrations were expressed on a total leaf area (N_a, P_a) and a mass basis (N_m, P_m).

Growth and fascicle size measurements

Plant diameter and height growth are reported at month 18. Estimates of leaf area were determined as the product of leaf mass and the leaf area to mass ratio. Foliage mass at month 18 was estimated from the following equations of foliage dry mass, W_F, (g) against plant diameter, D, (mm) developed at month 24: Clone A, $W_F = 0.0522\ D^{2.4498}$, $r^2 = 0.96$, $P < 0.001$, $n = 12$; and Clone B, $W_F = 0.0628\ D^{2.4229}$, $r^2 = 0.95$, $P < 0.001$, $n = 12$. Similarly, total plant mass (W_T) was determined based on the following equations developed at month 24: Clone A, $W_T = 0.3393\ D^{2.2948}$, $r^2 = 0.98$, $P < 0.001$, $n = 12$; and Clone B, $W_T = 0.3387\ D^{2.3430}$, $r^2 = 0.96$, $P < 0.001$, $n = 12$. Slopes ($F_{3,4} < 1.25$, $P > 0.40$) and intercepts ($F_{3,4} < 1.34$, $P > 0.37$) of the (log) linear relationships W_F/D and W_T/D for both clones were not significantly influenced by nutrient treatment. Reported measurements of fascicle mass were determined at month 24, by taking a random sample of 10 fascicles within each tree.

Data analysis

All subsequent analyses were made at the plant level with SAS software (1996; SAS Institute, Cary, NC). Variables were tested for normality and homogeneity of variance and transformations were made as necessary to meet the underlying statistical assumptions of the models used. The main and interactive effects of nitrogen and phosphorus supply and genotype on transfer conductance and associated variables were examined by analysis of variance. Tukey's least significant difference test was used to distinguish among individual means where applicable with a confidence level of $P \leq 0.05$. Differences in slopes and intercepts between clones in the linear relationship between transfer conductance against light-saturated photosynthetic rates and stomatal conductance were tested for significance by analysis of covariance.

RESULTS

Treatment influences on growth

Plant growth in diameter, height, plant mass, foliage mass and leaf area significantly increased with nitrogen and phosphorus supply ($F_{3,40} > 43$, $P < 0.001$) and all variables apart from leaf area growth ($F_{1,40} = 0.01$, $P = 0.92$) were greater in Clone A than Clone B ($F_{1,40} > 18$, $P < 0.001$) (Table 3.1). The main effects of nutrient treatment were generally much larger than the main effects of genotype as shown by F values. Similarities in leaf area growth between clones might be explained because the slow-growing clone had significantly larger ($F_{1,40} = 17.3$, $P < 0.001$) leaf area to mass ratio than the fast-growing clone i.e. 20 m^2 kg^{-1} for Clone B compared to 16 m^2 kg^{-1} for Clone A (Table 3.2). Average mass per fascicle conformed to plant growth increasing with nutrient supply ($F_{3,40} = 31.3$, $P < 0.001$) being significantly larger in the fast- (Clone A) compared with the slow-growing clone (Clone B) ($F_{1,40} = 57.3$, $P < 0.001$) (Table 3.1). All plant growth and foliage variables in imbalanced treatments N_0P_1 and N_1P_0 were intermediate between balanced N_0P_0 and N_1P_1 treatments, indicating an additive effect of mainly nitrogen but also phosphorus supply on these variables.

Table 3.1. Plant growth, leaf area and average mass per fascicle across nutrient treatments and clones. Nutrient treatments comprised two nitrogen supply regimes (N_0=1.43 and N_1=7.14 mM) and two phosphorus supply regimes (P_0=0.084 and P_1=0.420 mM). Values are presented as means (± 1 SE) for each treatment and clone. Significance of main effects of clones (C) and nutrient treatments (T) or the interaction between clones and treatments (C x T) are shown as F values, P values and P-range (ns: non significant, ***: significant at $P < 0.001$). Separation of means was determined by a Tukey test. Different letters within treatments or clones indicate that means were significantly different at $P < 0.05$.

	Diameter (mm)	Height (mm)	Plant mass (g)	Foliage mass (g)	Leaf area (m^2)	Mass per fascicle (mg)
Treatments						
N_0P_0	7.6 ± 0.3 a	633 ± 41 a	94 ± 4 a	21 ± 1 a	0.39 ± 0.02 a	31 ± 3 a
N_0P_1	8.6 ± 0.2 b	718 ± 48 ab	115 ± 5 b	27 ± 1 b	0.46 ± 0.02 a	35 ± 3 ab
N_1P_0	10.9 ± 0.3 c	811 ± 54 b	172 ± 8 c	40 ± 2 c	0.76 ± 0.05 b	39 ± 2 b
N_1P_1	17.6 ± 0.4 d	1180 ± 43 c	404 ± 18 d	99 ± 5 d	1.72 ± 0.16 c	56 ± 3 c
Clones						
A	11.8 ± 0.9 a	937 ± 51 a	211± 28 a	51 ± 7 a	0.81 ± 0.11 a	47 ± 3 a
B	10.5 ± 0.8 b	733 ± 49 b	181± 25 b	42 ± 6 b	0.85 ± 0.14 a	33 ± 2 b
ANOVA						
T	288.1,<0.001 ***	42.5,<0.001 ***	275.2,<0.001 ***	276.5,<0.001 ***	86.3,<0.001 ***	31.3,<0.001 ***
C	25.0,< 0.001 ***	30.4,<0.001 ***	17.8,<0.001 ***	24.4,<0.001 ***	0.01,0.92 ns	57.3,<0.001 ***
C × T	1.15,0.34 ns	0.25,0.86 ns	0.36,0.78 ns	0.38,0.77 ns	0.18,0.90 ns	0.25, 0.86 ns

Treatment influences on photosynthetic rates and foliage nutrient concentrations

Foliage nitrogen and phosphorus concentrations conformed to the nutrient treatments and were significantly affected by them ($F_{3,40} > 40$, $P < 0.001$) (Table 3.2). At the tree level, observed N_a ranged almost fourfold from 19 to 92 mmol m^{-2} whereas P_a ranged almost eightfold from 0.6 to 4.7 mmol m^{-2}. The N_a / P_a ratio ranged 18-fold from 5 to 91 (mole basis). Values of N_a / P_a did not significantly differ between the N_0P_0 and N_1P_1 treatment with an average (± 1 SE) value of 26.3 ± 1.1. However, N_a / P_a values of plants in the N_0P_1 and N_1P_0 treatments significantly differ with all other treatments being on average (± 1 SE) 8.7 ± 0.8 and 71.5 ± 2.6, respectively (data not shown). Values of N_a, P_a and their ratio were not influenced by clone ($F_{1,40} < 0.38$, $P > 0.54$).

Table 3.2. Leaf area to mass ratio (M), nitrogen and phosphorus concentration on an area basis (N_a, P_a), light-saturated rate of photosynthesis (A_{sat}) and photosynthetic nitrogen- (E_N) and phosphorus-use efficiency (E_P) across nutrient treatments and clones. Nutrient treatments comprised two nitrogen supply regimes (N_0=1.43 and N_1=7.14 mM) and two phosphorus supply regimes (P_0=0.084 and P_1=0.420 mM). Values are presented as means (± 1 SE) for each treatment and clone. Significance of main effects of clones (C) and nutrient treatments (T) or the interaction between clones and treatments (C × T) are shown as F values, P values and P-range (ns: non significant, ***: significant at $P < 0.001$). Separation of means was determined by a Tukey test. Different letters within treatments or clones indicate that means were significantly different at $P < 0.05$.

	M (m^2 kg^{-1})	N_a (mmol m^{-2})	P_a (mmol m^{-2})	A_{sat} (μmol m^{-2} s^{-1})	E_N (μmol mol^{-1} s^{-1})	E_P (μmol mol^{-1} s^{-1})
Treatments						
N_0P_0	18.6 ± 1.2 a	28.6 ± 1.7 a	1.2 ± 0.1 a	3.4 ± 0.2 a	125 ± 12 a	2910 ± 245 a
N_0P_1	17.5 ± 0.9 a	29.0 ± 2.7 a	3.4 ± 0.2 b	3.5 ± 0.2 a	119 ± 6 a	1027 ± 95 b
N_1P_0	19.0 ± 1.3 a	70.4 ± 4.5 b	1.0 ± 0.1 a	4.7 ± 0.3 b	70 ± 7 b	4894 ± 398 c
N_1P_1	17.5 ± 1.4 a	42.7 ± 2.4 c	1.5 ± 0.1 a	4.9 ± 0.2 b	117 ± 4 a	3335 ± 176 a
Clones						
A	16.3 ± 0.6 a	40.9 ± 4.3 a	1.8 ± 0.2 a	3.9 ± 0.2 a	108 ± 7 a	3001 ± 349 a
B	20.0 ± 0.9 b	45.2 ± 3.9 a	1.7 ± 0.2 a	4.4 ± 0.2 a	107 ± 7 a	3172 ± 325 a
ANOVA						
T	0.33,0.80 ns	39.6, <0.001 ***	56.7, <0.001 ***	9.2, <0.001 ***	9.9, <0.001 ***	34.2, <0.001 ***
C	17.3, <0.001 ***	0.38, 0.54 ns	0.07, 0.79 ns	3.20, 0.08 ns	0, 0.97 ns	0.12, 0.73 ns
C × T	0.99, 0.40 ns	1.54, 0.22 ns	1.35, 0.27 ns	0.81, 0.50 ns	0.63, 0.59 ns	0.47, 0.70 ns

The rate of photosynthesis at saturating irradiance and ambient CO_2 concentration, A_{sat}, increased significantly with nutrient supply ($F_{3,40} = 9.2$, $P < 0.001$) from about 3.4 to about 4.9 μmol m^{-2} s^{-1} in the low-nutrient compared with the high-nutrient supply treatment independent of clone ($F_{1,40} = 3.2$, $P = 0.08$) (Table 3.2). Values of A_{sat} resembled all plant growth and fascicle variables, with an additive effect of mainly nitrogen but also phosphorus supply on this

variable. Values of A_{sat} in imbalanced treatments were higher in plants growing with N only than P only

Photosynthetic nitrogen- (E_N) and phosphorus-use efficiency (E_P) were strongly influenced by nutrient supply ($F_{3,40} > 9.9$, $P < 0.001$) not differing between clones ($F_{1,40} < 0.12$, $P > 0.73$) (Table 3.2). Values of E_N were similar in nutrient treatments other than N_1P_0, being about 120 μmol CO_2 mol N^{-1} s^{-1}. Values of E_P were similar in balanced treatments N_0P_0 and N_1P_1 (about 3123 μmol CO_2 mol P^{-1} s^{-1}), and collectively intermediate between imbalanced treatments N_0P_1 and N_1P_0.

Transfer conductance, stomatal conductance and CO_2 gradients

Transfer conductance (g_m) significantly increased with nutrient supply ($F_{3,40} = 5.49$, $P = 0.003$) (Table 3.3) being 28% greater in the high-nutrient supply treatment (101 ± 4 mmol m^{-2} s^{-1} bar^{-1}) compared with the low-nutrient supply regime (79 ± 5 mmol m^{-2} s^{-1} bar^{-1}). Values of g_m also seemed to be influenced by the additive effect of nitrogen and phosphorus supply, with g_m values of plants growing with N only being greater than those of plants growing with P only.

Although stomatal conductance (g_s) was not significantly influenced by nutrient treatment, clone or their interaction, it tended to scale with nutrient supply from about 66 in the low-nutrient supply regime to about 86 mmol m^{-2} s^{-1} in the high-nutrient supply regime. Values of g_s in plants growing with N only also seemed to be greater than those of plants growing with P only. Average values of g_m were greater than those of g_s and their ratio (g_m / g_s) was on average (± 1 SE) 1.22 ± 0.06 not being significantly affected by nutrient treatment ($F_{3,29} = 0.68$, $P = 0.57$) or clone ($F_{1,29} = 0.22$, $P = 0.65$) (Table 3.3).

The intercellular (C_i) and chloroplastic (C_c) CO_2 concentration and their difference (C_i – C_c) were not influenced by nutrient treatment, clone or their interaction ($F_{7,40} < 1.16$, $P > 0.35$). Values of C_i were about 53 μmol mol^{-1} lower than ambient CO_2 concentration, C_a, being on average (± 1 SE) 303 ± 3 μmol mol^{-1}. Values of C_c were about 48 μmol mol^{-1} lower than C_i being on average (± 1 SE) 255 ± 4 μmol mol^{-1} (Table 3.3).

Table 3.3. Comparison of mesophyll (g_m) and stomatal (g_s) conductance to CO_2 diffusion, and their ratio (g_m / g_s), intercellular (C_i) and chloroplastic (C_c) CO_2 concentration and their gradient (C_i - C_c) across nutrient treatments and clones. Nutrient treatments comprised two nitrogen supply regimes (N_0=1.43 and N_1=7.14 mM) and two phosphorus supply regimes (P_0=0.084 and P_1=0.420 mM). Values are presented as means (± 1 SE) for each treatment and clone. Significance of main effects of clones (C) and nutrient treatments (T) or the interaction between clones and treatments (C × T) are shown as *F* values, *P* values and *P*-range (ns: non significant, **: significant at $P < 0.01$). Separation of means was determined by a Tukey test. Different letters within treatments or clones indicate that means were significantly different at $P < 0.05$. (‡) overall model was not significant.

	g_m	g_s	g_m / g_s	C_i	C_c	C_i - C_c
	(mmol m^{-2} s^{-1} bar^{-1})	(mmol m^{-2} s^{-1})		(µmol mol^{-1})	(µmol mol^{-1})	(µmol mol^{-1})
Treatments						
N_0P_0	79 ± 5 a	66 ± 7 a	1.12 ± 0.13 a	302 ± 7 a	257 ± 9 a	45 ± 4 a
N_0P_1	78 ± 5 a	61 ± 7 a	1.29 ± 0.19 a	306 ± 9 a	260 ± 10 a	46 ± 4 a
N_1P_0	88 ± 3 ab	72 ± 5 a	1.24 ± 0.08 a	302 ± 5 a	248 ± 6 a	54 ± 4 a
N_1P_1	101 ± 4 b	86 ± 6 a	1.21 ± 0.08 a	302 ± 5 a	254 ± 5 a	48 ± 2 a
Clones						
A	82 ± 3 a	71 ± 3 a	1.20 ± 0.07 a	305 ± 3 a	258 ± 5 a	47 ± 3 a
B	91 ± 4 a	71 ± 6 a	1.24 ± 0.11 a	300 ± 6 a	251 ± 6 a	49 ± 2 a
ANOVA						
T	5.49, 0.003	3.05, 0.05	0.68,0.57	0.08, 0.97	0.37, 0.77	1.22, 0.32
	**	ns(‡)	ns	ns	ns	ns
C	3.95, 0.053	1.73, 0.20	0.22,0.65	0.62, 0.44	0.98, 0.32	1.09, 0.30
	ns	ns	ns	ns	ns	ns
C × T	0.09, 0.97	1.47, 0.24	2.03,0.13	2.40, 0.08	1.88, 0.14	0.29, 0.83
	ns	ns	ns	ns	ns	ns

Predicting transfer conductance

Values of g_m scaled approximately with A_{sat} ($r^2 = 0.25$, $P < 0.001$) and g_s ($r^2 = 0.14$, $P < 0.001$) but not with foliage nitrogen ($F_{3,43} = 1.33$, $P = 0.27$) or phosphorus ($F_{3,43} = 1.87$, $P = 0.15$) concentrations. Slopes ($F_{1\text{-}3,40\text{-}44} < 1.96$, $P > 0.14$) and intercepts ($F_{1\text{-}3,40\text{-}44} < 0.77$, $P > 0.46$) of the linear relationship of g_m against A_{sat} and g_s were not influenced by nutrient treatment or clone (Figure 3.3). The g_m / A_{sat} and g_m / g_s relationships found in this study were very similar to the ones found by Loreto *et al.* (1992) using isotope fractionation, constant and variable *J* modeling, for 15 angiosperms species (dashed lines in Figure 3.3).

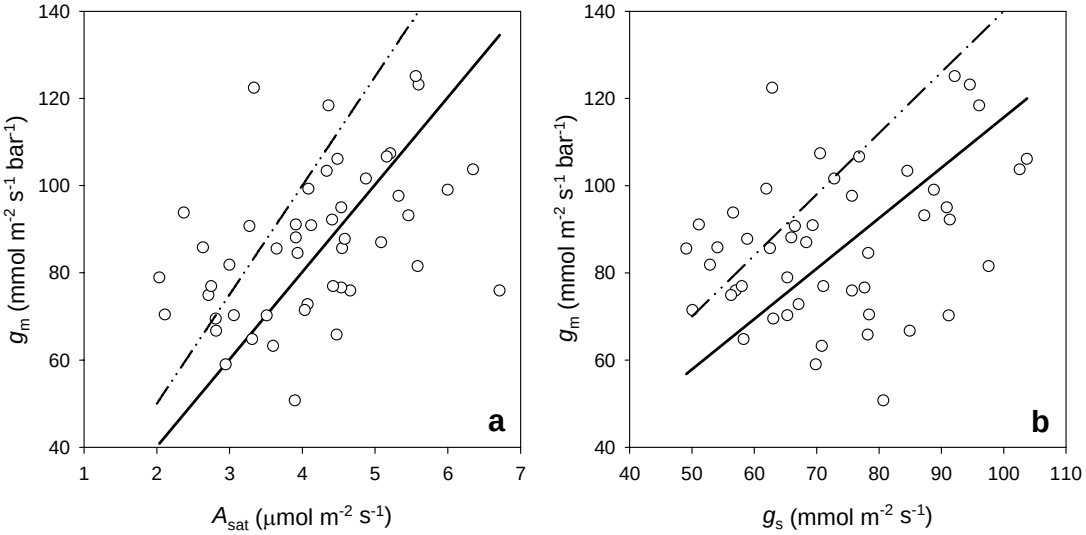


Figure 3.3. The response of transfer conductance (g_m) to the rate of photosynthesis at saturating irradiance and ambient CO_2 (A_{sat}) and stomatal conductance (g_s). Fitted lines are: (a) $g_m = 0.020\ A_{sat}$, $r^2 = 0.25$, $P < 0.001$; (b) $g_m = 1.16\ g_s$, $r^2 = 0.14$, $P < 0.001$. Slopes and intercepts of the g_m / A_{sat} and g_m / g_s linear relationships did not differ significantly between nutrient treatments or clones ($P > 0.14$). Dashed-lines are: (a) $g_m = 0.025\ A_{sat}$, $r^2 = 0.76$, (b) $g_m = 1.4\ g_s$, $r^2 = 0.80$, determined for 15 angiosperms species by Loreto *et al*. (1992).

Stomatal, mesophyll and biochemical limitations to photosynthesis

Relative stomatal (L_S), mesophyll (L_M) and biochemical (L_B) limitations to photosynthesis were not significantly influenced by main or interactive effects of nutrient treatment or clone ($F_{7,40} < 1.03$, $P > 0.43$), scaling as a fixed proportion of the light-saturated photosynthetic rate (Table 3.4). The relative limitation posed by g_m (L_M) was on average (± 1 SE) 0.16 ± 0.01 (range 0.07-0.25), and was generally greater than the relative limitation posed by g_s (L_S) which was on average (± 1 SE) 0.13 ± 0.01 (range 0.03-0.20). Biochemical limitations (L_B) were on average (± 1 SE) 0.71 ± 0.02 (range 0.57-0.87), and were predominant over g_s and g_m limitations (Table 3.4).

Table 3.4 Relative stomatal (L_S), mesophyl (L_M) and biochemical (L_B) limitations to photosynthesis across nutrient treatments and clones. Treatments comprised a combination of two nitrogen supply regimes (N_0=1.43 and N_1=7.14 mM) and two phosphorus supply regimes (P_0=0.084 and P_1=0.420 mM). Values are presented as means (± 1 SE) for each treatment and clone. Differences between clones and treatments were non significant.

	Relative Limitations (%)		
	Stomatal L_S	Mesophyll L_M	Biochemical L_B
Treatments			
N_0P_0	12 ± 2 a	13 ± 2 a	75 ± 4 a
N_0P_1	13 ± 3 a	16 ± 2 a	71 ± 4 a
N_1P_0	12 ± 1 a	18 ± 1 a	70 ± 2 a
N_1P_1	13 ± 1 a	15 ± 1 a	72 ± 2 a
Clones			
A	11 ± 1 a	15 ± 1 a	74 ± 2 a
B	14 ± 2 a	17 ± 1 a	70 ± 3 a
Mean	13 ± 1	16 ± 1	71 ± 2
ANOVA			
T	0.16, 0.92 ns	1.55, 0.22 ns	0.54, 0.66 ns
C	0.26, 0.61 ns	1.55,0.22 ns	1.79, 0.19 ns
C × T	0.74, 0.53 ns	0.39, 0.76 ns	0.82, 0.49 ns

The CO_2 response of photosynthesis

The mitochondrial CO_2 compensation point in the light (Γ^*) was remarkably stable across nutrient treatments and clones ($F_{7,40}$ = 0.52, P = 0.81) being on average (± 1 SE) 49.4 ± 1.4 μmol mol^{-1} (Table 3.5). This value was on average 7.2 μmol mol^{-1} greater than the intercellular CO_2 compensation in the light (C_i^*), which was on average (± 1 SE) 42.2 ± 1.4 μmol mol^{-1} and not influenced by nutrient treatments, clones or their interaction ($F_{7,40}$ = 0.66, P = 0.71). In contrast, the rate of mitochondrial respiration (R_d) varied five-fold (0.3-1.5 μmol m^{-2} s^{-1}) tending to increase with nutrient supply ($F_{3,40}$ = 1.87, P = 0.15) from 0.54 μmol m^{-2} s^{-1} in the low-nutrient supply regime compared to 0.70 μmol m^{-2} s^{-1} in the high-nutrient supply regime independent of clone ($F_{1,40}$ = 2.71, P 0.06).

The maximum rate of Rubisco carboxylation (V_{cmax}) calculated on a C_c basis varied from 9 to 28 μmol m^{-2} s^{-1} and significantly increased with nutrient supply ($F_{3,40}$ = 6.65, P < 0.001), being 65% greater in the high-nutrient supply regime (22.1 ± 1.6 μmol m^{-2} s^{-1}) compared with the low-nutrient supply treatment (13.4 ± 0.9 μmol m^{-2} s^{-1}). Similarly the rate of electron transport driving RuBP-regeneration on a C_c basis (J_{max}, 20-58 μmol electrons m^{-2} s^{-1}) increased significantly with nutrient supply ($F_{3,40}$ = 4.94, P = 0.005) from about 30 to about 47 μmol m^{-2} s^{-1} in the low- compared with the high-nutrient supply regime (57% increase). The

response of V_{cmax} and J_{max} in treatments N_0P_1 and N_1P_0 were intermediate between treatments N_0P_0 and N_1P_1, and the response was stronger in plants growing with N only than P only.

Table 3.5. The intercellular (C_i^*) and chloroplastic (Γ^*) CO_2 compensation concentration in the absence of mitochondrial respiration, the rate of mitochondrial respiration in the light (R_d), maximal rate of Rubisco carboxylation (V_{cmax}), maximal rate of electron transport driving regeneration of RuBP (J_{max}) and the ratio J_{max} / V_{cmax} across nutrient treatments and clones. The leaf area to mass ratio was used as a covariate in the analysis of V_{cmax} and J_{max}. Nutrient treatments comprised two nitrogen supply regimes (N_0=1.43 and N_1=7.14 mM) and two phosphorus supply regimes (P_0=0.084 and P_1=0.420 mM). Values are presented as means (± 1 SE) for each treatment and clone. Significance of main effects of clones (C) and nutrient treatments (T) or the interaction between clones and treatments (C x T) are shown as *F* values, *P* values and *P*-range (ns: non significant, **: significant at $P < 0.01$, ***: significant at $P < 0.001$). Separation of means was determined by a Tukey test where applicable. Different letters within treatments or clones indicate that means were significantly different at $P < 0.05$.

	C_i^*	Γ^*	R_d	V_{cmax}	J_{max}	J_{max} / V_{cmax}
	(μmol mol^{-1})	(μmol mol^{-1})	(μmol m^{-2} s^{-1})	(μmol m^{-2} s^{-1})	(μmol m^{-2} s^{-1})	
Treatments						
N_0P_0	40.7 ± 2.8 a	47.6 ± 2.9 a	0.54 ± 0.03 a	13.4 ± 0.8 a	30.1 ± 2.1 a	2.20 ± 0.08 a
N_0P_1	41.9 ± 2.5 a	49.7 ± 2.5 a	0.61 ± 0.09 a	15.4 ± 1.3 ab	33.1 ± 2.6 a	2.15 ± 0.03 a
N_1P_0	43.2 ± 3.2 a	50.2 ± 2.8 a	0.61 ± 0.06 a	19.9 ± 2.1 bc	41.1 ± 4.4 ab	2.07 ± 0.04 a
N_1P_1	43.1 ± 3.3 a	50.1 ± 3.6 a	0.70 ± 0.09 a	22.1 ± 1.6 c	46.7 ± 3.6 b	2.11 ± 0.05 a
Clones						
A	43.9 ± 2.0 a	50.3 ± 2.0 a	0.52 ± 0.04 a	18.2 ± 1.4 a	37.9 ± 2.9 a	2.11 ± 0.05 a
B	40.6 ± 2.1 a	48.5 ± 2.1 a	0.71 ± 0.06 a	17.2 ± 1.1 a	37.7 ± 2.4 a	2.18 ± 0.04 a
ANOVA						
T	0.12, 0.94 ns	0.17, 0.92 ns	1.87, 0.15 ns	6.65, <0.001 ***	4.94, 0.005 **	1.52, 0.22 ns
C	1.39, 0.25 ns	0.38, 0.54 ns	1.34, 0.25 ns	0.39, 0.53 ns	0, 0.95 ns	1.43, 0.23 ns
C × T	0.95, 0.42 ns	0.92, 0.44 ns	2.71, 0.06 ns	0.75, 0.53 ns	0.55, 0.,65 ns	0.70, 0.55 ns

Values of V_{cmax} and J_{max} were on average 15 % and 20% greater in the slow- (Clone B) compared to the fast-growing clone (Clone A). However differences were completely removed when the leaf area to mass ratio (*M*) was included as a covariate in the analysis, as Clone B had a significantly higher *M* than Clone A (Table 3.2). The ratio J_{max}/V_{cmax} was independent of nutrient treatment or clone ($F_{7,40} = 1.16$, $P = 0.35$) being on average (± 1 SE) 2.15 ± 0.03 (Table 3.5). Average values of V_{cmax} and J_{max} calculated on a C_c basis were respectively 15.4 % and 3.1 % greater than those on a C_i basis, which translated into different slopes of the J_{max} / V_{cmax} relationship (C_c basis: $J_{max} = 2.11\ V_{cmax}$, $r^2 = 0.88$, $P < 0.001$; C_i basis: $J_{max} = 2.43\ V_{cmax}$, $r^2 = 0.86$, $P < 0.001$). Slopes ($F_{3,40} < 0.24$, $P > 0.86$) and intercepts ($F_{3,40} < 0.23$, $P > 0.87$) of the linear J_{max} / V_{cmax} relationships did not significantly differ between nutrient treatments and clones (Figure 3.4).

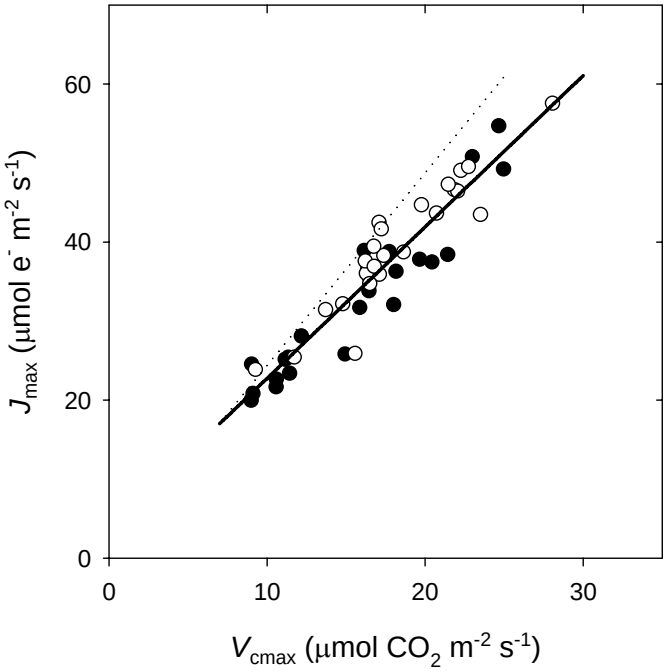


Figure 3.4. Relationship between the maximal rate of electron transport driving regeneration of RuBP (J_{max}) and the maximal rate of Rubisco carboxylation (V_{cmax}) calculated on a chloroplastic (solid line) and intercellular (dotted line) CO_2 concentration basis. On a C_c basis: $J_{max} = 2.1124\ V_{cmax}$, $r^2 = 0.88$, $P < 0.001$; on a C_i basis: $J_{max} = 2.4335\ V_{cmax}$, $r^2 = 0.86$, $P < 0.001$. Values of V_{cmax} and J_{max} calculated on a C_c basis were on average 15.4 % and 3.1 % greater than those on a C_i basis. Slopes and intercepts of the linear relationships between V_{cmax} and J_{max} did not differ significantly between clones ($P > 0.87$). Open symbols represent Clone A and closed symbols Clone B for V_{cmax} and J_{max} calculated on a C_c basis (omitted on a C_i basis).

DISCUSSION

Transfer conductance (g_m) scaled with nutrient supply, the light-saturated photosynthetic rate (A_{sat}) and stomatal conductance (g_s) but was not correlated with foliage nutrient concentrations. In relative terms, limitations posed by g_m were small (16%), similar to those posed by g_s (13%), and collectively much smaller than limitations posed by biochemical processes (71%), and no proportions were influenced by nutrient supply. Hence diffusion gradients posed by g_s (C_a-C_i) and g_m (C_i-C_c) were constant (about 50 µmol mol^{-1}), revealing homeostatic mechanisms regulating g_s and g_m so that diffusion gradients remained remarkably stable despite nutritional stresses. This proportional change in g_m and photosynthesis has been observed previously but mostly using data from different species by von Caemmerer and Evans (1991), Loreto *et al.* (1992) and Singsaas *et al.* (2003), among others, and suggests together with the results of this and the previous chapter (Bown *et al.* 2007), that nitrogen and phosphorus supply directly influence the rates of Rubisco carboxylation (V_{cmax}) and electron

transport (J_{max}), but only indirectly influence stomatal and transfer conductance processes, which are regulated to closely match V_{cmax} and J_{max} capacities.

Transfer conductance (g_m) and associated variables compared well with values reported in other studies. Transfer conductance values were on average 0.087 μmol m^{-2} s^{-1} bar^{-1} in this study (0.174 on a hemi-surface area basis) and similar to the 0.15 μmol m^{-2} s^{-1} bar^{-1} found in *Pinus radiata* by De Lucia *et al*. (2003). The g_m / g_s ratio found in this study was about 1.2, which compares well to the 1.1 given by De Lucia *et al* (2003), but considerably less than the 1.6-1.7 reported for Douglas-fir by Warren *et al*. (2003*b*). Also, transfer conductance limited photosynthesis by 16% in this study similarly to the 20% reported in 50-year-old *Pseudotsuga mensiezii* trees by Warren *et al*. (2003*b*), and 10-15 % reported for *Nicotiana tabacum* by Bernacchi *et al*. (2002). Finally, the CO_2 concentration gradient posed by g_m (C_i - C_c) in this study was about 48 μmol mol^{-1}, which is within the range given for *Pseudotsuga menziesii* (30-88 μmol mol^{-1}) by Warren and Adams (2006).

In this study, g_m correlated well with A_{sat} and g_s as reported previously (von Caemmerer and Evans 1991, Loreto *et al*. 1992, Singsaas *et al*. 2003, Warren *et al*. 2003*b*). Loreto *et al*. (1992) suggested that g_m can be incorporated into models of photosynthesis assuming g_m to be 1.4 times g_s or 0.025 A_{sat} based on data from 15 angiosperm species under non-stressed conditions. Similarly, transfer conductance (g_m) in *Pinus radiata* can be assumed to be 0.020 A_{sat} or 1.16 g_s, based on the results of this study, when measured at 20 °C, ambient CO_2 concentration and saturating irradiance using a VPD reference value between 0.5-1 kPa.

Some authors have argued that g_m might be at least partially determined by leaf structure characteristics such as leaf thickness (Singsaas *et al*. 2003), cell wall thickness (Hanba *et al*. 2002, Miyazawa and Terashima 2001) and the length of the liquid pathway that CO_2 must overcome to reach the chloroplasts (Evans and von Caemmerer 1996). In this study fascicle size increased with nutrient supply and this did not affect relative limitations imposed by g_m. Also the fast-growing clone had larger fascicles and a smaller leaf area to mass ratio than the slow-growing clone, and we did not find differences in g_m or the relative limitations imposed by g_m between clones. Bernacchi *et al*. (2002) argue that structural traits do not explain rapid responses of g_m to environmental stresses, and that responses of g_m to temperature resemble an enzyme mediated process. Our results support this hypothesis as differences in fascicle size (mass) and the leaf area to mass ratio did not influence the relative limitations posed by g_m.

Until recently, values of V_{cmax} and J_{max} were calculated based on responses of A to C_i rather than CO_2 concentration in the chloroplast, C_c, with the implicit assumption that mesophyll conductance, g_m, was infinite. However, g_m has been shown to be finite causing C_c at Rubisco to be lower than C_i and thus underestimating values of V_{cmax} and J_{max} (Harley *et al*.

1992, Loreto *et al*. 1992, von Caemmerer 2000, Long and Bernacchi 2003, Manter and Kerrigan 2004, Ethier *et al*. 2006). In this study, we found C_c to be about 50 μmol mol^{-1} lower than C_i independent of nutrient treatment or clone. This gradient changed the response of V_{cmax} and J_{max} to CO_2 concentration. Values of V_{cmax} and J_{max} calculated on a C_c basis were on average 15.4% and 3.1 % greater than those on a C_i basis, which translated into different slopes of the J_{max} / V_{cmax} relationship (C_c basis: J_{max} = 2.11 V_{cmax}, r^2 = 0.88, P < 0.001; C_i basis: J_{max} = 2.43 V_{cmax}, r^2 = 0.86, P < 0.001). Similarly, Singaas *et al* (2003) found V_{cmax} to be more affected by the recalculation than J_{max}, affecting the J_{max} / V_{cmax} relationship by reducing its slope.

Harley *et al*. (1992) showed that estimates of g_m using the constant J method are highly sensitive to errors in the estimation of Γ^* such that a plus or minus 10 % error in Γ^* led to 92 % and 32 % over and underestimations of g_m respectively. In this study values of Γ^* and R_d were determined using the approach followed by Warren (2006) i.e. values of C_i^* and R_d were calculated using the Laisk method (von Caemmerer 2000), and these used to solve for g_m and Γ^* using the constant J method (Harley *et al*. 1992). Our values of Γ^* for *Pinus radiata* were on average 49 μmol mol^{-1} and about 7 μmol mol^{-1} greater than those of C_i^*. Piel *et al*. (2002) found Γ^* to be about 51 μmol mol^{-1} and about 3 μmol mol^{-1} higher than those of C_i^* in *Juglans regia*, while Warren (2006) found Γ^* to be about 67 μmol mol^{-1} and about 15 to 24 μmol mol^{-1} greater than C_i^* in *Pinus pinaster*. Warren (2006) argues that these differences are large enough to influence g_m estimates, and the results of this thesis support this statement. As an example, we refitted values of g_m using values of C_i^* rather than Γ^*, and we found that trends were similar to those obtained using Γ^* (Table 3.5) with only treatment effects being significant ($F_{3,40}$ = 5.23, P = 0.004), but values of g_m calculated using C_i^* were on average 9% lower than values of g_m calculated using Γ^*. We are also aware that R_d could have been overestimated due to diffusion of CO_2 from foliage clamped under the gasket of the Li-6400-40 chamber (Pons and Welschen 2002). However, we consider the error in the estimation of g_m to be small because Harley *et al* (1992) reported that a ± 10 % error in R_d led to ± 3% error in the estimation of g_m using the constant J method and additionally we used a slow flow rate (200-300 μmol m^{-2} s^{-1}) in the determination of R_d as described by Warren (2006).

In conclusion, this paper examined the effects of nitrogen and phosphorus supply on transfer conductance using two contrasting clones of *Pinus radiata*. The study showed that transfer conductance limitations increased with nitrogen and phosphorus supply as a fixed proportion of the light-saturated photosynthesis rate (16%) independently of clone. Also, the gradient posed by g_m was constant and about 50 μmol mol^{-1}, which leads to an underestimation of 15% and 3% in V_{cmax} and J_{max} values when expressed on a C_i rather than C_c basis.

CHAPTER FOUR

INFLUENCE OF NITROGEN AND PHOSPHORUS SUPPLIES AND GENOTYPE ON NITROGEN UPTAKE, STORAGE AND INTERNAL CYCLING OF *PINUS RADIATA*

Summary One-year old *Pinus radiata* cuttings from four genotypes were cultivated in silica sand in a factorial combination of nitrogen (N_0=1.43 and N_1=7.14 mM) and phosphorus (P_0=0.084 and P_1=0.420 mM) supply for two years. N supply was enriched with ^{15}N to 2.5 ‰ (labelled N) during the first year, then plants transferred to clean sand and cultivated for another year with ^{15}N at levels close to natural abundance (0.3664899 atom percent ^{15}N, $\delta^{15}N$ 0.5115 ‰) provided by the source of N in nutrient solution. Recovery of labelled and unlabelled N was used to estimate N remobilization. Nitrogen was stored mainly in foliage (0.46-0.51) and roots (0.34-0.45) and to lesser extent in stems (0.09-0.14) at the end of the first year. Absolute values of N remobilization increased with N and P supply. N remobilization was about five-fold greater in the high-nutrient supply regime (953 mg plant^{-1}) compared to the low-nutrient supply regime (199 mg plant^{-1}) by the end of the experiment. N remobilization in imbalanced treatments N_0P_1 (228 mg plant^{-1}) and N_1P_0 (422 mg plant^{-1}) was intermediate between balanced treatments N_0P_0 and N_1P_1. In relative terms, however, 65% of all stored N was remobilized in the high-nutrient supply regime compared to 42-48% at lower N and P addition rates. The ratio of N uptake to N remobilization was greater in high-N supply regimes (3.4) compared to low-N supply regimes (2.1), indicating that trees rely progressively more on remobilization than uptake as fertility drops. Most N remobilization occurred during spring-summer (77%), coincidently with the largest proportion of needle development (80%), suggesting that N remobilization is driven by sink-strength. Old-foliage was by far the main source for internal cycling (87%) while roots were the main sink (40%). Clones exhibited differences in N remobilization but these differences were not associated with growth performance, and were completely explained by the size of the N pool before remobilization took place, suggesting that N remobilization performance was similar among clones.

Keywords: *internal cycling, genotypes, nitrogen, phosphorus, Pinus radiata*.

INTRODUCTION

Nutritional stresses are commonly associated with nutrient imbalances rather than single nutrient deficiencies (Reich and Schoettle 1988, Marschner 1995, Aerts and Chapin, 2000). The mechanisms leading to nutrient imbalances can be difficult to unravel because factors affecting nutrient uptake and internal cycling are poorly understood (Proe *et al.* 2000). Trees rely heavily on their capacity for internal cycling of mobile nutrients for growth and survival (Nambiar and Fife 1987, 1991, Millard and Proe 1993, Proe *et al*. 2000). For instance, Nambiar and Fife (1987) found that 32-57% of nitrogen required for leaf growth in young trees of *Pinus radiata* might come from remobilization. Similarly, for the same species, Fife and Nambiar (1982) estimated that 40-86% of phosphorus required to support new growth was provided by remobilization.

Nutrient remobilization in conifers has been determined by sequential sampling of needle masses and nutrient contents (nutrient budgets) and by using stable isotopes (Proe *et al*. 2000). Nutrient budgets underestimate the relative contribution of remobilization because uptake from the soil and remobilization can not be separated (Mead and Preston 1994, Proe and Millard 1994, Proe *et al*. 2000). Stable isotopes, on the other hand, have been frequently and reliably used to quantify nutrient uptake and remobilization. Such studies have shown that trees separate nutrients from current uptake and storage from the previous year (Proe *et al*. 2000). This has been shown for both N (Proe and Millard 1994) and P (Proe and Millard 1995), but investigations on the interactive influence of nitrogen and phosphorus supply on nitrogen remobilization in pines have apparently not been conducted. Although foliage is the main source for nutrient remobilization, the use of stable isotopes has shown that woody tissues also contribute to nutrient remobilization in *Pinus contorta* (Mead and Preston 1994), whereas budget studies in *Pinus radiata* have not (Nambiar 1987), providing a sound hypothesis to be tested for the latter species using stable isotopes.

Genetics is emerging as an important factor controlling internal nutrient efficiency and remobilization. For instance, Miller and Hawkins (2003) compared nitrogen uptake and utilization by slow- and fast-growing families of interior spruce in a greenhouse experiment with different nitrogen supply regimes. Fast growing families developed more rapidly and were more efficient in the utilization of internal nitrogen at all fertility levels, suggesting a potential for tree improvement based on nitrogen-use-efficiency traits. Similarly Fife and Nambiar (1995) found large differences in the growth responses of *Pinus radiata* families to

nitrogen additions independently of water relations, and therefore there is a possibility that nutrient remobilization capacity can at least partially account for these differences.

This chapter examines the interactive influences of nitrogen and phosphorus supply on nitrogen storage and remobilization in *Pinus radiata*, and also the extent to which four genotypes with contrasting growth patterns may exhibit differences in N remobilization response to combined nitrogen and phosphorus limitations. The hypotheses for the study were that (1) nitrogen remobilization increases with N supply but is constrained by N or P imbalances, (2) the contribution of woody tissues to N remobilization is minimal, and (3) greater genotypic growth performance is explained by greater storage and remobilization capacity.

MATERIALS AND METHODS

Plant material

One-year old *Pinus radiata* cuttings from four clones (Clones P26C2, P26C5, P08C9 and S11C3 referred to hereafter as clones A, B, C and D), raised under standard ENSIS (formerly New Zealand Forest Research Institute) nursery conditions, were cultivated in silica sand for 24 months ending July 2006. Plants were grown under a factorial combination of nitrogen (N_0=1.43 and N_1=7.14 mM) and phosphorus (P_0=0.084 and P_1=0.420 mM) supply with nine replicates per clone per treatment (144 plants). Plants were irrigated with nutrient solutions in increasing amounts (from 0.5 to 1.0 dm^3 per tree) and frequencies (from once weekly to twice weekly) to approximately account for increasing nutrient demands with plant size (Ingestad 1982, 1986) and were watered daily in excess of plant requirements. Nutrients other than N and P were provided at 1.023 mol m^{-3} K, 0.250 mol m^{-3} Ca, 0.411 mol m^{-3} Mg, 0.281 mol m^{-3} S, 12.532 mmol m^{-3} Fe, 0.445 mmol m^{-3} Zn, 0.473 mmol m^{-3} Cu, 7.281 mmol m^{-3} Mn, 0.073 mmol m^{-3} Mo, 18.502 mmol m^{-3} B, 0.946 mmol m^{-3} Cl and 0.145 mmol m^{-3} Na following Ingestad (1979). Nitrogen was applied as $^{15}NH_4{}^{15}NO_3$ enriched to 2.5 atom percent ^{15}N for a year until plants were dormant in July 2005 (winter), and then was applied as NH_4NO_3 with ^{15}N at 0.3664899 atom percent ^{15}N ($\delta^{15}N$ 0.5115 ‰) for the rest of the experiment.

Trees were grown in a thermostatically controlled greenhouse where temperature fluctuated between 7 and 38 °C (mean ± 1 SD: 19 ± 5 °C) during the day, and 1 and 26 ºC (15 ± 4 °C) during the night depending on weather conditions. Vapour pressure deficit during the day fluctuated between 0 and 4.6 kPa (0.76 ± 0.65 kPa) and between 0 and 1.5 kPa (0.25 ±

0.42 kPa) during the night (Figure 4.2 a). Roots of all plants were artificially inoculated with spores of *Rhizopogon rubescens* Tul. and confirmed as mycorrhizal either by visual inspection of roots or by the presence of fruiting bodies.

Plants were arranged in a greenhouse in three replicate blocks of 48 plants (9 replicate plants per clone per treatment), and cultivated for a year in clean silica sand (< 0.1 % organic matter) using labelled ^{15}N. At the end of the first year of growth, while plants were still dormant (Figure 4.2c, indicated as harvest 1), three replicate plants per clone per treatment were randomly selected and harvested, (except for 3 out of 48 plants in which mortality precluded their harvest), and remaining plants removed from their pots, the sand carefully washed from roots, and trees transferred into pots 30 cm in diameter and 60 cm deep (42-dm^3) with clean sand. Hence, any carry-over effect of labelled N was eliminated during the second year of growth (Millard and Proe 1992).

During the second year, the remaining 6 replicate trees per clone per treatment (96 plants) were randomly selected to be harvested in equal amounts in two stages at months 18 and 24 (Figure 4.2c, indicated as harvest 2 and 3). Shoots were separated into stems, branches and foliage from the first and second year of growth. Pot content was spread over a tarpaulin, bulk root system collected and sand sieved to recover loose roots. Sand was thoroughly mixed and weighed and a 2.5 kg sample was taken to recover remaining roots by flotation. Roots were washed and separated into fine roots (≤ 2mm), coarse roots (>2mm), and below-ground stems. Fine roots were separated as first or second year growth during harvests at months 18 and 24. All samples from current year tissues used in the determination of nitrogen isotopic composition were known with certainty to be produced during the second year. All plant components were oven-dried to constant mass and dry mass recorded. Additionally a random sample of 10 three-needle fascicles from each age class for each tree was randomly selected and weighed, in order to calculate mean fascicle dry mass and total number of fascicles.

Oven-dried samples from foliage, stems and roots from first and second year growth from all plants and harvests (765 samples) were ground to a fine powder using first a rotary mill (Thomas-Wiley Laboratory Mill, Model 4, Philadelphia, PA., USA) and then a vibratory ball mill (Retsch MM-2000, Haan, Germany) and total nitrogen and its isotopic composition ($\delta^{15}N$) was determined using a mass spectrometer at the Stable Isotope Laboratory at the University of Waikato, New Zealand. The δ values were calculated as: $\delta\ (‰) = [(R_{sp} / R_{st}) - 1] \times 1000$, where R_{sp} and R_{st} are the $^{15}N/^{14}N$ ratios of the sample and standard (N_2 in air).

Reconciliation of ^{15}N recovery at harvests

The total labelled ^{15}N provided during the first year of growth ranged from 5 to 59 mg per tree (harvest 1). These amounts were expected to be fully recovered in harvests at months 18 and 24 because ^{15}N labelling stopped during the second year of growth. This was verified by comparing ^{15}N recovery at months 12, 18 and 24. However, these comparisons were not direct as trees harvested at months 12, 18 and 24 were different. Therefore, allometric equations fitted to tree diameters harvested at month 12 were used to predict tree mass at month 12 of those trees harvested at months 18 and 24. Then, tissue N concentrations and Atom % ^{15}N values at month 12 were used to predict ^{15}N content at month 12 in trees harvested at months 18 and 24.

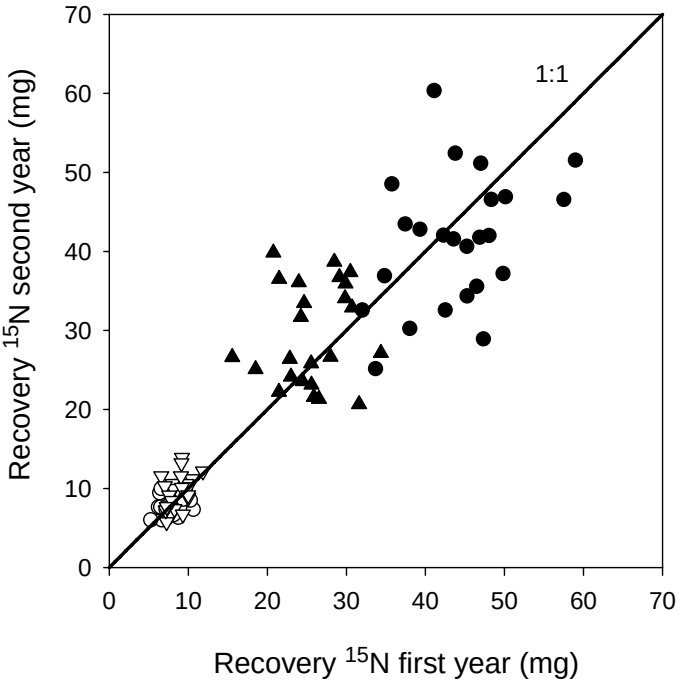


Figure 4.1. ^{15}N recovery (modelled) at the end of the first year of growth compared to actual ^{15}N recovered at harvests during the second year of growth over the background level (0.3664899 atom% ^{15}N, $\delta^{15}N$ = 0.51 ‰). Fitted model: $y = 0.98\ x$, $r^2 = 0.84$, $P < 0.001$.

There was good agreement between ^{15}N content modeled at month 12 and the actual ^{15}N content recovered in plants harvested at months 18 and 24 (Figure 4.1), which confirms that ^{15}N additions stopped during the second year of growth, and that any movement of ^{15}N from old to new tissues must had been the result of remobilization. Slopes ($F_{3,88} < 1.92$, $P > 0.13$) and intercepts ($F_{3,88} < 2.56$, $P > 0.06$) of the linear relationship between actual ^{15}N

recovery in plants harvested during the second year compared to the one modeled at month 12 were not significantly different among nutrient treatments and clones (Figure 4.1). This shows that ^{15}N was not lost from the system and that reliable estimators of overall remobilization were obtained.

Calculation of nutrient storage and remobilization

A ^{15}N recovery and mass-balance approach was used to determine N remobilization for each tree. N remobilization was calculated as the ^{15}N content recovered in new tissues at months 18 and 24 compared to total tree ^{15}N content at month 12. Tissue ^{15}N content was calculated as the product of tissue mass, tissue N concentration and tissue ^{15}N atom percent (excess). Tissue ^{15}N atom percent (excess) was calculated as the difference between the ^{15}N atom percent determined on plant tissue by mass spectrometry and the background level of the N source applied during the second year (0.3664899 atom% ^{15}N, $\delta^{15}N = 0.51$ ‰).

Data analysis

All analyses were made with SAS software (1996; SAS Institute, Cary, NC). Variables were tested for normality and homogeneity of variance and transformations were made as necessary to meet the underlying statistical assumptions of the models used. Analysis of variance was used to examine the main and interactive effects of nitrogen and phosphorus supply and genotype on internal cycling variables. Tukey's least significant difference test was used to distinguish among individual means where applicable with a confidence level of $P \leq 0.05$. Differences in slopes and intercepts between nutrient treatments and clones in the linear relationships between nitrogen remobilization during the second year against plant N pool accumulated during the previous year were tested for significance by analysis of covariance.

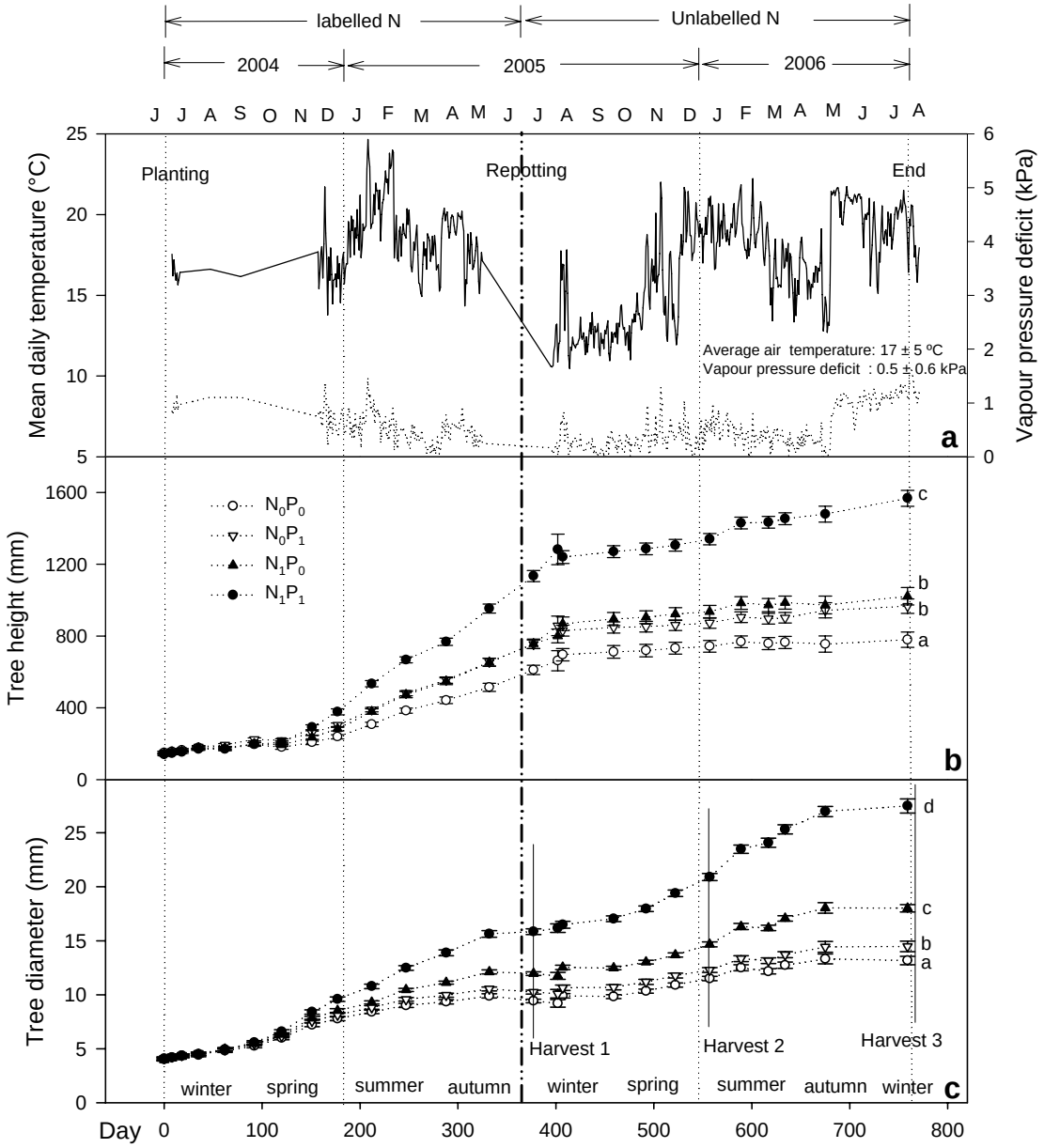


Figure 4.2. Climate and plant development in the nitrogen remobilization experiment over twenty-four months. (a) Average daily air temperature (solid line) and vapour pressure deficit (dotted line) during the 24-months experiment, (b) tree heights and (c) basal diameters across nutrient treatments. Nutrient treatments comprised two nitrogen supply regimes (N_0=1.43 and N_1=7.14 mM) and two phosphorus supply regimes (P_0=0.084 and P_1=0.420 mM). Figure (c) shows times of harvest at months 12, 18 and 24. Letters at the far right of the each curve in figures (b) and (c) indicate significant differences in tree height and diameter growth at the end of the experiment ($P < 0.05$).

RESULTS

Treatment influences on tree growth

Tree growth was strongly influenced by nutrient supply and to a lesser extent by genotype. The growth response in plant diameter, height and mass significantly increased with N and P supply ($F_{3,30} > 59$, $P < 0.001$), and this response was consistent within clones and at all harvests (Table 4.1, Figure 4.2b,c). Plant mass growth by the end of the experiment was 5.6 times greater in the high-nutrient supply regime (798 g) than in the low-nutrient supply regime (143 g) (Figure 4.3). The growth responses in imbalanced treatments N_0P_1 and N_1P_0 were intermediate between balanced treatments N_0P_0 and N_1P_1 and trees supplied with N only developed better than those with P only.

Although significant at all time intervals, the main effect of clone was relatively minor compared to the main effect of nutrient treatment as evidenced by *F* values (Table 4.1), and the variation between extremes in growth, which ranged 5.6 fold for nutrition, and 1.2 fold for clones. As the clone by nutrient treatment interaction was significant for mass growth at months 12 and 18 ($F_{9,30} > 2.3$, $P < 0.042$), there was variation in the growth response to nutrient treatments between clones during this time. However, by month 24 the interaction was insignificant, and growth in clone B, significantly exceeded that of other clones, in all nutrient treatments (Table 4.1).

The seasonality of plant growth varied with nutrient treatment (Figure 4.3). Plant mass growth during the first year was about 45-49% of that accumulated at the end of two years in all treatments except N_1P_1, in which the proportion was much lower (about 28%). During the second year, most growth occurred between months 12 and 18 which corresponded to spring-summer, while a smaller proportion of growth was observed between months 18-24 (autumn-winter) (see Figure 4.2 for seasonal effect, Figure 4.3 for mass values).

Initial plant mass did not confound the influence of genotype on plant growth throughout the experiment. Plant diameter, height and mass at the time of planting were not influenced by nutrient treatment ($F_{3,30} < 1.25$, $P > 0.31$), but were significantly different among clones ($F_{3,30} > 7.6$, $P < 0.001$) on a scale B ≥ C ≥ D ≥ A (data not shown). However, covariate analysis showed that initial plant mass was non-significant as a predictor of plant mass growth at months 12, 18 and 24 ($F_{1,29} < 0.23$, $P > 0.63$).

Table 4.1. Accumulated plant mass growth at months 12, 18 and 24 across nutrient treatments and clones. Nutrient treatments comprised two nitrogen supply regimes (N_0=1.43 and N_1=7.14 mM) and two phosphorus supply regimes (P_0=0.084 and P_1=0.420 mM). Values are presented as means (± 1 SE) for each treatment and clone; *n* =3. Significance of main effects of clones (C) and nutrient treatments (T) or the interaction between clones and treatments (C × T) are shown as *F* values, *P* values and *P* range: ns, non significant; *, significant at $P < 0.05$; **, significant at $P < 0.01$; ***, significant at $P < 0.001$. Separation of means was determined by a Tukey test when applicable. Different letters indicate significant differences at $P < 0.05$.

Clone	Treatment	Plant mass growth accumulated at month: 12 (g)	18 (g)	24 (g)
A	N_0P_0	63 ± 6 a	105 ± 4 a	119 ± 11 a
	N_0P_1	66 ± 5 a	137 ± 11 a	144 ± 16 a
	N_1P_0	125 ± 7 b	192 ± 12 b	225 ± 16 b
	N_1P_1	235 ± 6 c	619 ± 34 c	795 ± 78 c
	Mean	**122 ± 15 b**	**263 ± 63 b**	**321 ± 85 a**
B	N_0P_0	89 ± 4 a	141 ± 9 a	172 ± 10 a
	N_0P_1	109 ± 6 ab	136 ± 5 a	205 ± 14 ab
	N_1P_0	142 ± 8 b	215 ± 9 b	281 ± 10 b
	N_1P_1	264 ± 11 c	658 ± 24 c	881 ± 41 c
	Mean	**151 ± 15 c**	**287 ± 65 c**	**385 ± 88 b**
C	N_0P_0	69 ± 3 a	93 ± 3 a	143 ± 9 a
	N_0P_1	89 ± 3 ab	116 ± 3 a	170 ± 13 a
	N_1P_0	110 ± 5 b	194 ± 8 b	280 ± 8 b
	N_1P_1	205 ± 19 c	437 ± 15 c	734 ± 26 c
	Mean	**118 ± 12 b**	**210 ± 41 a**	**332 ± 72 a**
D	N_0P_0	61 ± 4 a	108 ± 2 a	137 ± 6 a
	N_0P_1	70 ± 4 a	116 ± 11 a	176 ± 10 a
	N_1P_0	102 ± 5 b	201 ± 11 b	270 ± 17 b
	N_1P_1	176 ± 11 c	640 ± 37 c	783 ± 23 c
	Mean	**102 ± 10 a**	**266 ± 67 b**	**341 ± 78 ab**
All Clones	N_0P_0	70 ± 3 a	112 ± 6 a	143 ± 7 a
	N_0P_1	84 ± 4 b	126 ± 5 b	174 ± 9 b
	N_1P_0	119 ± 4 c	200 ± 5 c	264 ± 9 c
	N_1P_1	220 ± 9 d	589 ± 29 d	798 ± 26 d
Overall	**Mean**	**123 ± 7**	**257 ± 29**	**345 ± 39**
Anova				
C		23.6, <0.001 ***	17.7, <0.001 ***	10.3, <0.001 ***
T		224.0, <0.001 ***	823.8, <0.001 ***	508.6, <0.001 ***
C × T		2.30, 0.042 *	3.46, 0.005 **	1.05, 0.42 ns

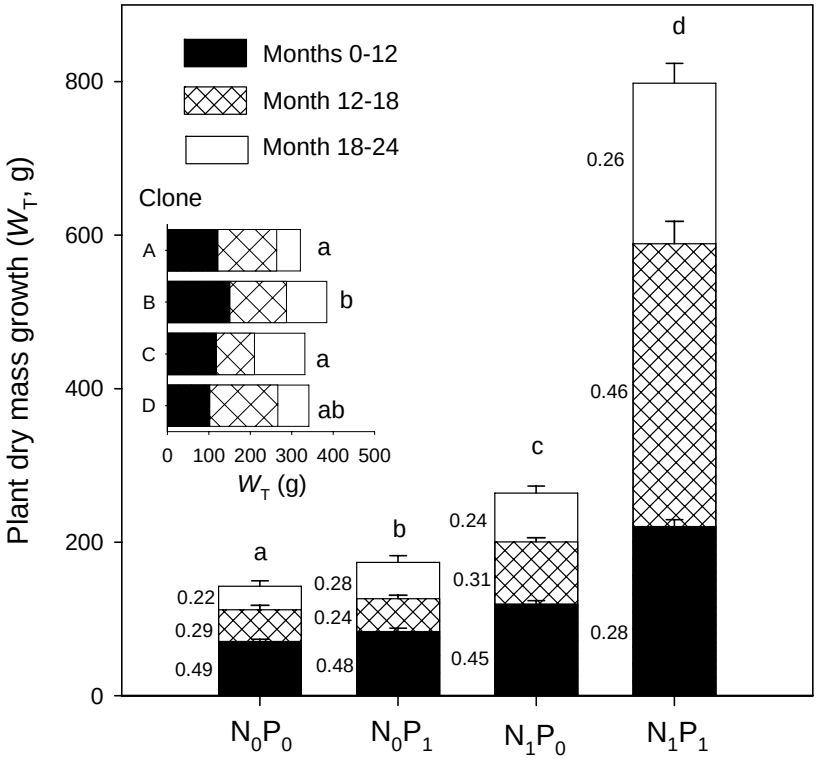


Figure 4.3. Comparison of plant dry mass growth across nutrient treatments and clones at months 12, 18 and 24. Treatments comprised two nitrogen supply regimes (N_0=1.43 and N_1=7.14 mM) and two phosphorus supply regimes (P_0=0.084 and P_1=0.420 mM). Values are presented as means (± 1 SE) for each treatment, clone and harvest. Different letters indicate significant differences at $P < 0.05$. Numbers in the left-hand side of each vertical column of the main graph are fractions of total mass growth.

Fascicle size and number resembled closely treatment influences on plant growth (Appendix E). Average mass per fascicle scaled with nitrogen and phosphorus supply, being 1.5 to 1.9 times larger in the high-nutrient supply regime (70-81 mg) compared to the low-nutrient supply regime (37-55 mg). Average mass per fascicle in imbalanced treatments N_0P_1 and N_1P_0 were intermediate between balanced treatments N_0P_0 and N_1P_1. The number of fascicles per plant also scaled with nutrient supply being about three-fold larger in the high-nutrient supply regime (2322) compared to the low-nutrient supply regime (746), exhibiting also an additive effect of mainly N but also P supply on this variable. Fascicle growth was concentrated mostly in spring-summer (0.67-0.91) compared to autumn-winter (0.09-0.33), with this trend being consistent across nutrient treatments and clones.

Fascicle size and number also tended to explain differences in growth performance among clones (Appendix E). The fastest growing clone (B) exhibited substantially larger fascicles without a considerable lower number of fascicles per plant than Clones C and D, which at least may partially explain their differences in growth performance. In relation to this

pattern, Clone A was atypical, because it had 1.2-1.8 times larger fascicles with only 0.56-0.62 of the total number of fascicles per plant compared to the other clones, which may at least partially explain its poorer growth performance.

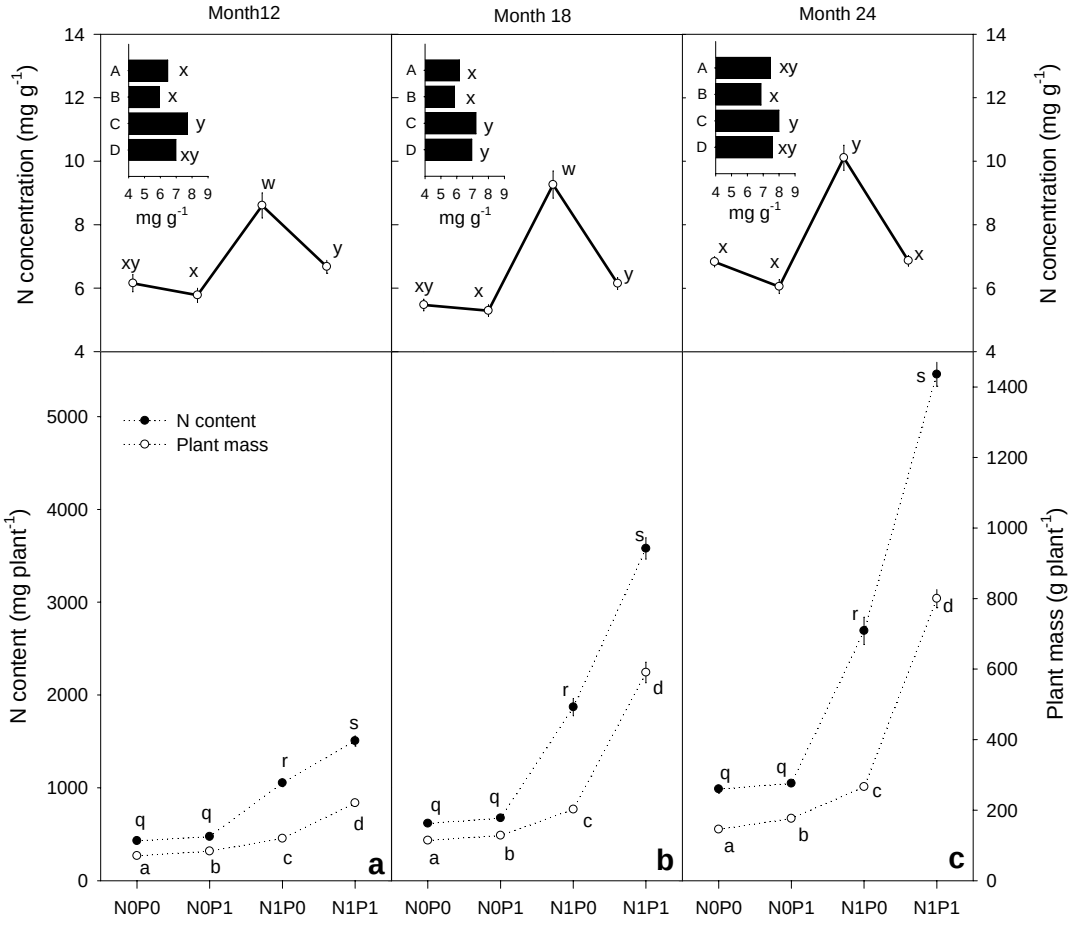


Figure 4.4. Average whole-tree N concentration, N content and plant mass across nutrient treatments at months 12 (a), 18 (b) and 24 (c). Upper graphs corresponds to whole-tree nitrogen concentrations (mg g^{-1}). The bar graph insert in upper graphs represents main effects of clones (A,B,C,D) on average whole-plant N concentration. Nutrient treatments comprised two nitrogen supply regimes (N_0=1.43 and N_1=7.14 mM) and two phosphorus supply regimes (P_0=0.084 and P_1=0.420 mM). Values are presented as means (± 1 SE) for each treatment and harvest. Different letters indicate significant differences at $P < 0.05$ (plant mass *a* to *d*, N content *q* to *s*, N concentration *x* to *w*). Main effects of nutrient treatments and clones on N concentration were significant at months 12, 18 and 24, while the interactive effect was significant only at month 12. The interactive effects between nutrient treatments and clones were significant for N content at month 18 and 24 (but not 12), and for plant mass at months 12 and 18 (but not 24).

Treatment influences on N concentration and content

Patterns of average whole-tree nitrogen concentration, N content and plant mass conformed to nutrient supply regimes and differed between clones at all harvests (Figure 4.4). Average whole-tree nitrogen concentration (N content divided by plant mass) was lowest in treatment N_0P_1 (e.g. 6.05 ± 0.22 SE, mg g^{-1}, month 24), possibly because high-P supply enhanced tree growth inducing a dilution effect on N concentration. In contrast, N

concentration was highest in treatment N_1P_0 (e.g. 10.11 ± 0.39 SE, mg g^{-1}, month 24) compared to all other treatments, possibly because low-P supply holds tree growth inducing N accumulation (luxurious consumption). N concentration did not differ significantly between balanced treatments N_0P_0 and N_1P_1, and collectively were intermediate between treatments N_0P_1 and N_1P_0. Clones A and B exhibited generally lower whole-tree N concentrations than clones C and D, and clones B and C always differed significantly (Figure 4.4).

Treatment influences on N remobilization

Using a mass balance approach, plant ^{15}N content at month 12 and the proportion of that content remobilized to new tissues were determined. Total N remobilization at month 24 significantly increased with nutrient supply from 199 mg $plant^{-1}$ in the low- compared to 953 mg $plant^{-1}$ in the high-nutrient supply regime (Table 4.2). Nitrogen remobilization in imbalanced treatments N_0P_1 and N_1P_0 was intermediate between balanced treatments N_0P_0 and N_1P_1. However, trees supplied with abundant N only (N_1P_0) remobilized about twice the amount (422 mg $plant^{-1}$) of those supplied with abundant P only (228 mg $plant^{-1}$) (N_0P_1), and this difference was correlated with the size of the N pool before remobilization took place.

Nitrogen remobilization efficiency, calculated as the proportion of N content remobilized to new tissues during the following year, scaled with nutrient supply, being significantly greater in the high-nutrient (N_1P_1) supply regime (0.65) than for trees growing at lower N and P addition rates (0.42-0.48) (Table 4.2). Most N remobilization occurred during the first half of the second year of growth (> 66%), and this proportion was slightly higher in the high-nutrient supply regime (0.83) compared to lower N and P addition regimes (0.66-0.75).

N remobilization efficiency, but not N remobilization, was significantly greater in Clone D (0.59) than in other clones (0.46-0.48) at month 24 (Table 4.2). Also, clone B grew faster and did not show enhanced capacity for N remobilization, suggesting that N remobilization and growth performance were not matched. The sizes of plants and N pools may confound the interpretation of N remobilization. This confounding effect can be removed by analysis of covariance. N remobilized during the second year significantly increased with the size of the N pool (content) before remobilization took place ($F_{7,40}$ = 179, $P < 0.001$). Slopes ($F_{3,40} < 1.26$, $P > 0.30$) and intercepts ($F_{3,40} < 1.80$, $P > 0.16$) of this linear relationship were not influenced by nutrient treatment or clone (Figure 4.5). However, a single linear model was biased in relation to nutrient regimes, and therefore two linear models were preferred, one for the high-nutrient supply regime and the other for the other nutrient

treatments (Figure 4.5). The non-significance of clone on this linear relationship indicated that N remobilization performance was similar among all clones.

Table 4.2. Plant N contents and N remobilization efficiencies for all nutrient-supply regimes and clones. Nutrient treatments comprised two nitrogen supply regimes (N_0=1.43 and N_1=7.14 mM) and two phosphorus supply regimes (P_0=0.084 and P_1=0.420 mM). Values are presented as means (± 1 SE) for each treatment and clone. Significance of main effects of clones (C) and nutrient treatments (T) or the interaction between clones and treatments (C × T) are shown as *F* values, *P* values and *P* range: ns, non significant; **, significant at $P < 0.01$; ***, significant at $P < 0.001$. Separation of means was determined by a Tukey test when applicable. Different letters indicate significant differences at $P < 0.05$.

Clone	Treatment	N content (1) at month 12 (mg plant^{-1})	N remob. (2) months 12-18 (mg plant^{-1})	N remob. (3) months 12-24 (mg plant^{-1})	N remob. efficiency months 12-18 (2) / (1)	N remob. efficiency months 12-24 (3) / (1)
A	N_0P_0	375 ± 36 a	146 ± 3 a	154 ± 18 a	0.40 ± 0.05 ab	0.43 ± 0.09 ab
	N_0P_1	386 ± 31 a	208 ± 19 a	205 ± 18 ab	0.55 ± 0.04 b	0.53 ± 0.01 ab
	N_1P_0	956 ± 55 b	199 ± 28 a	288 ± 21 b	0.20 ± 0.03 a	0.33 ± 0.02 a
	N_1P_1	1466 ± 38 c	844 ± 29 b	933 ± 45 c	0.58 ± 0.04 b	0.64 ± 0.04 b
	Mean	**795 ± 96 a**	**350 ± 87 ab**	**395 ± 95 a**	**0.43 ± 0.05**	**0.48 ± 0.04 a**
B	N_0P_0	439 ± 20 a	171 ± 20 a	208 ± 22 a	0.37 ± 0.04 ab	0.52 ± 0.08 ab
	N_0P_1	541 ± 29 a	99 ± 30 a	239 ± 37 a	0.20 ± 0.07 a	0.44 ± 0.08 ab
	N_1P_0	1222 ± 65 b	298 ± 31 b	453 ± 36 b	0.27 ± 0.06 ab	0.36 ± 0.02 a
	N_1P_1	1794 ± 71 c	919 ± 58 c	1064 ± 32 c	0.49 ± 0.05 b	0.63 ± 0.01 b
	Mean	**999 ± 117 b**	**372 ± 99 b**	**491 ± 105 b**	**0.33 ± 0.04**	**0.49 ± 0.04 a**
C	N_0P_0	472 ± 23 a	130 ± 3 a	220 ± 12 a	0.30 ± 0.02 a	0.44 ± 0.04 a
	N_0P_1	552 ± 20 a	130 ± 28 a	231 ± 25 a	0.25 ± 0.07 a	0.42 ± 0.06 a
	N_1P_0	1192 ± 53 b	370 ± 13 b	503 ± 40 b	0.31 ± 0.01 a	0.43 ± 0.04 a
	N_1P_1	1485 ± 137 b	604 ± 23 c	940 ± 16 c	0.49 ± 0.1 a	0.58 ± 0.02 a
	Mean	**925 ± 96 b**	**308 ± 60 a**	**474 ± 89 b**	**0.34 ± 0.04**	**0.46 ± 0.03 a**
D	N_0P_0	428 ± 28 a	164 ± 12 a	215 ± 15 a	0.38 ± 0.06 a	0.53 ± 0.03 a
	N_0P_1	416 ± 26 a	161 ± 20 a	239 ± 17 a	0.41 ± 0.03 a	0.55 ± 0.03 a
	N_1P_0	845 ± 38 b	315 ± 30 b	443 ± 15 b	0.35 ± 0.04 a	0.56 ± 0.02 a
	N_1P_1	1283 ± 79 c	830 ± 34 c	873 ± 30 c	0.62 ± 0.05 b	0.73 ± 0.01 a
	Mean	**743 ± 78 a**	**368 ± 83 b**	**442 ± 80 ab**	**0.44 ± 0.04**	**0.59 ± 0.03 b**
All Clones	N_0P_0	429 ± 15 a	153 ± 7 a	199 ± 11 a	0.36 ± 0.02 a	0.48 ± 0.03 a
	N_0P_1	474 ± 20 a	150 ± 16 a	228 ± 12 a	0.35 ± 0.05 a	0.48 ± 0.03 a
	N_1P_0	1054 ± 41 b	296 ± 22 b	422 ± 27 b	0.28 ± 0.03 a	0.42 ± 0.03 a
	N_1P_1	1507 ± 56 c	799 ± 39 c	953 ± 25 c	0.54 ± 0.03 b	0.65 ± 0.02 b
Overall	**Mean**	**866 ± 49**	**349 ± 41**	**451 ± 45**	**0.39 ± 0.02**	**0.51 ± 0.02**
Anova						
C		19.28,<0.001 ***	4.62, 0.0085 **	9.91,<0.001 ***	4.63, 0.0085 **	6.82, 0.0011 **
T		409.39,<0.001 ***	518.63, <0.001 ***	677.90,<0.001 ***	17.07, <0.001 ***	18.06, <0.001 ***
C × T		2.35,0.021 *	10.40, <0.001 ***	4.23,<0.0011 **	2.59, 0.0229 *	1.31, 0.2717 ns

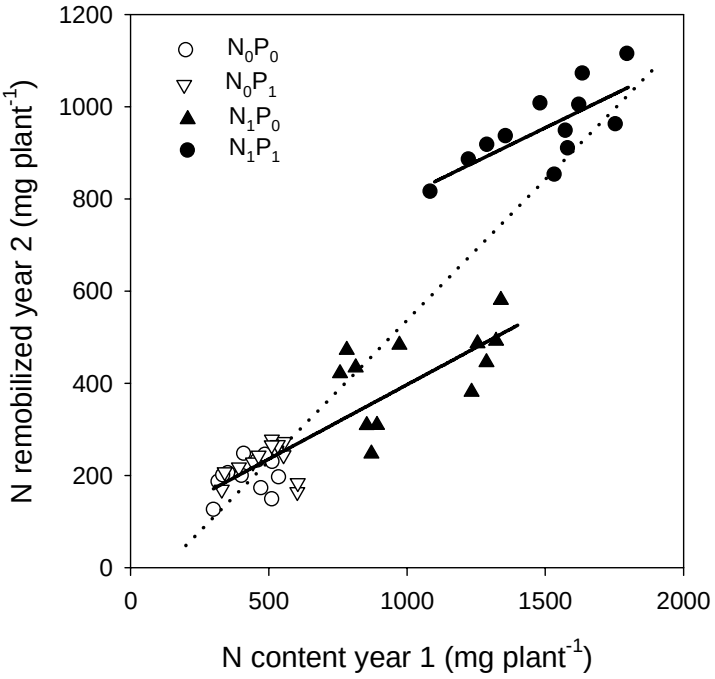


Figure 4.5. Total nitrogen remobilized during the second year of growth against total nitrogen content at the end of the first year of growth. For treatment N_1P_1: $y = 515.7970 + 0.2923\ x$, $r^2 = 0.53$, $P = 0.007$. For all other treatments: $y = 74.1501 + 0.3227\ x$, $r^2 = 0.74$, $P < 0.001$. Covariance analysis showed that nutrient treatment and clone did not influence slopes and intercepts of these linear relationships. However the model was clearly biased ($y = -74.6201 + 0.6112\ x$, $r^2 = 0.84$, $P < 0.001$, dotted line) in relation to nutrient treatment and therefore two equations were preferred, which produced an apparently unbiased model.

Comparison between uptake and remobilization

N uptake and N remobilization and their ratio increased mainly with N supply (Figure 4.6). Despite the fact that N uptake and N remobilization differed between clones, possibly as a result of differences in plant size, their ratios were only influenced by the main effect of nutrient treatments ($F_{3,30} > 14.7$, $P > 0.001$) but not clones ($F_{3,30} < 1.68$, $P > 0.19$) or their interaction ($F_{9,30} < 1.94$, $P > 0.09$). On average, the ratio of N uptake to N remobilization was greater in high-N supply regimes (3.4) compared to low-N supply regimes (2.1), indicating that trees rely progressively more on remobilization than uptake as fertility drops. The ratio of N uptake to remobilization also increased from the first to the second term of the second year of growth from 1.5 to 2.7 in low-N supply regimes and from 2.7 to 4.1 in high-N supply regimes. This indicates that most remobilization occurred early in the growing season and that thereafter plants depended progressively more on uptake than remobilization.

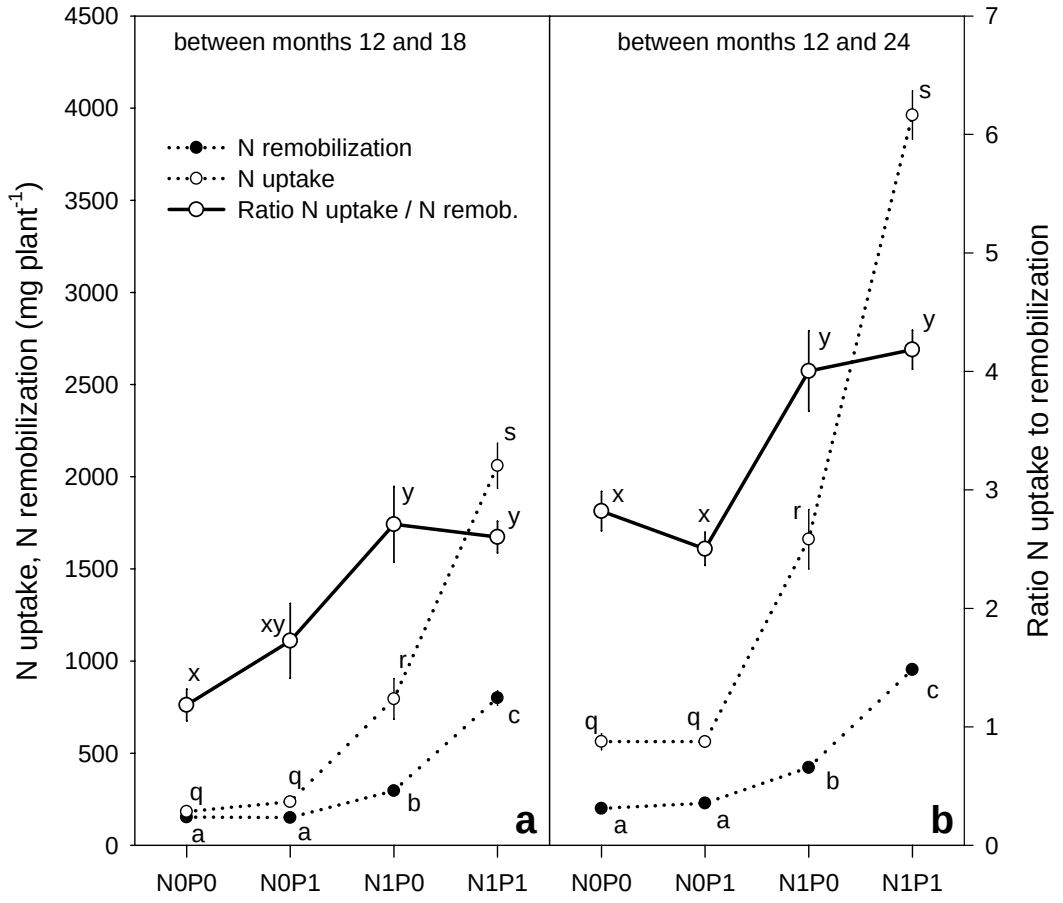


Figure 4.6. N uptake, N remobilization and their ratio for all nutrient-supply regimes for two time intervals. (a) months 12 to 18, (b) months 12 to 24. Nutrient treatments comprised two nitrogen supply regimes (N_0=1.43 and N_1=7.14 mM) and two phosphorus supply regimes (P_0=0.084 and P_1=0.420 mM). Values are presented as means (± 1 SE) for each treatment and remobilization period. Different letters indicate significant differences at $P < 0.05$ (N remobilization *a* to *c*, N uptake *q* to *s*, Ratio N uptake to remobilization *x* to *y*). Despite significant differences in N uptake and N remobilization between clones, their ratios were only influenced by nutrient treatment.

Sources and sinks for N remobilization

The methods of the study did not directly assess whether roots were a source for N remobilization. However, an indirect approach was used to assess whether foliage could account for all remobilization to new tissues. If foliage could account for most N remobilization then by difference it would follow that remobilization from roots and stems would be minimal. However, if foliage could not account for all N remobilized then stems and mainly roots would be candidate sources for N remobilization. Depletion of ^{15}N from old foliage and litter was used to calculate total N remobilized from foliage, and compared with total N remobilized to all tissues (Figure 4.7).

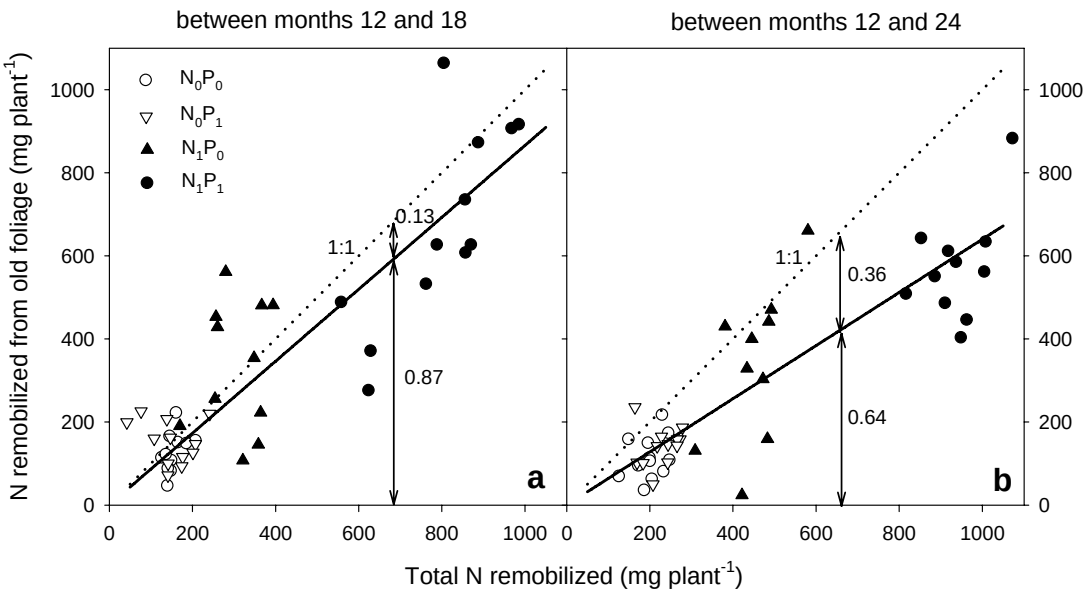


Figure 4.7. Comparison of N remobilized (depleted) from old foliage against total N remobilized during months 12-18 (a) and 12-24 (b). For Figure a: $y = 0.8659\ x$, $r^2 = 0.75$, $P < 0.001$. For Figure b: $y = 0.6394\ x$, $r^2 = 0.75$, $P < 0.001$. Values of N remobilized from old-foliage are greater than total N remobilized in some cases. This is because N remobilized from old-foliage was calculated as ^{15}N depletion from old-foliage plus litterfall, while total N remobilized as ^{15}N enrichment in new tissues, being these two measurements independent of each other, but overall indicating the proportion of N remobilized from foliage.

On average 87% of total N remobilized came from one-year old foliage between months 12-18 (Figure 4.7a), and this proportion was significantly reduced ($F_{1,83} = 9.88$, $P = 0.002$) to 64% when considering the whole second year of growth (Figure 4.7b). Neither slopes ($F_{3,39} < 2.51$, $P > 0.07$) nor intercepts ($F_{3,39} < 1.94$, $P > 0.13$) of both relationships were significantly influenced by nutrient treatment or clone. These results indicate that foliage was the primary source of N remobilization to new tissues, that old-foliage N was depleted first, and then progressively replaced by stems and roots which accounted for 36 % of all N remobilized to new tissues. As most N was stored in foliage (33-61%) and roots (23-58%) and only a small proportion in stems (6-22%) (Appendix E), it is likely that most of that 36% of N collectively remobilized from stems and roots must have been provided by roots during autumn-winter of the second year of growth.

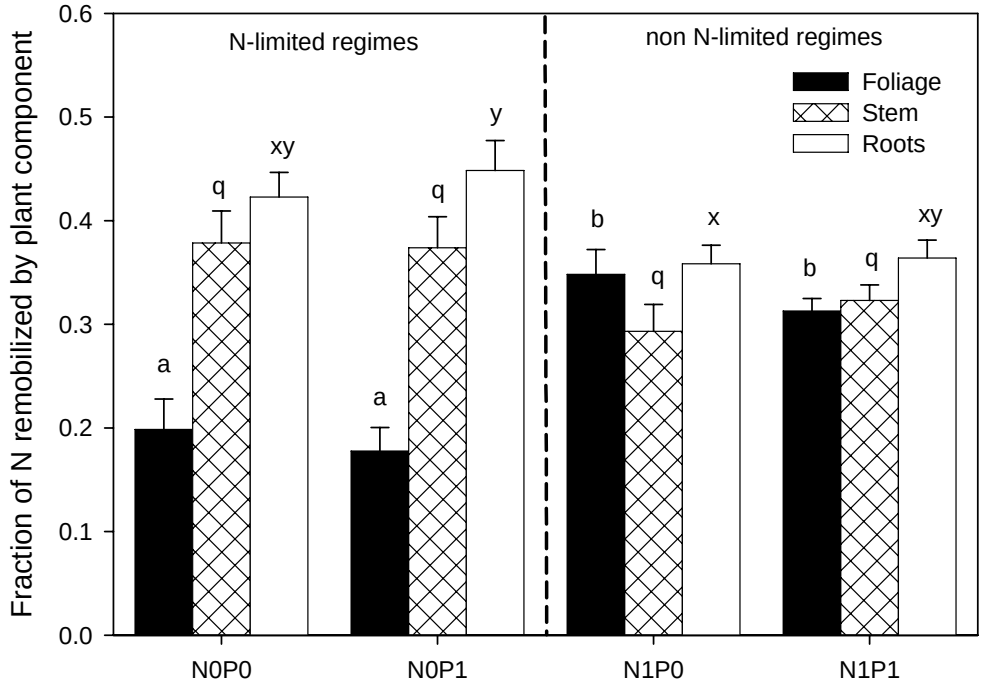


Figure 4.8. Fractions of N remobilized to new foliage, new stems and new roots at the end of the N remobilization experiment. Nutrient treatments comprised two nitrogen supply regimes (N_0=1.43 and N_1=7.14 mM) and two phosphorus supply regimes (P_0=0.084 and P_1=0.420 mM). Values are presented as means (± 1 SE) for each treatment and tree component. Different letters indicate significant differences at $P < 0.05$ (Foliage *a* to *b*, Stems *q*, Roots *x* to *y*). N remobilization fractions were only influenced by nutrient treatments, but not clone or their interaction.

Sinks for N remobilization differed between low-N and high-N supply regimes (Figure 4.8). Fractions of N remobilization allocated to roots and foliage significantly differed between nutrient supply regimes ($F_{3,30} > 4.04$, $P < 0.016$) but not clones ($F_{3,30} < 2.03$, $P > 0.13$) or their interaction ($F_{9,30} < 1.66$, $P > 0.14$). The fraction of N remobilized to stems was not significantly influenced by nutrient treatments, clones or their interaction (Overall model: $F_{17,30} = 1.30$, $P = 0.26$). In the low-N supply regimes, N was remobilized mainly to roots (44%) and stems (37%) and to less extent to foliage (19%). In contrast, in the high N supply regimes, N remobilization was similarly allocated to foliage (33%), stems (31%) and roots (36%). This trend was mainly explained because more biomass was partitioned to roots at the expense of foliage in the low-N supply regimes (Appendix E).

DISCUSSION

In this study, nutrient supply increased the absolute capacity of plants for N remobilization on a scale $N_1P_1 > N_1P_0 > N_0P_1 > N_0P_0$. Nambiar and Fife (1987, 1991) showed that nutrient remobilization increased with fertility in *Pinus radiata*, and similar results have been reported for *Pinus sylvestris* (Helmisaari 1992), *Pseudotsuga menziesii* (Hawkins *et al*. 1999) and for the New Zealand conifer *Prumnopitys ferruginea* (Carswell *et al*. 2003). The first hypothesis for this study was that nitrogen remobilization will increase with N supply but will be constrained by N or P imbalances. In absolute terms, N remobilization scaled with plant size, growth and N content, and so was not constrained by single N or P deficiencies except to the extent that N or P deficiencies constrained plant growth. However, in relative terms, N remobilization efficiency was 65% in the high N high P supply regime, compared to 42-48 % at lower N and P addition rates, indicating that single or joint N and P deficiencies constrained further increases in N remobilization efficiency. These results extend previous work emphasizing that plants growing with balanced nutrition only would achieve maximal nutrient remobilization efficiencies.

In this study, almost all N remobilized during spring-summer came from foliage (87%) and little from stems and roots (13%). However the proportion remobilized from stems and roots increased to 36% of all N remobilized by the end of winter (second hypothesis). In conifers, Millard and Proe (1993) and Nambiar and Fife (1987) found that foliage was the main source for nutrient remobilization, while Nambiar (1987) found that monthly variations in nutrient concentrations in fine roots were minor with no seasonal pattern, suggesting little or no remobilization. Our results extend previous work in that once foliage N was depleted, alternative sources might be used including coarse and fine roots particularly during autumn and winter which may account for as much as 36% of all N remobilized.

There was a clear seasonal effect on N remobilization. Most N remobilization occurred during spring and summer (66-83%) compared to autumn and winter (17-34%). Fife and Nambiar (1982) showed that in young trees of *Pinus radiata* most needle growth occurred in a period of 4-5 months after bud break in spring and early summer in South Australia. Similarly in this study most needle growth occurred in spring-summer (67-91%), supporting the hypothesis that N remobilization is driven by sink strength (Nambiar and Fife 1987, 1991). Supporting evidence was also found in that the relative importance of N remobilization compared to N uptake to support new growth decreased from spring-summer to autumn-

winter. This has been previously observed in *Picea sitchensis* (Millard and Proe 1993) and *Juglans nigra* × *regia* (Frak *et al*. 2005).

Millard and Proe (1993) showed that the initial growth of *Picea sitchensis* was not influenced by current N supply, but by N provided during the previous year. This was also observed by Carswell *et al.* (2003) in the New Zealand conifer *Prumnopitys ferruginea*. The methods used in this study prevented us from concluding that initial growth was mostly dependent of N remobilization as N uptake was also observed during this period. However, it seems remarkable that more N remobilization both in absolute and relative terms was observed in the high nutrient supply regime, even through plants had plenty of nutrients and took up proportionally more than in other treatments. This may suggest independence between the processes of N remobilization and uptake. This coincides with Nambiar (1990) who argues that shoot production and growth, rather than nutrient supply, is the key determinant of nutrient remobilization.

Forest seedlings are usually planted in winter when root regeneration and nutrient uptake is restricted by low soil temperatures (Nordborg *et al*. 2003). Under these conditions foliage nitrogen readily remobilizes to support root growth (Nambiar and Fife 1991), and this effect has been reported to be enhanced by a nursery practice known as nutrient loading that improves seedling growth and survival in the field (Salifu and Timmer 2001, 2003). Similarly, plants in this study were young (1-2 years), N remobilization was apportioned mainly to roots (44% and 36% in low-N and high-N supply regimes respectively), and this capacity increased with the nutrient status of plants. Despite that trees were not switched from one nutrient supply regime to another in this experiment, our results would suggest that balanced nutrient loading of *Pinus radiata* in the nursery may have beneficial effects in growth and survival particularly in early-establishment in poor fertility soils.

Although nutrient remobilization is recognized as an important factor in the plant nutrient economy, the biochemical paths by which these nutrients are stored and remobilized are not clearly understood (Nambiar and Fife, 1991). Conroy (1992) argued that a large proportion of leaf N was associated with proteins located in the chloroplast and mostly involved in photosynthesis, with Rubisco accounting for 25% of the total leaf N, and proteins associated with photosynthetic electron transport accounting for a further 25%. Nasholm and McDonald (1990) found that the proportion of aminoacids in birch tissues significantly increased with N supply (1 to 7%), so that they might be transport and storage compounds, in addition to being the primary products of nitrogen assimilation and precursors for protein and nucleic acids. Warren *et al*. (2003*a*), working in pot trials with *Pinus sylvestris*, found that

Rubisco content was in excess of the amount required for photosynthesis and this excess was positively correlated to foliage nitrogen concentration across an N supply gradient, suggesting that Rubisco functions as a storage protein in addition to its catalytic role. Similarly, Warren and Adams (2002) found a positive correlation between foliage P and Rubisco concentrations in *Pinus pinaster* across a P fertility gradient suggesting that P availability controls the partitioning of N to Rubisco when P is limiting. The partitioning of foliage N to storage compounds was not measured in this study. However, N remobilization efficiency was constrained by P deficiency in treatment N_1P_0 (0.48) from realizing the maximal value (N_1P_1, 0.65). This may have been brought about because P deficiencies controlled the proportion of N allocated to storage proteins (Warren and Adams 2002). Nitrogen remobilization efficiency was also constrained by N deficiency in treatment N_0P_1 (0.43), possibly because the reduced N pool was tied up in compounds required for minimal leaf function. These results emphasize the need for a balanced nutrition for trees to realize their maximum potential for timber growth and carbon sequestration.

Nutrient remobilization efficiency has been observed to vary across and within species. For instance, Bothwell *et al*. (2001) showed that N remobilization efficiency was higher in *Pinus contorta* (50-52%) than in *Picea sitchensis* (24-36%) in Vancouver Island (Canada), whereas Oleksyn *et al*. (2003), comparing *Pinus sylvestris* sourced from seeds from six populations across Europe in a common-garden experiment, showed that northern populations from colder environments exhibited higher internal nutrient cycling efficiency than southern populations, indicating that genetics, soil and environmental factors jointly control nutrient remobilization. In this study nitrogen remobilization did not explain differences in productivity among genotypes (third hypothesis), probably as a result of a narrower genetic pool compared to other studies (e.g. Miller and Hawkins 2003, Oleksyn *et al*. 2003). Clones in this study differed in fascicle size and number, and foliage nitrogen concentration and content as previously found by Cotterill and Nambiar (1981) in growth contrasting families of *Pinus radiata*, suggesting that differences in growth performance might be at least partially attributed to leaf area and phenology.

In conclusion, we examined the effects of nitrogen and phosphorus supply on nitrogen remobilization in four contrasting clones of *Pinus radiata*. The study showed that trees growing with abundant and balanced nutrient supply exhibited enhanced capacity for nitrogen remobilization (0.65) compared to trees exposed to single or joint N and P deficiencies (0.42-0.48), that woody tissues remobilized a substantial amount of N (36%) after foliage N was depleted and that faster growing clones did not show enhanced capacity for N remobilization.

CHAPTER FIVE

INFLUENCE OF NITROGEN AND PHOSPHORUS SUPPLY ON PATTERNS OF CARBON ALLOCATION OF *PINUS RADIATA* CLONES

Summary Nitrogen (N) and phosphorus (P) deficiencies commonly limit growth of *Pinus radiata* in New Zealand, and nutrient imbalances might be more important than single straightforward nutrient deficiencies. Patterns of carbon allocation were examined in *Pinus radiata* clones cultivated in a greenhouse with a factorial combination of nitrogen and phosphorus supply for ten months using a carbon balance approach (Giardina *et al*. 2003). Gross-primary productivity (GPP) increased with nitrogen and phosphorus supply, being 3.6 times greater in the high-nutrient supply regime (109 g C plant^{-1}) compared to the low-nutrient supply regime (30 g C plant^{-1}). Values of GPP in imbalanced treatments N_0P_1 (53 g C plant^{-1}) and N_1P_0 (54 g C plant^{-1}) were intermediate between balanced treatments N_0P_0 and N_1P_1. The fraction of GPP allocated to above-ground components (ANPP) increased mainly with N but also P supply at the expense of total-below ground C allocation (TBCA) with no apparent effect on the fraction of GPP partitioned to above-ground plant respiration (APR). At low-nutrient supply, the fraction of GPP represented by ANPP, APR and TBCA were 26%, 30% and 44%, respectively, compared to 35%, 33% and 32% in the high-nutrient supply regime. Carbon-use efficiency (NPP:GPP) significantly increased with N supply whereas the effect of P supply was always smaller. Soil respiration accounted for 62% of TBCA in the low-nutrient supply regime, decreasing to 48% of TBCA when single or joint N and P additions were applied, suggesting that in severely deficient environments a larger proportion of the C budget is respired. Patterns of carbon allocation may help to explain differences in genotypic growth performance. The slowest-growing clone allocated consistently less carbon to above-ground components (about 2-4%) compared to other clones, suggesting that faster-growing genotypes allocate more carbon to light capture and photosynthesis that may compound and increase overall carbon assimilation over time. The study suggests that a balanced soil and plant nutrition may play a key role in enhancing carbon sequestration in forest ecosystems.

Keywords: clones, above-ground plant respiration, gross-primary productivity, net primary productivity, nutrient limitations, Pinus radiata, total belowground carbon allocation,

INTRODUCTION

Ecosystem physiological models are required to assess global environmental change, carbon balance, net primary production and the use of limiting resources such as water and nutrients (Jarvis 1995). These models are needed as traditional approaches to productivity in land-related sciences have provided large numbers of field trials and information yet little understanding of the mechanisms explaining primary productivity responses to environmental change (McMurtrie and Landsberg 1992). Waring *et al* (1998) point out the need for robust simplifications for modeling ecosystem responses to changing environments, particularly in relation to carbon assimilation, utilization and allocation, while Landsberg *et al*. (1991) argue that greatest confidence is achieved in modeling water but progressively less for carbon and nutrient processes.

Most studies have looked into ecosystem productivity based on standing but mainly above-ground biomass, with a reduced fraction of studies looking at carbon allocation to roots, even though above-ground net primary production may account for only 25-30 % of gross-primary productivity (Giardina *et al*. 2003). Ryan (1991*b*) argues that gross-primary productivity estimates are difficult to determine because of uncertainties in scaling flux measurements made on small samples over short periods of time and also because of the inherent difficulties in estimating carbon allocation to roots. Nevertheless, most studies in forests have shown that improved nutrition increases the proportion of dry-matter partitioned to above-ground at the expense of below-ground components (e.g. Albaugh *et al*. 1998, Haynes and Gower 1995), while a few have found that this proportion does not change with nutrient availability (e.g. Nadelhoffer *et al*. 1985). Contradictory evidence may arise because a large proportion of fixed carbon is respired, allocated to mycorrhizae, exuded by roots or released as above- and below ground litter (Ryan 1991*a*, Ryan *et al*. 1996, Giardina *et al*. 2003), and therefore not accounted for in biomass studies. Whole carbon budgets are a relatively recent development, which was greatly assisted by a carbon balance protocol developed by Raich and Nadelhoffer (1989), and methods for scaling plant respiration (Ryan 1991*a*, 1991*b*, Ryan *et al*. 1996) and carbon allocation to roots (Giardina and Ryan 2002). Particularly this last approach provides estimates of known variance and overcomes many of the limitations and assumptions that were difficult to test associated with previous root production assessment methods (Giardina and Ryan 2002). The carbon balance approach has helped us to understand environmental controls on productivity, and to determine patterns of carbon allocation in a so

far reduced range of forest ecosystems (e.g. Giardina *et al*. 2002, Hamilton *et al*. 2002, Stape 2002, Giardina *et al*. 2003).

Pinus radiata is commonly planted in New Zealand and the southern hemisphere, and has been shown to be growth limited by nitrogen and phosphorus availability in the New Zealand soils (Watt *et al*. 2005). Forest productivity is commonly limited by nutrient availability (Nambiar 1984, Aerts and Chapin 2000), and genetically improved trees may potentially contribute to alleviating forest nutritional problems (Turner and Lambert 1986). However an incomplete understanding of the mechanisms that make certain genotypes grow faster has prevented defining selection criteria for tree improvement (Turner and Lambert 1986). Therefore, investigations into genetic controls on productivity and their interaction particularly with nitrogen and phosphorus supply are required. Understanding the influence of these two elements on carbon assimilation and partitioning would greatly enhance our ability to predict patterns of productivity and allocation (using physiological models) across environments which differ widely in fertility. Many studies have suggested that carbon allocation to belowground processes decreased with nitrogen availability (Giardina *et al*. 2003), whereas only a few have looked at the effects of phosphorus deficiencies, and investigations assessing the interactive influence of nitrogen and phosphorus supply on carbon allocation in pines have apparently not been conducted.

The aim of the study was to examine concurrent influences of nitrogen and phosphorus supply on patterns of carbon allocation for four *Pinus radiata* clones. We examined how concurrent influences of N and P supply affected absolute C fluxes allocated to ANPP, APR and TBCA, and the relative partitioning of GPP to ANPP, APR and TBCA. We also explored whether carbon-use efficiency (NPP:GPP) was affected by N and P supply and whether faster-growing genotypes exhibited greater C allocation to aboveground at the expense of belowground processes.

MATERIALS AND METHODS

Plant material

A greenhouse experiment was laid out in a factorial design with four clones, two nitrogen supply regimes (N_0=1.43 and N_1=7.14 mM) and two phosphorus supply regimes (P_0=0.084 and P_1=0.420 mM) with six replicates per clone per treatment (96 plants). Clones were selected to represent a gradient in growth performance within a set of 400 genotypes planted in the Purokohukohu Experimental Basin (Beets *et al*. 2004).

One-year old *Pinus radiata* cuttings from four clones (Clones P26C2, P26C5, P02C7 and S02C1 referred to hereafter as clones A, B, C and D) were raised under standard ENSIS (formerly New Zealand Forest Research Institute) nursery conditions and transplanted to 42-dm^3 pots containing silica sand (< 0.1 % organic matter). Nutrient treatments were randomly allocated to the plants and applied for ten months from September 2004 until plants were harvested in July 7, 2005. Plants received 1 dm^3 of nutrient solution per week and were watered twice daily ensuring that always there was water draining from the largest plants and therefore plants were only limited by nutrients and not by water. Nutrients other than N and P were provided at 1.023 mol m^{-3} K, 0.250 mol m^{-3} Ca, 0.411 mol m^{-3} Mg, 0.281 mol m^{-3} S, 12.532 mmol m^{-3} Fe, 0.445 mmol m^{-3} Zn, 0.473 mmol m^{-3} Cu, 7.281 mmol m^{-3} Mn, 0.073 mmol m^{-3} Mo, 18.502 mmol m^{-3} B, 0.946 mmol m^{-3} Cl and 0.145 mmol m^{-3} Na following Ingestad (1979).

Plants were grown in a thermostatically controlled greenhouse where air temperature fluctuated between 7 and 38 °C during the day (mean ± 1 SD: 21 ± 5 °C), and between 7 and 26 ºC during the night (16 ± 3 °C) depending on weather conditions (Figure 5.1a). Soil temperatures resembled closely air temperatures, and differed mostly in the extremes i.e. soil temperatures were up to 5 ºC higher and 5.5 ºC lower than the daily minimum and maximum, respectively (data not shown). Vapour pressure deficit fluctuated between 0 and 4.6 kPa (0.84 ± 0.77 kPa) during the day and 0 to 1.2 kPa (0.16 ± 0.18 kPa) during the night (Figure 5.1a). All these variables were monitored using temperature and relative humidity sensors connected to a HOBO micro weather station data logger (ONSET Computer Corporation, Bourne, MA, USA). Roots of all plants were artificially inoculated with spores of *Rhizopogon rubescens* Tul. and confirmed as mycorrhizal either by visual inspection of roots or by the presence of fruiting bodies.

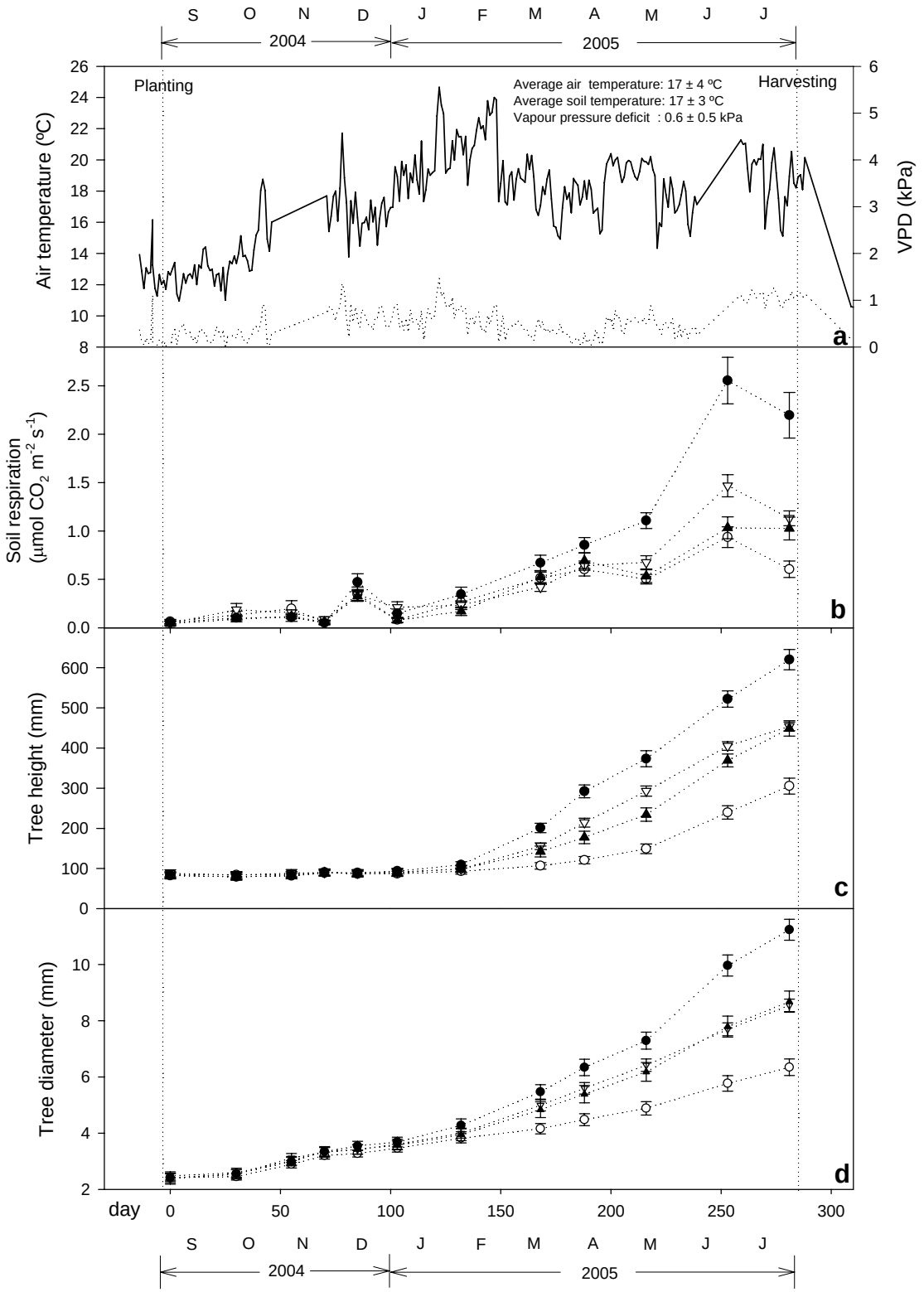


Figure 5.1. Variation in climatic conditions and tree dimensions over the duration of the carbon allocation experiment. (a) Average daily air temperatures (solid line) and vapour pressure deficit (dotted line) during the 10-months experiment, (b) soil respiration CO_2 efflux, (c) tree heights and (d) diameters across nutrient treatments. Nutrient treatments comprised two nitrogen supply regimes (N_0=1.43 and N_1=7.14 mM) and two phosphorus supply regimes (P_0=0.084 and P_1=0.420 mM). Symbols: N_0P_0 (open circles), N_0P_1 (inverted open triangles), N_1P_0 (solid triangles) and N_1P_1 (solid circles).

Carbon balance method

The methods and nomenclature used to examine patterns of carbon allocation in this study follow the carbon balance protocol described by Giardina *et al*. (2003), based on previous work by Raich and Nadelhoffer (1989), Ryan (1991*a*, 1991*b*), Ryan *et al*. (1996) and Giardina and Ryan (2002). Gross-primary production (GPP) corresponds to the integration of plant net photosynthesis over a given period of time, but can be more easily estimated as the sum of carbon in dry matter production plus respiration,

$$\mathrm{GPP} = \mathrm{ANPP} + \mathrm{APR} + \mathrm{TBCA} \qquad (5.1)$$

where ANPP is above-ground net primary production, APR is above-ground plant respiration and TBCA is total below-ground carbon allocation (Giardina *et al*. 2003). ANPP is estimated as,

$$\mathrm{ANPP} = F_A + F_W + \Delta C_C + \Delta C_w \qquad (5.2)$$

where F_A is the carbon content of above-ground litterfall, F_W is the C content associated with tree mortality, ΔC_C is the change in C content of live foliage, and ΔCw is C content change in live branches, bark and wood, over a given period of time (Giardina *et al* 2003). These fluxes can be partitioned as leaf NPP (ΔC_C plus foliage component of F_A) and stems and branch NPP (F_W plus ΔC_W plus the branch and twigs component of F_A).

Above-ground plant respiration, APR, can be estimated as a sum of foliage construction (L_{RC}), foliage maintenance respiration (L_{RM}) and wood construction and maintenance respiration (W_R) from,

$$\mathrm{APR} = L_{RC} + L_{RM} + W_R \qquad (5.3)$$

Total C allocated belowground for root and mycorrhizal construction and maintenance respiration, and C released through root exudates and root turnover (TBCA), can be estimated using a carbon mass balance approach (Raich and Nadelhoffer 1989, Giardina and Ryan 2002). The method is based in that all C allocated belowground must be either respired, leached or stored,

$$\mathrm{TBCA} = F_S + F_E - F_A + \Delta C_S + \Delta C_R + \Delta C_L \qquad (5.4)$$

where F_S is the soil respiration C efflux, F_E is the C flux off the system by leaching or erosion, ΔC_S is change in C content in the mineral soil, ΔC_R is the change in C content of root biomass, and ΔC_L is the change in C content in the litter layer.

Aboveground net primary productivity

Values of ANPP (leaf plus wood NPP) were calculated based on Eqn. (5.2). Litterfall (F_A) was collected monthly, and oven-dried mass aggregated over time to yield F_A for each tree. As there was no mortality (F_W) over the course of the experiment F_W was set to zero. Initial and final dry mass by plant component (foliage, stems, branches and roots) were used to calculate ΔC_C and ΔC_w. For all calculations C content was assumed to be 50% of tree dry mass.

Aboveground plant respiration

Values of APR (foliage and wood maintenance and construction respiration) were calculated based on Eqn. (5.3). Construction respiration costs for leaves (L_{RC}) and wood were assumed to be 25% of leaf NPP and wood NPP respectively (Penning de Vries 1972, 1975, Ryan 1991*a*, 1991*b*). Wood maintenance respiration was assumed 7 % of wood NPP based on data from Giardina *et al*. (2003). This is a reasonable assumption since wood maintenance respiration is usually less than 10% of GPP (Ryan *et al*. 1995, Waring *et al* 1998). Wood construction plus maintenance respiration yielded W_R. Maintenance respiration for leaves (L_{RM}) was measured as CO_2 efflux at night and scaled over the whole growing season using a Q_{10} equal to 2 (Ryan 1991*a*) and hourly monthly air temperature averages.

The rate of foliage maintenance respiration at night (R_d) was measured on fully expanded foliage of all 96 plants which comprised 6 plants per treatment per clone. All gas exchange measurements were carried out at night from 9 pm to 2 am from April 2 to 11, 2005. Values of R_d were measured using a portable photosynthesis system (Li-6400, Li-Cor, Lincoln, NE). For each plant, three to six fascicles were arranged in a 6-cm^2 cuvette avoiding shading between needles. Temperature in the cuvette was maintained at 20 ºC while leaf-to-air vapour pressure deficit (VPD) was always lower than 1 kPa. External CO_2 concentration (C_a) was maintained at 360 μmol mol^{-1} using a CO_2 mixer. Gas exchange measurements were left to stabilize for at least 10 minutes until values of A, C_i and g_s were stable (coefficient of variation $\leq$ 2%), and then values were recorded every 20 seconds for 2 minutes.

Following the completion of dark-respiration rates, foliage samples were carefully removed from the cuvette and cut to match the leaf area exposed to gas exchange. Total surface area of needles was determined based on water volume displacement as described by Johnson (1984). All measurements and analyses are reported on a total leaf area basis. Foliage samples were dried at 70 °C to constant mass and dry mass recorded. For foliar chemical analysis, leaf samples were finely ground and acid-digested by a Kjeldahl method (Blakemore *et al*. 1987). Nitrogen and phosphorus in the digests were determined colorimetrically by the Landcare Research Laboratories, Palmerston North, New Zealand. Nitrogen and phosphorus concentrations were expressed on a total leaf area basis (N_a, P_a).

Total belowground carbon allocation

The components of TBCA were calculated based on Eqn. (5.4). Soil surface CO_2 efflux and mineral soil temperature were measured monthly in all plants using a soil respiration chamber (100 mm inner diameter, Model SRC-1, PP Systems, Herts, UK) connected to an infrared gas analyzer (Model EGM-4, PP Systems, Herts, UK). Soil collars made of polyvinyl chloride (100 mm inner diameter and 50 mm length) were placed in each of the 96 pots at the start of the experiment. Monthly soil respiration measurements were made within the same day between 10 am and 6 pm. Additional to the 96 experimental units, four pots filled with silica sand without plants were set as controls for soil respiration and mineral soil carbon. These controls were subject to the same regimes of nutrition and irrigation as those applied in the experimental pots carrying plants (one pot per nutrient treatment). Monthly soil respiration values in control pots were very small (<0.02) or negative suggesting that CO_2 efflux was zero and that values measured in containers with plants were true measures of root and mycorrhizal respiration. Soil respiration (F_S) was measured monthly as CO_2 efflux during the day and scaled over the whole growing season as the product of actual F_S values and the number of hours between intervening monthly measurements. This approach was used as soil respiration positively scaled with tree size but was insensitive to soil temperature (Figure 5.2). Carbon lost by leaching (F_E) was assumed to be zero.

Initial and final C content in the silica sand was determined by loss on ignition to yield ΔC_S. Mineral sand C concentration at the end of the experiment did not differ significantly between nutrient treatments and clones ($F_{47,48} = 1.16$, $P = 0.30$) being on average 0.60 ± 0.01 mg g^{-1}(± 1 SE, $n = 96$), and this value was not significantly higher than the one observed at the beginning of the experiment (0.58 ± 0.02 mg g^{-1}, $n = 20$). Therefore ΔC_S was considered to be

zero. Values of ΔC_R were determined as the difference between initial and final root mass multiplied by 0.5. As the litter layer was inexistent ΔC_L was set to zero. In summary, values of F_E, F_A, ΔC_S and ΔC_L were zero or assumed zero, so that Eqn. 5.4 simplified to: TBCA = F_S + ΔC_R.

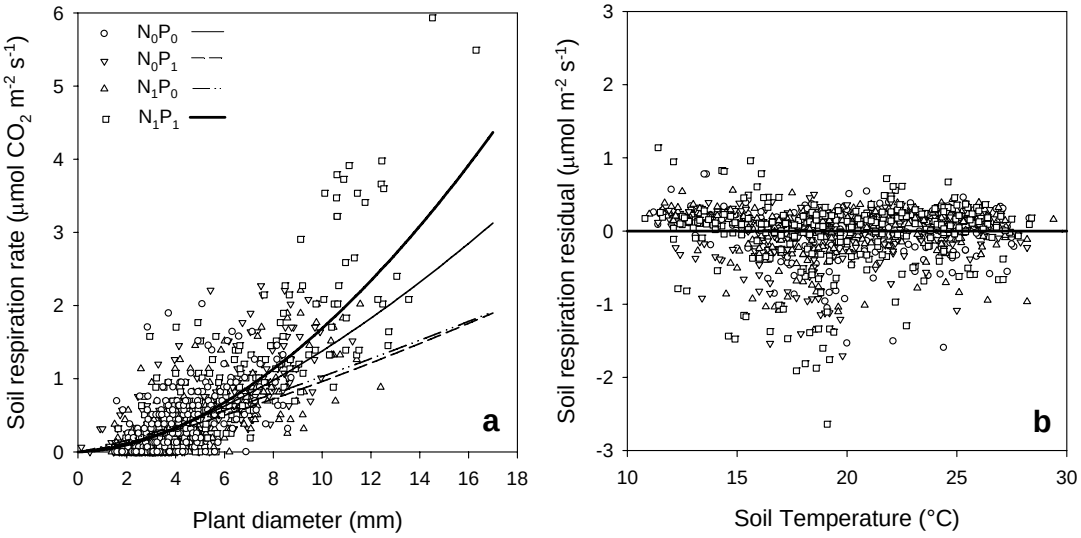


Figure 5.2. The relationship between the rate of soil respiration (F_S, µmol m^{-2} s^{-1}) and (a) plant diameter (D, mm) and (b) soil temperature (T, °C). The R_s/D log-linear relationship was fitted by analysis of covariance, where both slopes and intercepts were affected by nutrient treatment: $F_S = 0.0394\ D^{1.5438}$ (N_0P_0), $F_S = 0.0698\ D^{1.1685}$ (N_0P_1), $F_S = 0.0503\ D^{1.2813}$ (N_1P_0), $F_S = 0.0272\ D^{1.7925}$ (N_1P_1), $r^2 = 0.49$, $P < 0.001$, $n = 861$. Residuals from this model were plotted against soil temperature (b) and their correlation was found to be insignificant ($F_{1,859} = 1.39$, $P = 0.23$).

Tree harvesting

Total and component tree oven-dried masses and leaf areas at the start of the experiment were estimated using allometric equations developed with ten plants per clone from unplanted stock in September 2004 (Appendix E). At the end of the experiment, all plants (96 in total) were harvested over a 10 day period ending July 7, 2005. Tree diameter, height and crown diameter were measured and above-ground tree components dissected before roots were removed. The bulk root system was collected and sand sieved to recover loose roots. Sieved sand was thoroughly mixed, weighted, and a 2.5 kg subsample was taken to recover remaining roots by flotation. All tree components were oven-dried at 70˚C to constant mass and dry mass recorded. Estimates of leaf area were determined separately for each tree as the product of leaf mass and the leaf area to mass ratio from measurements obtained at month 10.

Data analysis

All analyses were undertaken with SAS software (1996; SAS Institute, Cary, NC). Variables were tested for normality and homogeneity of variance and transformations were made as necessary to meet the underlying statistical assumptions of the models used. Main and interactive effects of nitrogen and phosphorus supply and genotype on carbon allocation patterns were examined by analysis of variance. Tukey's least significant difference test was used to distinguish among individual means where applicable with a confidence level of $P \leq 0.05$. Differences in slopes and intercepts between clones and nutrient treatments in the linear relationships between GPP fractions (ANPP, APR, TBCA) and GPP were tested for significance by analysis of covariance.

RESULTS

Treatment influences on growth

Plant growth was strongly influenced by nutrient supply and to a lesser extent by genotype. Growth responses in plant diameter, height and mass significantly increased with N and P supply ($F_{3,30} > 74$, $P < 0.001$), and these responses were consistent within clones (Table 5.1). Plant mass growth by the end of the experiment was 4.3 times greater in the high-nutrient supply regime (113 g plant^{-1}) compared to the low-nutrient supply regime (26 g plant^{-1}) (Table 5.1). The growth responses in imbalanced treatments N_0P_1 and N_1P_0 were intermediate between balanced treatments N_0P_0 and N_1P_1.

The main effect of clone was relatively minor compared to the main effect of nutrient treatment as evidenced by *F* values (11 cf. 95) and the variation between extremes in growth, which ranged 4.3 fold for nutrition, and 1.5 fold for clones. The interaction between nutrient treatment and clone was significant for plant mass growth ($F_{9,30} = 3.4$, $P = 0.005$) and involved clone D developing faster in the low-nutrient supply regime than at higher N and P addition rates than Clones B and C (Table 5.1). Clone A was consistently smaller in all treatments. Clones A and B, but not C and D, were the same ones used in the N remobilization experiment (Chapter *Four*). Clone A had large needles but a drastically smaller number of fascicles per plant than other clones. This may also explain its poorer growth performance in this experiment.

At time of planting none of the tree dimensions examined were significantly influenced by nutrient treatment ($F_{3,30} < 0.21$, $P > 0.88$), but they were significantly different among clones ($F_{3,30} > 6.4$, $P < 0.002$) on a scale D ≥ C ≥ B ≥ A (data not shown). However, initial plant size did not influence any of the plant growth or carbon balance variables as shown by analysis of covariance.

Table 5.1. Plant growth in diameter, height and dry mass for all combinations of nutrient treatments and clones. Nutrient treatments comprised two nitrogen supply regimes (N_0=1.43 and N_1=7.14 mM) and two phosphorus supply regimes (P_0=0.084 and P_1=0.420 mM). Values are presented as means (± 1 SE) for each treatment and clone; n = 6. Significance of main effects of clones (C) and nutrient treatments (T) or the interaction between clones and treatments (C × T) are shown as F values, P values and P range: ns, non significant; **, significant at $P < 0.01$; ***, significant at $P < 0.001$. Separation of means was determined by a Tukey test when applicable. Different letters indicate significant differences at $P < 0.05$. Significance of main effects of nutrient treatments and clones are indicated by letters when the C × T interaction was in-significant (omitted otherwise).

Clone	Treatment	Plant growth in:		
		diameter (mm)	height (mm)	dry mass (g)
A	N_0P_0	3.2 ± 0.3a	192 ± 33a	17 ± 3a
	N_0P_1	6.1 ± 0.3b	431 ± 20b	42 ± 2b
	N_1P_0	5.0 ± 0.3b	412 ± 53b	33 ± 6b
	N_1P_1	8.7 ± 0.4c	730 ± 45c	87 ± 10c
	Mean	**5.7 ± 0.4a**	**441 ± 44**	**45 ± 6**
B	N_0P_0	4.2 ± 0.4a	356 ± 27a	23 ± 2a
	N_0P_1	6.3 ± 0.4b	468 ± 28b	47 ± 3b
	N_1P_0	7.6 ± 0.4b	500 ± 36b	67 ± 8b
	N_1P_1	10.4 ± 0.4c	778 ± 37c	131 ± 14c
	Mean	**7.1 ± 0.5b**	**526 ± 36**	**67 ± 9**
C	N_0P_0	3.4 ± 0.6a	188 ± 29a	21 ± 4a
	N_0P_1	6.4 ± 0.4b	363 ± 18b	53 ± 5b
	N_1P_0	7.3 ± 0.6b	350 ± 39b	63 ± 10b
	N_1P_1	10.2 ± 0.8c	517 ± 31c	137 ± 18c
	Mean	**6.8 ± 0.6b**	**355 ± 28**	**68 ± 10**
D	N_0P_0	5 ± 0.8a	355 ± 29a	40 ± 5a
	N_0P_1	6.6 ± 0.3b	379 ± 13a	58 ± 5a
	N_1P_0	7.0 ± 0.7b	507 ± 50b	60 ± 9a
	N_1P_1	9.6 ± 0.5c	501 ± 50b	94 ± 10b
	Mean	**7.0 ± 0.5b**	**435 ± 23**	**63 ± 5**
All Clones	N_0P_0	3.9 ± 0.3 a	273 ± 22	26 ± 3
	N_0P_1	6.3 ± 0.2 b	410 ± 13	51 ± 2
	N_1P_0	6.7 ± 0.3 b	442 ± 25	56 ± 5
	N_1P_1	9.7 ± 0.3 c	631 ± 32	113 ± 8
Overall	**Mean**	**6.7 ± 0.3**	**439 ± 18**	**61 ± 4**
Anova				
C		**	***	***
		6.4,0.002	16.4,<0.001	10.5,<0.001
T		***	***	***
		88.1,<0.001	73.5,<0.001	94.7,<0.001
C × T		ns	***	***
		1.37,0.25	5.04,<0.001	3.4, 0.005

Treatment influences on gross-primary productivity

Gross-primary productivity (GPP) scaled with nutrient supply ($F_{3,30} = 71.5$, $P < 0.001$), being about 3-fold greater in the high-nutrient supply regime (109 g C plant^{-1}) compared to the low-nutrient supply regime (30 g C plant^{-1}) (Table 5.2). As for plant mass growth, values of GPP in imbalanced treatments N_0P_1 (53 g plant^{-1}) and N_1P_0 (54 g plant^{-1}) were intermediate between balanced treatments N_0P_0 and N_1P_1.

The main effect of genotype on GPP was significant but relatively minor compared to the main effect of nutrient treatments as evidenced by F values (7.6 versus 71.5), and their interaction was only marginally significant ($F_{9,30} = 2.3$, $P = 0.05$) manifested by clone D developing faster in the low- and slower in the high-nutrient supply regime than Clones B and C (Table 5.2). Tukey's test on GPP clearly separated clones in two groups: a slower-growing Clone A, compared to faster-growing genotypes B,C and D (Table 5.2).

Net primary productivity (NPP), calculated as GPP minus autotrophic respiration (R_a), closely resembled the response of GPP to nutrient treatment and genotype. Carbon-use efficiency, defined as the ratio of NPP to GPP, significantly increased from 0.43 in the low-nutrient supply regime (N_0P_0) to 0.52 in the N_1P_0 and N_1P_1 treatment. The imbalanced treatment N_0P_1 had a carbon use efficiency of intermediate value (0.48) between these extremes. Overall this ranking was relatively consistent across clones. The marginally significant interaction between clone and treatment was attributable to the relatively low carbon use efficiency of N_1P_1 for clone D. Although the influence of clone on NPP : GPP ratio (carbon use efficiency) was marginally insignificant it is worth noting that the slowest growing clone, also displayed the lowest carbon use efficiency (0.46).

The fraction of GPP allocated to ANPP increased with N and P supply at the expense of TBCA, while the APR:GPP ratio remained relatively constant. At low-nutrient supply, the fraction of GPP represented by ANPP, APR and TBCA were 26%, 30% and 44%, respectively, compared to 35%, 33% and 32% in the high-nutrient supply regime. All these GPP fractions, except for APR, were strongly influenced by nutrient supply ($F_{3,30} > 27.3$, $P < 0.001$) and to less extent by genotype ($F_{3,30} > 3.2$, $P < 0.04$). Values of ANPP:GPP and TBCA:GPP in imbalanced treatments N_0P_1 and N_1P_0 were intermediate between balanced treatments N_0P_0 and N_1P_1. However, the effect was more pronounced in those plants growing with N only than P only (Table 5.2).

Table 5.2. Partitioning gross-primary productivity (GPP) into above-ground net primary productivity (ANPP), above-ground plant respiration (APR) and total below-ground C allocation (TBCA) for all combinations of nutrient treatments and clones (GPP = ANPP + APR + TBCA). Also shown is total net-primary productivity (NPP) and the fraction of GPP represented by NPP across nutrient treatments and clones. Nutrient treatments comprised two nitrogen supply regimes (N_0=1.43 and N_1=7.14 mM) and two phosphorus supply regimes (P_0=0.084 and P_1=0.420 mM). Values are presented as means (± 1 SE) for each treatment and clone; n = 6. Significance of main effects of clones (C) and nutrient treatments (T) or the interaction between clones and treatments (C × T) are shown as F values, P values and P range: ns, non significant; *, significant at $P < 0.05$; **, significant at $P < 0.01$; ***, significant at $P < 0.001$. Separation of means was determined by a Tukey test when applicable. Different letters indicate significant differences at $P < 0.05$. Significance of main effects of nutrient treatments and clones are indicated by letters when the C × T interaction showed to be non-significant (omitted otherwise).

Clone	Treat.	ANPP/GPP (%)	APR/GPP (%)	TBCA/GPP (%)	GPP (g C)	NPP (g C)	NPP/GPP (%)
A	N_0P_0	21.8 ± 1.7 a	30.9 ± 3.5 a	47.3 ± 3.0 a	23 ± 3.4 a	8.8 ± 1.6 a	37.6 ± 2.2 a
	N_0P_1	26.7 ± 1.0 ab	32.3 ± 1.8 a	41.0 ± 2.2 ab	47.3 ± 2.6 b	21.5 ± 0.9 b	45.6 ± 1.1 ab
	N_1P_0	32.8 ± 1.3 b	30.9 ± 2.5 a	36.2 ± 2.6 ab	32.7 ± 6.4 a	16.5 ± 3.0 b	50.8 ± 1.0 b
	N_1P_1	33.3 ± 1.5 b	32.7 ± 1.6 a	34.0 ± 1.6 b	86.3 ± 7.0 c	43.8 ± 5.0 c	50.2 ± 2.7 b
	Mean	**28.7 ± 1.2 a**	**31.7 ± 1.2 a**	**39.6 ± 1.5 b**	**47.3 ± 5.6 a**	**22.6 ± 3.1**	**46 ± 1.4 a**
B	N_0P_0	25.8 ± 1.7 a	33.5 ± 1.4 a	40.6 ± 2.5 a	28.4 ± 1.7 a	12 ± 1.2 a	41.9 ± 2.3 a
	N_0P_1	28.6 ± 1.4 ab	32.8 ± 1.2 a	38.7 ± 1.5 a	50.5 ± 3.5 b	23.6 ± 1.6 b	47.0 ± 2.0 ab
	N_1P_0	34.1 ± 1.5 bc	28.1 ± 1.7 a	37.7 ± 2.5 a	64.6 ± 6.8 b	34.1 ± 4.0 b	52.5 ± 0.9 b
	N_1P_1	38.4 ± 1.2 c	30.0 ± 2.7 a	31.6 ± 1.8 a	119.3 ± 9.6 c	66 ± 6.9 c	54.7 ± 2.3 b
	Mean	**31.7 ± 1.2 ab**	**31.1 ± 1 a**	**37.2 ± 1.2 ab**	**65.7 ± 7.6 b**	**33.9 ± 4.6**	**49 ± 1.4 a**
C	N_0P_0	25.1 ± 1.5 a	26.8 ± 3.5 a	48.1 ± 2.7 a	25.5 ± 5.4 a	10.5 ± 2.2 a	41.8 ± 1.2 a
	N_0P_1	26.8 ± 0.7 ab	35.0 ± 2.6 a	38.2 ± 3.2 ab	58.7 ± 6.8 b	27.2 ± 2.8 b	46.7 ± 1.2 ab
	N_1P_0	31.5 ± 1.7 bc	33.5 ± 1.3 a	35.0 ± 2.2 b	60.5 ± 8.8 b	31.9 ± 4.8 b	52.5 ± 1.6 b
	N_1P_1	35.4 ± 1.2 c	29.7 ± 1.0 a	34.8 ± 1.6 b	125.4 ± 16.8 c	69.2 ± 9.3 c	55.1 ± 1.2 b
	Mean	**29.7 ± 1 ab**	**31.3 ± 1.3 a**	**39 ± 1.6 ab**	**67.5 ± 9 b**	**34.7 ± 5.2**	**49 ± 1.2 a**
D	N0P0	29.2 ± 0.8 a	27.2 ± 1.2 a	43.6 ± 1.4 a	43.5 ± 5.0 a	21.1 ± 2.4 a	48.6 ± 1.4 a
	N0P1	30.9 ± 0.8 a	29.3 ± 1.9 a	39.8 ± 2.3 ab	57.0 ± 4.4 a	29.7 ± 2.7 a	51.8 ± 1.4 a
	N1P0	36.4 ± 1.2 a	32.1 ± 1.8 a	31.5 ± 1.0 b	58.2 ± 7.6 a	30.8 ± 4.7 a	52.4 ± 1.6 a
	N1P1	34.0 ± 3.4 a	39.7 ± 3.5 a	26.3 ± 1.8 b	105.7 ± 15.1 b	47.7 ± 5.2 b	46.8 ± 4.4 a
	Mean	**32.6 ± 1.1 b**	**32.1 ± 1.4 a**	**35.3 ± 1.6 a**	**66.1 ± 6.5 b**	**32.3 ± 2.7**	**49.9 ± 1.3 a**
All	N_0P_0	25.5 ± 0.9 a	29.6 ± 1.4 a	44.9 ± 1.3 c	30.1 ± 2.5 a	13.1 ± 1.3	42.5 ± 1.2 a
	N_0P_1	28.2 ± 0.6 a	32.3 ± 1.0 a	39.4 ± 1.1 b	53.4 ± 2.3 b	25.5 ± 1.2	47.8 ± 0.8 b
	N_1P_0	33.7 ± 0.8 b	31.2 ± 1.0 a	35.1 ± 1.1 a	54.0 ± 4.3 b	28.3 ± 2.4	52.1 ± 0.6 c
	N_1P_1	35.3 ± 1.0 b	33.0 ± 1.4 a	31.7 ± 1.1 a	109.2 ± 6.7 c	56.7 ± 3.9	51.7 ± 1.5 c
Overall Mean		**30.7 ± 0.6**	**31.5 ± 0.6**	**37.8 ± 0.8**	**61.7 ± 3.7**	**30.9 ± 2.0**	**48.5 ± 0.7**
Anova							
C		** 5.44, 0.004	ns 0.12, 0.95	* 3.20, 0.037	*** 7.62, <0.001	*** 11.39, <0.001	ns 2.56, 0.074
T		*** 35.28, <0.001	ns 1.81, 0.17	*** 27.25, < 0.001	*** 71.48, < 0.001	*** 96.15, <0.001	*** 17.70, <0.001
C × T		ns 1.30, 0.28	* 2.27, 0.045	ns 1.41, 0.23	* 2.26, 0.046	** 3.56, 0.0042	* 2.48, 0.030

Carbon allocation patterns at least partially explained differences in genotypic growth performance. Total carbon assimilation (GPP) was significantly less in Clone A than other clones (Table 5.2), and in this clone a significantly lower proportion of assimilates were partitioned to the photosynthetic apparatus (ANPP : GPP).

All GPP fractions (ANPP:GPP, APR:GPP and TBCA:GPP) were plotted against GPP to test whether plant size may confound the influence of nutrient treatment and genotype on carbon allocation (Figure 5.3). The linear relationship between APR : GPP against GPP was non-significant and neither slopes nor intercepts differed between nutrient treatment ($F_{7,88}$ = 0.72, P = 0.65).Values of ANPP:GPP and TBCA:GPP were not correlated with GPP ($F_{1,88}$ < 0.37, P > 0.54) and slopes were unaffected by nutrient treatments ($F_{3,88}$ <1.7, P < 0.18). However intercepts ($F_{3,88}$ > 4.4, P < 0.001) were significantly different between nutrient treatments, and therefore changes between treatments were represented by lines with different intercepts and zero slope (intercepts being equal to the treatment means), with equivalent results to those presented in Table 5.2. Therefore, values of ANPP:GPP were significantly greater and TBCA:GPP significantly smaller in the high-nutrient supply regime compared to lower N and P addition rates (Figure 5.3a and 5.3c), and plant size did not confound the effect of nutrient treatment on GPP partitioning.

Analysis of covariance also revealed that plant size did not affect the influence of genotype on GPP partitioning. The effect of clone was superimposed on the previous analysis of covariance with nutrient treatment as the only effect, yielding a three dimensional analysis of covariance with clone and treatment as independent effects. Only the intercept of the ANPP : GPP against GPP linear relationship was significantly influenced by genotype ($F_{1,82}$ = 6.20, P < 0.001), and therefore resulted in effects equivalent to those presented in Table 5.2 i.e. clones B,C and D exhibited greater GPP partitioning to ANPP than clone A.

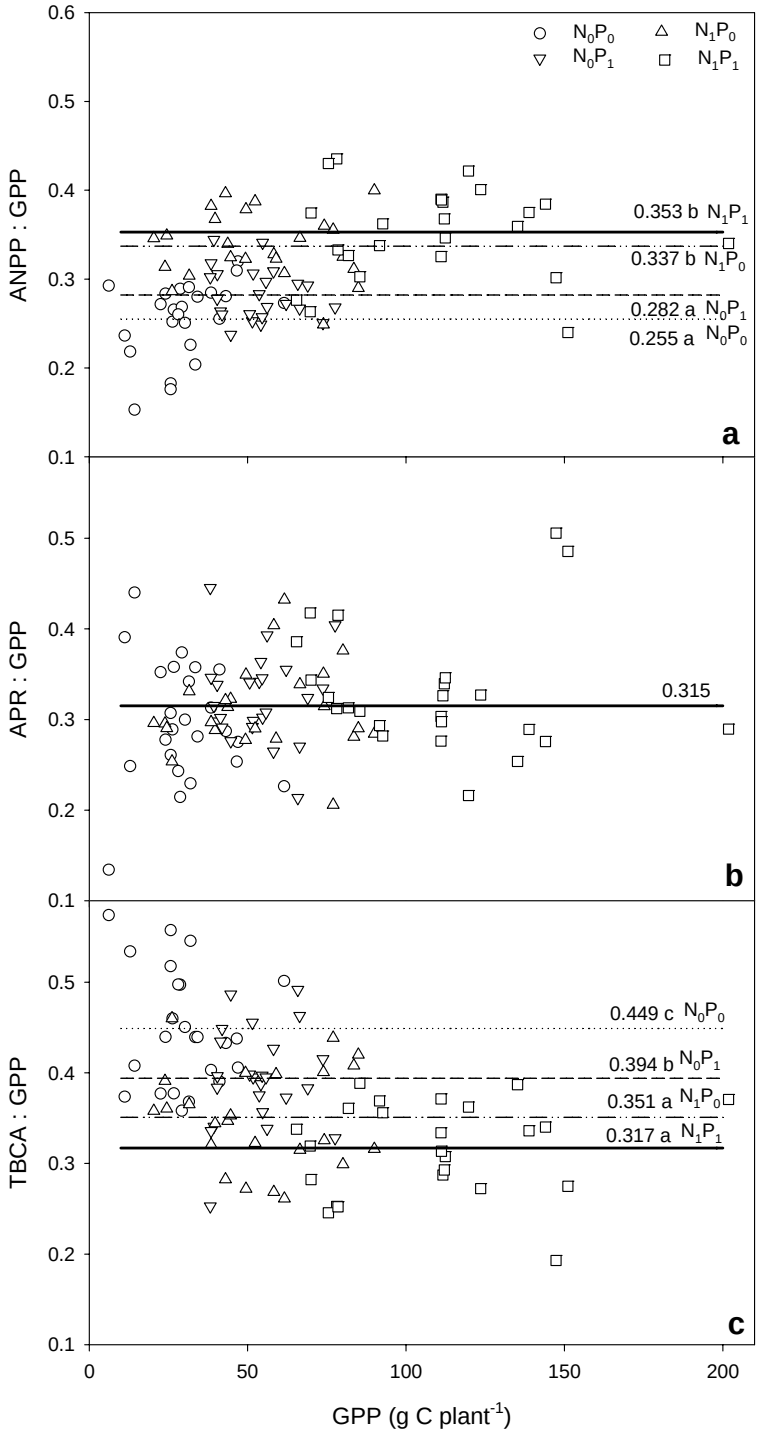


Figure 5.3. The relationships between (a) ANPP: GPP, (b) APR : GPP and (c) TBCA : GPP against GPP. Nutrient treatments comprised two nitrogen supply regimes (N_0=1.43 and N_1=7.14 mM) and two phosphorus supply regimes (P_0=0.084 and P_1=0.420 mM). Analysis of covariance showed that the ANPP : GPP, APR : GPP and TBCA : GPP ratios were uncorrelated to GPP, but intercepts differed between nutrient treatments except for APR : TBCA . This is represented by single lines with zero slope and intercept equal to the treatment means. Different letters indicate significant differences among intercepts at $P < 0.05$.

Treatment influences on above-ground net primary productivity

Above-ground net primary productivity (ANPP) scaled positively with nutrient supply ($F_{3,30}$ =125, $P < 0.001$) being about five-fold greater in the high-nutrient supply regime (39 g C plant^{-1}) compared to the low-nutrient supply regime (8 g C plant^{-1}) (Table 5.3). The components of ANPP, Litterfall C content (F_A), live foliage (ΔC_C) and wood and branch (ΔC_W) C content growth all significantly increased in absolute value with nutrient supply ($F_{3,30} > 3.4$, $P < 0.03$). As proportions, F_A, ΔC_C and ΔC_W represented about 3.2%, 69.3% and 27.5% of ANPP, respectively. Although absolute litterfall significantly increased with increasing nutrient supply (Table 5.3), there was a strongly significant decline ($F_{3,30}$ = 11.1, $P < 0.001$) in the ratio of litterfall to ANPP as nutrient availability increased (Figure 5.4) from 5.4 % in the low-nutrient supply regime to 1.9 % in the high-nutrient supply regime. The fractions of ANPP represented by ΔC_C and ΔC_W remained largely constant across nutrient treatments despite small but significant reductions for ΔC_C and increases for ΔC_W ($F_{3,30} > 3.19$, $P < 0.037$) with increasing P but not N supply (Figure 5.4).

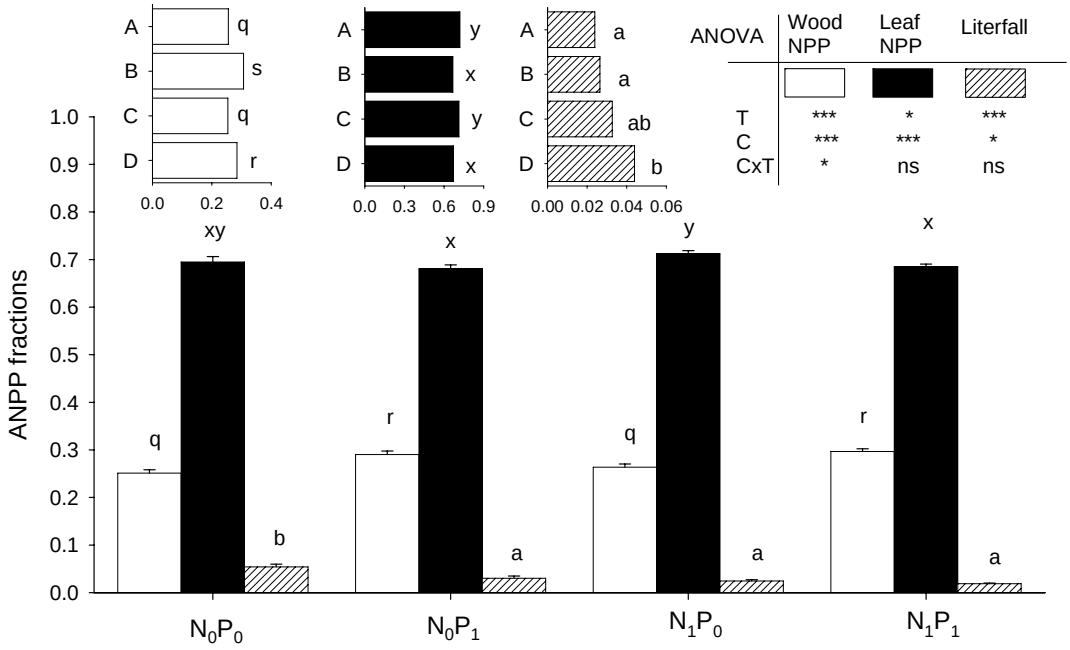


Figure 5.4. ANPP partitioning into litterfall production (F_A), leaf NPP and wood NPP, across nutrient treatments and clones. Nutrient treatments comprised two nitrogen supply regimes (N_0=1.43 and N_1=7.14 mM) and two phosphorus supply regimes (P_0=0.084 and P_1=0.420 mM). Different letters indicate significant differences among treatments at $P < 0.05$ (litterfall *a* to *b*, wood NPP *q* to *s*, leaf NPP *x* to *y*). Significance of main effects of clones (C) and nutrient treatments (T) or the interaction between clones and treatments (C × T) are shown as *P* range: ns, non significant; *, significant at $P < 0.05$; **, significant at $P < 0.01$; ***, significant at $P < 0.001$.

Table 5.3. Above-ground net primary productivity (ANPP = F_A + ΔC_C + ΔC_w, g C) components for all nutrient supply regimes and clones. Components of ANPP are: litterfall C content (F_A, g C), foliage C content increment (ΔC_C, g C), and stem and branch C content increment (ΔCw, g C). Nutrient treatments comprised two nitrogen supply regimes (N_0=1.43 and N_1=7.14 mM) and two phosphorus supply regimes (P_0=0.084 and P_1=0.420 mM). Values are presented as means (± 1 SE) for each treatment and clone; n = 6. Significance of main effects of clones (C) and nutrient treatments (T) or the interaction between clones and treatments (C × T) are shown as F values, P values and P range: ns, non significant; *, significant at $P < 0.05$; **, significant at $P < 0.01$; ***, significant at $P < 0.001$. Separation of means was determined by a Tukey test when applicable. Different letters indicate significant differences at $P < 0.05$. Significance of main effects of nutrient treatments or clones are indicated by letters when the C × T interaction was insignificant (omitted otherwise).

Clone	Treatment	F_A Litterfall C (g C)	ΔC_C Foliage C change (g C)	ΔC_W Wood C change (g C)	ANPP (g C)
A	N_0P_0	0.19 ± 0.04 a	3.8 ± 0.7a	1.1 ± 0.2 a	5.1 ± 0.9 a
	N_0P_1	0.22 ± 0.03 a	8.9 ± 0.4 b	3.5 ± 0.3 c	12.6 ± 0.6 b
	N_1P_0	0.2 ± 0.03 a	8 ± 1.4 b	2.5 ± 0.5 b	10.7 ± 2 b
	N_1P_1	0.43 ± 0.08 a	20.2 ± 2 c	8.3 ± 0.9 d	28.9 ± 2.9 c
	Mean	**0.26 ± 0.03 a**	**10.2 ± 1.4 a**	**3.9 ± 0.6**	**14.3 ± 2**
B	N_0P_0	0.34 ± 0.04 a	5.2 ± 0.6 a	1.9 ± 0.2 a	7.4 ± 0.8 a
	N_0P_1	0.32 ± 0.03 a	9.1 ± 0.5 b	4.9 ± 0.4 b	14.3 ± 0.9 b
	N_1P_0	0.45 ± 0.11 ab	14.8 ± 1.4 c	6.5 ± 0.7 b	21.8 ± 2.1 c
	N_1P_1	0.7 ± 0.1 b	29.9 ± 2.7 d	15.6 ± 1.5 c	46.1 ± 4.3 d
	Mean	**0.45 ± 0.05 ab**	**14.8 ± 2.1 b**	**7.2 ± 1.1**	**22.4 ± 3.3**
C	N_0P_0	0.28 ± 0.05 a	4.6 ± 1.1 a	1.4 ± 0.2 a	6.3 ± 1.4 a
	N_0P_1	0.55 ± 0.15 ab	11.1 ± 1.2 b	4 ± 0.4 b	15.6 ± 1.6 b
	N_1P_0	0.45 ± 0.08 a	13.7 ± 2 b	4.8 ± 0.7 b	18.9 ± 2.6 b
	N_1P_1	0.67 ± 0.11 b	31.6 ± 3.9 c	12.2 ± 1.7 c	44.4 ± 5.7 c
	Mean	**0.49 ± 0.06 b**	**15.2 ± 2.4 b**	**5.6 ± 0.9**	**21.3 ± 3.3**
D	N_0P_0	0.93 ± 0.19 a	8.1 ± 0.8 a	3.6 ± 0.4 a	12.7 ± 1.4 a
	N_0P_1	0.84 ± 0.3 a	11.5 ± 0.7 b	5.2 ± 0.4 ab	17.5 ± 1.2 ab
	N_1P_0	0.68 ± 0.14 a	14.8 ± 2.1 b	5.9 ± 1.0 b	21.3 ± 3.2 b
	N_1P_1	0.96 ± 0.12 a	23.5 ± 2.6 c	10 ± 1.1 c	34.5 ± 3.7 c
	Mean	**0.85 ± 0.1 c**	**14.5 ± 1.4 b**	**6.2 ± 0.6**	**21.5 ± 2.1**
All Clones	N_0P_0	0.43 ± 0.08 a	5.4 ± 0.5 a	2 ± 0.2	7.9 ± 0.8
	N_0P_1	0.48 ± 0.09 a	10.2 ± 0.4 b	4.4 ± 0.2	15 ± 0.7
	N_1P_0	0.44 ± 0.06 a	12.8 ± 1.0 b	4.9 ± 0.5	18.2 ± 1.5
	N_1P_1	0.69 ± 0.06 b	26.3 ± 1.7 c	11.5 ± 0.9	38.5 ± 2.5
Overall	**Mean**	**0.51 ± 0.04**	**13.7 ± 0.9**	**5.7 ± 0.4**	**19.9 ± 1.4**
Anova					
C		*** 14.3,<0.001	*** 8.92,<0.001	*** 29.9,<0.001	*** 14.98,<0.001
T		* 3.39,0.03	*** 99.0,<0.001	*** 179.0,<0.001	*** 125.0<0.001
C × T		ns 0.56,0.82	* 2.40,<0.03	*** 5.41, <0.001	** 3.29,0.007

Treatment influences on above-ground plant respiration

Foliar maintenance respiration (L_{RM}) represented 73% of APR, whereas foliage construction respiration (L_{RC}) and wood construction and maintenance respiration (W_R), represented 18% and 9% of APR, respectively (Table 5.4). Although absolute values of APR positively scaled with nutrient supply and were significantly influenced by clone, the APR : GPP ratio was not influenced by nutrient treatment or clone (Table 5.2), indicating that differences in APR were attributable to variation in plant size rather than differences in specific tissue respiration rates.

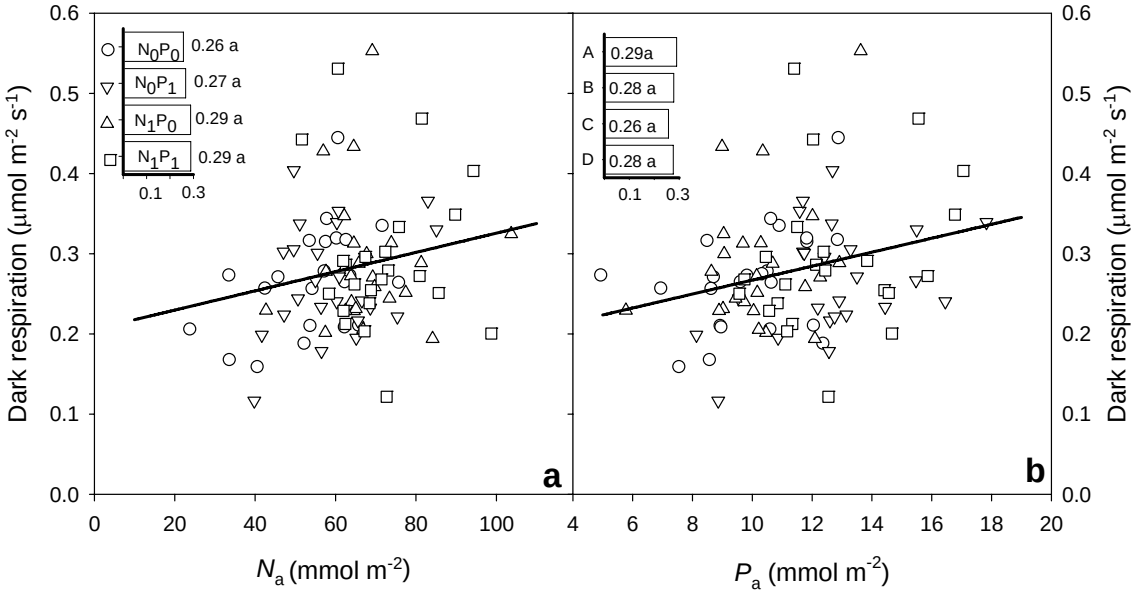


Figure 5.5. The relationships between the rate of foliage respiration at night (R_d) and foliage nitrogen (N_a) and phosphorus concentration (P_a) on an area basis. Slopes and intercepts of these linear relationships were not influenced by nutrient treatment or clone: $R_d = 0.2056 + 0.0012\ N_a$, $r^2 = 0.04$, $P = 0.05$, $n = 94$; $R_d = 0.1800 + 0.0087\ P_a$, $r^2 = 0.07$, $P = 0.10$. Horizontal bar graph inserts show main effects of nutrient treatment (a) and clone (b). Values of R_d were (± 1 SE, $n = 24$): 0.26 ± 0.02, 0.27 ± 0.01, 0.29 ± 0.02 and 0.29 ± 0.02 for treatments N_0P_0, N_0P_1, N_1P_0 and N_1P_1, respectively. Values of R_d were (± 1 SE, $n = 24$): 0.29 ± 0.02, 0.28 ± 0.01, 0.26 ± 0.02, 0.28 ± 0.02 for clones A,B,C and D respectively. Main or interactive effects between nutrient treatments and clones on R_d were not significant.

The main component of above-ground plant respiration (APR), foliage maintenance respiration, was calculated by scaling the rate of respiration at night (R_d) over time using air temperature records (Figure 5.1a) and an exponential response of night respiration to temperature ($Q_{10} = 2$, $R_d = e^{0.0693\ T}$, with T being air temperature in °C). Values of R_d ranged from 0.11 to 0.55 μmol m^{-2} s^{-1} (average 0.28 ± 0.01 SE, $n = 96$). Although neither the main or interactive effects of nutrient treatments and clones significantly affected R_d ($F_{47,48} = 1.58$, $P = 0.06$), values showed weak positive correlations with foliage nitrogen and phosphorus concentrations (Figure 5.5; $r^2 < 0.07$), which were only marginally significant in the case of

foliage nitrogen ($P = 0.05$). Slopes ($F_{3,86} < 0.73$, $P > 0.53$) and intercepts ($F_{3,86} < 0.78$, $P > 0.51$) of these linear relationships were not influenced by nutrient treatment or clone.

Table 5.4. Above-ground plant respiration (APR = L_{RC} + L_{RM} + W_R) components for all nutrient-supply regimes and clones. Components of APR are: wood respiration (W_R), foliage construction (L_{RC}) and foliage maintenance respiration (L_{RM}). Nutrient treatments comprised two nitrogen supply regimes (N_0=1.43 and N_1=7.14 mM) and two phosphorus supply regimes (P_0=0.084 and P_1=0.420 mM). Values are presented as means (± 1 SE) for each treatment and clone; $n = 6$. Significance of main effects of clones (C) and nutrient treatments (T) or the interaction between clones and treatments (C × T) are shown as F values, P values and P range: ns, non significant; *, significant at $P < 0.05$; **, significant at $P < 0.01$; ***, significant at $P < 0.001$. Different letters indicate significant differences at $P < 0.05$ (Tukey test) when the C × T interaction was insignificant (omitted otherwise).

Clone	Treatment	L_{RC} Fol. Const. Resp. (g C)	L_{RM} Fol. Maint. Resp. (g C)	W_R Wood Const. & Maint. Resp. (g C)	APR Above-ground plant Resp. (g C)	L_{RM} / APR (%)
A	N_0P_0	1 ± 0.2 a	5.2 ± 0.3 a	0.4 ± 0.1 a	6.5 ± 0.5 a	79.7 ± 2.9 a
	N_0P_1	2.3 ± 0.1 b	12 ± 1.4 ab	1.1 ± 0.1 b	15.4 ± 1.5 b	77.5 ± 1.2 a
	N_1P_0	2 ± 0.4 b	8 ± 2.8 a	0.8 ± 0.2 b	10.8 ± 3.3 ab	71.0 ± 2.2 a
	N_1P_1	5.1 ± 0.5 c	20.1 ± 1.7 b	2.7 ± 0.3 c	27.9 ± 2.2 b	72.0 ± 2.1 a
	Mean	**2.6 ± 0.4 a**	**11.3 ± 1.4 a**	**1.2 ± 0.2**	**15.2 ± 1.9 a**	**75 ± 1.3 a**
B	N_0P_0	1.4 ± 0.1 a	7.5 ± 0.4 a	0.6 ± 0.1 a	9.5 ± 0.5 a	79.2 ± 1.6 b
	N_0P_1	2.4 ± 0.1 ab	12.8 ± 1.5 ab	1.5 ± 0.1 b	16.7 ± 1.6 a	75.9 ± 1.8 ab
	N_1P_0	3.8 ± 0.4 b	12.1 ± 1.6 ab	2.1 ± 0.2 b	18 ± 2 ab	66.5 ± 2.8 ab
	N_1P_1	7.6 ± 0.7 c	22.3 ± 2.4 b	5 ± 0.5 c	34.9 ± 2.5 b	63.1 ± 4.2 a
	Mean	**3.8 ± 0.5 b**	**13.6 ± 1.4 a**	**2.3 ± 0.4**	**19.8 ± 2.1 b**	**71.2 ± 1.9 a**
C	N_0P_0	1.2 ± 0.3 a	6 ± 1.8a	0.4 ± 0.1 a	7.6 ± 2.1 a	70.9 ± 6.6 a
	N_0P_1	2.9 ± 0.3 b	16.2 ± 2.3 b	1.3 ± 0.1 b	20.4 ± 2.7 b	79.0 ± 1.4 a
	N_1P_0	3.5 ± 0.5 b	15.2 ± 2.5 b	1.5 ± 0.2 b	20.3 ± 3 b	74.6 ± 1.6 a
	N_1P_1	8.1 ± 1 c	25 ± 3.1 b	3.9 ± 0.6 c	36.9 ± 4.5 b	67.7 ± 1.6 a
	Mean	**3.9 ± 0.6 b**	**15.6 ± 1.8 a**	**1.8 ± 0.3**	**21.3 ± 2.6 b**	**73.1 ± 1.9 a**
D	N_0P_0	2.3 ± 0.3 a	8.2 ± 0.7 a	1.1 ± 0.1 a	11.6 ± 1a	70.8 ± 1.4 a
	N_0P_1	3.1 ± 0.2 a	11.7 ± 1.2 a	1.6 ± 0.1 ab	16.5 ± 1.3 a	70.9 ± 2.0 a
	N_1P_0	3.9 ± 0.5 ab	12.9 ± 1.8 a	1.9 ± 0.3 ab	18.7 ± 2.4 a	68.8 ± 2.5 a
	N_1P_1	6.1 ± 0.7 b	34.7 ± 9.2 b	3.2 ± 0.4 b	44 ± 9.7 b	75.1 ± 4.4 a
	Mean	**3.8 ± 0.4 b**	**16.9 ± 3.1 a**	**2 ± 0.2**	**22.7 ± 3.5 b**	**71.4 ± 1.4 a**
All Clones	N_0P_0	1.5 ± 0.1 a	6.7 ± 0.5 a	0.6 ± 0.1 a	8.8 ± 0.7 a	75.2 ± 2.0 ab
	N_0P_1	2.7 ± 0.1 b	13.2 ± 0.9 b	1.4 ± 0.1 b	17.2 ± 1 b	75.8 ± 1.0 a
	N_1P_0	3.3 ± 0.3 b	12 ± 1.2 b	1.6 ± 0.2 b	16.9 ± 1.5 b	70.2 ± 1.2 bc
	N_1P_1	6.7 ± 0.4 c	25.5 ± 2.6 c	3.7 ± 0.3 c	35.9 ± 2.9 c	69.5 ± 1.8 c
Overall	**Mean**	**3.5 ± 0.2**	**14.4 ± 1**	**1.8 ± 0.1**	**19.7 ± 1.3**	**72.7 ± 0.8 a**
Anova						
C		*** 11.28, <0.001	ns 2.06, 0.13	*** 29.86, <0.001	* 4.03, 0.0160	ns 1.44, 0.24
T		*** 106.0, <0.001	*** 28.2, <0.001	*** 179.0, <0.001	*** 45.47, <0.001	** 4.82, 0.007
C × T		* 2.75, 0181	Ns 1.37, 0.24	*** 5.41,<0.001	ns 1.42, 0.22	* 2.38. 0.036

Treatment Influences on total-below-ground carbon allocation

Absolute values for total below-ground carbon allocation (TBCA) significantly increased with nutrient supply ($F_{3,30} = 16.6$, $P < 0.001$), from 13 g C plant^{-1} in the low nutrient supply treatment to 35 g C plant^{-1} in the high-nutrient supply regime, with plants in imbalanced treatments N_0P_1 and N_1P_0 showing similar intermediate values between these two extremes (Table 5.5). Values of TBCA were much larger in Clones B,C and D than clone A, an effect which was mainly attributable to tree size, as the TBCA:GPP ratio showed the opposite trend i.e. Clone A showed greater TBCA : GPP ratio than clones B,C and D (Table 5.2).

When averaged across all treatments, soil respiration (F_S) and root C content increment (ΔC_R) constituted a similar proportion of TBCA. Although the ratio of of F_S / TBCA was relatively constant for all three treatments in which nutrients were added (N_1P_0, N_0P_1,N_1P_1), at an average of 49%, it significantly increased to 61% for the treatment with low nutrient additions, N_0P_0 (Table 5.5). Genotype did not change the partitioning of TBCA between F_S and ΔC_R ($F_{3,30} = 2.87$, $P = 0.053$).

Table 5.5. Total-belowground carbon allocation (TBCA = F_S + ΔC_R) components for all nutrient-supply regimes and clones. Components of TBCA are: soil respiration C efflux (F_S) and change in C content of root biomass (ΔC_R). The ratio F_S/TBCA ratio represents the amount C efflux respired by roots and mycorrhizae and root turnover. Nutrient treatments comprised two nitrogen supply regimes (N_0=1.43 and N_1=7.14 mM) and two phosphorus supply regimes (P_0=0.084 and P_1=0.420 mM). Values are presented as means (± 1 SE) for each treatment and clone; n = 6. Significance of main effects of clones (C) and nutrient treatments (T) or the interaction between clones and treatments (C × T) are shown as F values and P range: ns, non significant; *, significant at P < 0.05; **, significant at P < 0.01; ***, significant at P < 0.001. Separation of means was determined by a Tukey test when applicable. Different letters indicate significant differences at P < 0.05. Significance of main effects of clones are indicated by letters when the C × T interaction was insignificant (omitted otherwise).

Clone	Treatment	F_S Soil Resp. C efflux (g C)	ΔC_R Root C change (g C)	TBCA C allocation below-ground (g C)	F_S / TBCA (%)
A	N_0P_0	7.7 ± 1.5 ab	3.7 ± 0.7 a	11.3 ± 2.1 a	66.0 ± 2.6 b
	N_0P_1	10.5 ± 0.7 ab	8.9 ± 0.6 bc	19.3 ± 1.3 ab	54.1 ± 0.7 ab
	N_1P_0	5.4 ± 0.5 a	5.8 ± 1 ab	11.2 ± 1.4 a	49.6 ± 2.8 a
	N_1P_1	14.5 ± 1.6 b	14.9 ± 2.3 c	29.4 ± 2.7 b	50.2 ± 4.6 a
	Mean	9.5 ± 0.9	8.3 ± 1.1 a	17.8 ± 1.8 a	55 ± 2.0 a
B	N_0P_0	6.9 ± 0.8 a	4.6 ± 0.4 a	11.5 ± 1 a	59.7 ± 3.0 a
	N_0P_1	10.2 ± 1.1 ab	9.3 ± 0.9 ab	19.5 ± 1.4 ab	51.8 ± 4.0 a
	N_1P_0	12.5 ± 1.6 ab	12.4 ± 2.3 b	24.8 ± 3.6 ab	51.6 ± 3.2 a
	N_1P_1	18.4 ± 2.0 b	19.9 ± 2.7 b	38.2 ± 4.4 b	48.5 ± 2.3 a
	Mean	12 ± 1.1	11.5 ± 1.4 b	23.5 ± 2.5 b	52.9 ± 1.7 a
C	N_0P_0	7.3 ± 1.2 a	4.2 ± 0.9 a	11.5 ± 2.1 a	64.8 ± 2.4 b
	N_0P_1	11.1 ± 2.1 ab	11.6 ± 1.3 b	22.7 ± 3.3 a	47.1 ± 3.3 a
	N_1P_0	8.4 ± 1.4 a	13 ± 2.4 b	21.4 ± 3.8 a	39.6 ± 3.0 a
	N_1P_1	19.3 ± 3.4 b	24.8 ± 3.7 b	44.1 ± 7 b	43.4 ± 1.8 a
	Mean	11.5 ± 1.4	13.4 ± 1.9 b	24.9 ± 3.2 ab	48.7 ± 2.4 a
D	N_0P_0	10.8 ± 1.9 a	8.4 ± 1 a	19.2 ± 2.7 a	55.3 ± 2.2 a
	N_0P_1	10.8 ± 1.0 a	12.2 ± 1.7 a	23.0 ± 2.7 a	47.9 ± 1.6 a
	N_1P_0	8.8 ± 1.0 a	9.5 ± 1.5 a	18.2 ± 2.3 a	49.0 ± 2.7 a
	N_1P_1	13.9 ± 2.3 a	13.3 ± 1.8 a	27.2 ± 3.6 a	50.5 ± 4.9 a
	Mean	11.1 ± 0.9	10.8 ± 0.8 b	21.9 ± 1.5 b	50.6 ± 1.6 a
All Clones	N_0P_0	8.2 ± 0.7 a	5.2 ± 0.5 a	13.4 ± 1.2 a	61.5 ± 1.5 b
	N_0P_1	10.7 ± 0.6 a	10.5 ± 0.6 b	21.1 ± 1.1 b	50.2 ± 1.4 a
	N_1P_0	8.8 ± 0.8 a	10.2 ± 1.1 b	18.9 ± 1.7 b	47.4 ± 1.7 a
	N_1P_1	16.5 ± 1.2 b	18.2 ± 1.6 c	34.7 ± 2.6 c	48.2 ± 1.8 a
Overall	**Mean**	11 ± 0.5	11 ± 0.7	22.1 ± 1.2	51.8 ± 1
Anova					
C		1.99, 0.1367 ns	6.44, 0.0017 **	4.24, 0.0130 *	2.87, 0.0527 ns
T		25.02, <0.001 ***	51.15, <0.001 ***	36.95, <0.001 ***	16.59, <0.001 ***
C × T		2.04, 0.070 ns	3.70, 0.0032 **	2.90, 0.0135 *	1.47, 0.2041 ns

DISCUSSION

Although N and P deficiencies similarly decreased GPP and NPP, N supply had a stronger effect on TBCA:GPP, ANPP:GPP and NPP:GPP than P supply. All clones conformed to this general pattern, but faster growing genotypes allocated about 2-4% more carbon to aboveground components providing a mechanistic explanation for their enhanced growth performance. One key finding in this study was that the effects of N and P on C fluxes were most marked if both were present.

It has been argued that ANPP may increase with nutrient supply at the expense of TBCA without major effects on GPP (e.g. Keyes and Grier 1981). The results of this study revealed that GPP, ANPP, APR and TBCA increased together with single or joint N and P supply, and also that increased nutrition increased ANPP at the expense of TBCA. Nadelhoffer and Raich (1992) found similar trends for a variety of forest ecosystems around the world, and Giardina *et al*. (2003) for *Eucalyptus saligna* plantations in Hawaii.

Nutrient availability drastically reduced the proportion of GPP partitioned to TBCA from about 0.45 in the low-nutrient supply regime compared to 0.32 in the high-nutrient supply regime. These values are similar to those found by Giardina *et al*. (2003) in control (0.45) compared to fertilized plots (0.30) of *Eucalyptus saligna* plantations. Similarly, Ryan *et al*. (1996) found that the TBCA : GPP ratio decreased from 42% in control plots compared to 22% in fertilized plots of *Pinus radiata* plantations in Australia. Our results extend previous studies by demonstrating that reductions in TBCA were triggered mainly by N rather than P supply, and that TBCA was lowest when both N and P were supplied simultaneously.

The ratio of TBCA to (ANPP+APR), which is an ecosystem measurement analogous to root : shoot ratio, substantially decreased with nutrient supply from 0.84 at low- compared to 0.47 at high-nutrient supply, while similarly Giardina *et al*. (2003) found that this ratio was about 1 for control plots compared to 0.57 in fertilized plots of *Eucalyptus saligna* plantations. Concomitantly with reductions in partitioning to TBCA, nutrient supply increased the fraction of GPP partitioned to ANPP from about 0.26 to 0.35, which are similar to those found by Giardina *et al*. (2003) in control (0.26) compared to fertilized plots (0.36).

The fraction of GPP partitioned to APR in this study remained largely unaffected (about 0.31) by nutrient supply, whereas Giardina *et al*. (2003) and Ryan *et al*. (1996) found that proportion increased from control (0.25, 0.29) to fertilized plots (0.29, 0.41) in *Eucalyptus saligna* and *Pinus radiata* plantations in Hawaii and Canberra, respectively. Dark respiration values found in this study (0.552 μmol m^{-2} s^{-1} hemi-surface area basis) were within the range for

mature stands of black spruce (0.21-0.64 μmol m^{-2} s^{-1}) and jack pine (0.37-0.61 μmol m^{-2} s^{-1}) in Manitoba, Canada, found by Ryan *et al*. (1997). There was a slight tendency for dark respiration to increase with nitrogen and phosphorus concentration, but this did not translate into a greater proportion of total carbon assimilated being partitioned to above-ground plant respiration.

Carbon use efficiency (NPP:GPP) was expected to be highly conservative and not influenced by nutrient deficiencies. Carbon use efficiency (NPP:GPP) is an important parameter in many ecosystems process-based models used in assessing the influence of climate change on the carbon budget of ecosystems from community to planetary scales (e.g. Ichii *et al*. 2005, Turner *et al*. 2006). Waring *et al*. (1998) found in a survey of annual carbon budgets from six evergreen and one deciduous forests in New Zealand, Australia and United States, that the total NPP : GPP ratio was conservative and about 0.47 ± 0.04 SD, being also similar to the 0.45 ± 0.05 SD reported by Landsberg and Waring (1997). Our values of carbon use efficiency fall within reported ranges, but they significantly increased from 0.43 ± 0.01 SE in the low nutrient supply regime compared to 0.52 ± 0.02 SE in the high nutrient supply regime. Giardina *et al*. (2003) found that carbon use efficiency was slightly higher in fertilized (0.53) compared with control plots (0.51) of *Eucalyptus saligna* plantations, providing further evidence that carbon use efficiency increases with fertility. Our results extend previous research by demonstrating that reductions in carbon use efficiency are primarily attributable to nitrogen rather than phosphorus deficiencies. We suggest that nutrient management and fertilization of plantation forestry, particularly in poor fertility environments, may contribute in reducing CO_2 emissions to the atmosphere. This is also important as differences between measured and modelled NPP : GPP ratios has been observed in the application of global ecosystems models such as the Biome-BGC carbon cycle process model (Turner *et al*. 2006). Jarvis (1995) pointed out the need to continually improve on assumptions of ecosystems process-based models. Recognizing that carbon-use-efficiency increases with fertility may be a way to contribute to that aim.

We hypothesized that carbon allocation patterns may help to explain differences in genotypic growth performance. Raison and Myers (1992) argued that genotype, additionally to fertility and silviculture, may influence carbon allocation patterns in *Pinus radiata*, while similarly Miller and Hawkins (2003, 2007) found that fast-growing families of interior spruce in Canada exhibited a greater plasticity in dry mass allocation between shoots and roots than slow-growing families, suggesting that carbon allocation might be partially controlled by genotype. In this study, significant clonal differences in carbon allocation to above-ground components (about 4%) at the expense of below-ground processes were found. Thus, marginally greater allocation to foliage, and hence leaf area, may compound increasing overall carbon assimilation

over time in faster-growing genotypes. Our results extend on studies reported above by confirming their hypotheses using a complete C budget approach. Also the genotype × fertility interactions were minor compared to the main effects of nutrient treatments and clones, suggesting that selecting genotypes for better growth performance in poor fertility sites would not be substantially better than selecting for growth on all sites irrespective of nutrient availability as suggested by Carson *et al.* (2004).

Many ecosystem-process based carbon budget models assume that TBCA is evenly allocated between root production and respiration (Ryan 1991*a*, Landsberg and Waring 1997, Waring *et al.* 1998). McDowell *et al.* (2001) found that soil respiration was 36-47% while root production 53-64% of TBCA in two *Pseudotsuga menziesii* stands in Washington. Giardina and Ryan (2002) found that annual soil respiration (F_S) was lower in fertilized than control plots of *Eucalyptus saligna* plantations, suggesting a proportional decrease in F_S with nutrient availability. This study extends this research by demonstrating the interactive effect of N and P on the F_S / TBCA ratio. Both the imbalanced and high nutrient supply regimes exhibited very similar values for this ratio, which were significantly lower than in the low nutrient supply regime. This suggests that as nutrient supply declines, plant roots and mycorrhizae respire proportionally more than at higher N and P addition rates, and this is a result that can be readily incorporated in ecosystem physiological models.

Several assumptions were made in the estimation of gross- and net-primary productivity and its partitioning, but they proved not to change the main conclusions of the study. First of all, we assumed that leaf and wood construction respiration were 25% of leaf and wood net primary productivity. Ryan (1991*b*), based on Penning de Vries (1972, 1975), suggested that a reasonable approximation is to assume that construction respiration equals 0.25 g per gram incorporated to new tissues. However, foliage construction and total wood respiration were 5% and 3% of the gross-primary productivity, and we consider these amounts to be unlikely to change the main conclusions of the study. A second assumption of the study was to scale foliage respiration using a Q_{10} equal to 2, which seems to be fairly conservative (Landsberg and Gower 1997). For instance, Ryan *et al.* (1997) found Q_{10} values of 2 and 2.1 for jack pine and black spruce, respectively, in Canada. A third assumption was to scale leaf dark respiration over the entire growing season, assuming that leaf respiration in the light and dark were equal, despite that respiration rates in the light might be less than in darkness (Brooks and Farquhar 1985), but as pointed out by Atkin *et al.* (2000) respiration in the light is highly variable and dependent on irradiance which is the reason why we preferred to use dark respiration projected over the whole growing season. However, if we assumed day respiration to be 50% of dark

respiration (Villar *et al*. 1995), then values of foliage maintenance respiration (L_{RM}) would be about 25% less than estimated values, and the difference will reduce gross-primary productivity by 6%, which would be unlikely to change the main conclusions of the study.

In conclusion, we examined concurrent effects of nitrogen and phosphorus supply on carbon allocation patterns of *Pinus radiata* clones. The study revealed that N and P deficiencies similarly decreased GPP and NPP, but N deficiencies had a stronger effect on the TBCA:GPP, ANPP:GPP and NPP:GPP than P deficiencies, and that greater allocation above-ground explained at least partially greater growth performance in faster-growing genotypes.

CHAPTER SIX

CARBON ALLOCATION PATTERNS IN CONTROL AND FERTILIZED MINI-PLOTS OF *PINUS RADIATA*: A PILOT STUDY AT FIVE SITES IN THE SOUTH ISLAND OF NEW ZEALAND

Summary Patterns of carbon allocation were examined in highly stocked (40 000 stems ha^{-1}) control and fertilized mini-plots of *Pinus radiata* in five sites covering a wide climatic and edaphic gradient in the South Island of New Zealand. Gross-primary productivity (GPP) and the partitioning of GPP to above- and belowground production and respiration were calculated using a carbon budget approach (Giardina *et al*. 2003). All components of GPP: above-ground net primary productivity, ANPP (r = 0.82, P <0.01); above-ground plant respiration, APR (r = 0.81, P < 0.01) and total below-ground carbon allocation, TBCA (r = 0.64, P < 0.01) scaled positively and significantly with GPP, but the ANPP:GPP, APR:GPP and TBCA:GPP ratios were not significantly correlated with GPP (P > 0.41). The TBCA:GPP ratio significantly increased with the soil C:N ratio (r^2 = 0.93, P < 0.01) with a concomitant decrease in the APR:GPP ratio (r^2 = -0.88, P < 0.001) without a significant effect on the ANPP:GPP ratio (P > 0.32). None of these fractions were correlated to soil total or extractable phosphorus (P > 0.32). There was a strong correlation between the soil C:N ratio and the ratio of exchangeable ammonium to nitrate measured using ion exchange membranes and soil extracts in the field. Because the soil C:N ratio was strongly correlated to carbon assimilation and partitioning, and also correlated with the ratio of inorganic nitrogen fractions, it may provide a potential predictor of site quality in ecophysiological models. Although periodic and intensive measurements were required to determine the whole-carbon budget, by necessity the number of sites was limited and therefore our results would require further confirmation using whole-carbon budgets from a wider range of soil and environmental conditions.

Keywords: gross- primary productivity, net-primary productivity, Pinus radiata, total below ground carbon allocation, nitrogen, phosphorus, whole-carbon budget

INTRODUCTION

Pinus radiata is the most widely planted forest species in the southern hemisphere (Lewis and Ferguson 1993), and represents 89% of the 1.8 million ha of plantation forests in New Zealand. Its predominance is attributable to higher productivity, greater adaptability to soil and environmental conditions, better response to tree breeding and silviculture, and a wider end-use range than most other plantation species (Turner and Lambert 1986, Cown 1997). Such outstanding characteristics contribute to the success of the New Zealand forest industry that provides 1.1% of world timber production from only 0.05% of the global forest cover (N.Z.F.O.A. 2007). Additionally plantation forestry is considered to be critical to meet New Zealand environmental commitments and particularly obligations related to the Kyoto protocol (M.F.E. 2007).

Pinus radiata plantations have commonly been reported to be limited by nutrients in New Zealand (Will 1978, 1985). Major nutrient deficiencies include nitrogen, phosphorus, magnesium and boron, although localized deficiencies of potassium, manganese, copper, zinc and molybdenum have also been recorded (Will 1985, MacLaren 1993, Hunter *et al*. 1991, Mead 2005*b*). Among these nutrients, nitrogen and phosphorus are the strongest soil chemistry determinants of productivity of *Pinus radiata* in New Zealand (Watt *et al*. 2005).

Fertilization has been routinely used to ameliorate nutrient deficiencies of *Pinus radiata* since the 1950s. As plantation forestry was historically often relegated to land with low agricultural potential (Boomsma and Hunter 1990, Hunter and Smith 1996), fertilization has been an effective management tool permitting the New Zealand forestry sector to produce fast-growing radiata pine plantations in nutrient deficient areas (Mead and Gadgil 1978, Mead 2005*a*). There are a large number of empirical studies reporting growth responses to fertilization, recommended products, doses, and environmental risks associated with the use of fertilizers. However mechanisms by which plant and soil nutrition influence growth are not well enough understood to provide a good framework for modelling (Landsberg and Gower 1997).

Tree productivity hybrid models integrate fundamental physiological processes to predict canopy photosynthesis and carbon allocation to plant and soil processes (Landsberg and Gower 1997, Johnsen *et al*. 2001, Landsberg 2003). These models play a major role in assessing global environmental change, carbon balance, primary production, and water and nutrient use-efficiency (Jarvis 1995). Although models such as 3-PG, Promod and CABALA (Landsberg 2003, Battaglia and Sands 1997, Battaglia *et al*. 2004) have enhanced our

understanding in these areas, plant and soil nutrition is still poorly represented in physiological and hybrid models. The development and application of these models has highlighted new directions for nutritional research. Areas of greatest uncertainty include carbon allocation processes, nutrient availability in soils and nutrient uptake and cycling within the plant (Raison and Myers 1992, Waring *et al*. 1998, Johnsen *et al*. 2001, Landsberg 2003).

Whole carbon budgets are a relatively recent development, which have enabled the accurate determination of carbon allocation. This methodology has been defined based on a carbon balance protocol (Raich and Nadelhoffer, 1989), and methods for scaling plant respiration (Ryan1991*a*, 1991*b*, Ryan *et al.* 1996) and carbon allocation to roots (Giardina and Ryan 2002, Giardina *et al.* 2003). Using these methods gross primary production (GPP), defined as net photosynthesis summed over an annual time step, can be estimated as the annual sum of C allocated to dry matter production and respiration:

$$GPP = ANPP + APR + TBCA \qquad (6.1)$$

where ANPP is above-ground net primary production, APR is above-ground plant respiration and TBCA is total below-ground carbon allocation. The carbon balance approach has been used in a so far reduced range of forest ecosystems, and mostly within limited geographic areas, and studies looking at the effects of fertilization and soil chemical properties on whole-carbon budgets over wider geographic areas in pines have apparently not been conducted.

Using five experimental sites covering a wide climatic and edaphic gradient, and a within site fertilisation treatment, the aims of this study were to (i) quantify the influence of fertilisation and water balance on carbon allocation both within and across the site gradient and (ii) identify standard soil chemical properties (soil C, N, C:N, Bray-2 P, Olsen P, pH, CEC, among others) which are most strongly correlated with carbon allocation. Using pre-existing highly stocked three-year old mini-plots of *Pinus radiata*, we examined for the fourth year after planting how absolute C fluxes were allocated to ANPP, APR and TBCA, and the relative partitioning of GPP to ANPP, APR and TBCA. The hypotheses for the study were that, (1) The fraction of GPP allocated belowground would increase with nutrient deficiencies at the expense of both ANPP and APR, (2) carbon allocation would be relatively insensitive to water availability, (3) Carbon use efficiency (NPP:GPP) would be highly conservative and not influenced by nutrient deficiencies and water availability and (4) standard soil chemical property measurements would be correlated with observed patterns of carbon allocation. This

chapter provides a field component that complements results found in Chapter *Five*, and expands on current understanding of carbon allocation as influenced by nutrient and water availability.

MATERIALS AND METHODS

Sites Description

The study was conducted at five locations across an environmental gradient which form part of a national trial series (Figure 6.1). At each location a series of eight plots was installed during 2001 using a factorial design with the following three factors: species (*P. radiata* and *Cupressus lusitanica* Mill.), fertiliser (no fertiliser and nutrients supplied in excess of crop requirements) and disturbance (low and high disturbance). In this chapter measurements and modelling were restricted to only the *Pinus radiata* subplots. Samples were taken from fertilised and unfertilised subplots in the undisturbed treatments at sites 1, 2 and 3. At site 4 measurements were taken in the disturbed plots as the undisturbed plots exhibited a permanent white mottling of the foliage. At site 5, the disturbed fertilized plot was chosen as mortality prevented the use of the undisturbed fertilized plot. Despite that selecting plots other than the undisturbed might have introduced a confounding effect in the analysis; disturbance was showed to be insignificant for the whole trial (Watt *et al*. 2005).

Each plot was small in size (3 x 3 m) and contained nine measurement trees spaced at 0.5 m x 0.5 m (40 000 trees ha^{-1}) surrounded by a two-row buffer. Regular applications of herbicide were made to ensure weed-free conditions. All sites were planted with one-year-old *Pinus radiata* seedlings with a growth and form factor of 19 (Vincent and Dunstan 1989), sourced from the Scion nursery in Rotorua. All sites were planted in the winter of 2001 and harvested at the end of winter of 2005. It was hypothesized that these highly stocked plots, even at young age, would cause high demand on nutrient resources permitting the development of relationships between soil properties and tree productivity and nutrition (Watt *et al*. 2005, Davis *et al*. 2007).

In fertilised plots a base dressing was applied at the time of planting in doses of 18, 6, 16.8, 4.8, 1.2 and 4.8 kg ha^{-1} of total elemental N, P, K, S, Mg and Ca, respectively. Fertilization (Hydrogreen; 14% N, 5% P, 15% K, 1% S, 1.2% Mg) was applied in prescribed plots in the spring of every year from 2002 to 2004 at 612 g per plot (9 m^2) equivalent to 95, 34, 102, 7 and 8 kg ha^{-1} of elemental N, P, K, S and Mg, respectively. Nitrogen in fertilizer

was provided as 44% NO_3^- -N and 56% NH_4^+ -N. Fertilization was not applied in 2005 as plots were harvested at the end of the winter 2005.

A comprehensive set of soil physical and chemical properties was taken within each plot following the methods fully described in Watt *et al*. (2005). Measurements of air temperature and relative humidity were taken from sensors installed on a 3 m tower located adjacent to the experimental plots. A tipping bucket rain gauge positioned on top of the tower was used to measure above-canopy rainfall. Soil temperature was measured in each plot by sensors installed to a depth of 0-10 cm. Estimates of solar radiation were provided by the National Institute of Water and Atmospheric Research (NIWA).

Figure 6.1. Location of experimental sites within the South Island of New Zealand. Sites are Rai Valley (41° 12.637 S, 173° 28.006 E) (1), Golden Downs (41° 30.451' S, 172° 53.962' E) (2), Tekapo (44° 2.226' S, 170° 25.565' E) (3), Longwoods (46° 9.857' S, 167° 57.053' E) (4) and Catlins (46° 24.495' S, 169° 28.014' E) (5).

During the fourth year after planting, monthly measurements of volumetric water content, θ, were made to a depth of 30 cm at all study sites. Average values of volumetric water content were used as an index of water availability. During the same period, soil nitrogen and phosphorus availability was measured at monthly intervals using ion exchange membranes and soil extracts. Cation and anion exchange membranes (BDH Laboratory Supplies, Product No 55164-65, Poole England) of 2 × 6 cm dimension, were fixed separately within two 4 × 10 cm plastic frames with a central 7.5 cm^2 slot, exposing membranes to both sides (15 cm^2). Four cation and four anion exchange membranes were buried randomly within each plot at 10-20 cm depth and removed in the following visit (inserting a new set in different places). Collected membranes were washed in the field with distilled water and a soft brush, and placed in plastic containers with 50 ml 2M KCl. Four soil samples 0-30 cm depth were randomly cored from each plot, placing 10 g of fresh soil from each sample in 50 ml 2M KCl (Bremner 1965). Exchange membranes and soil extracts were shaken for 2 hours to eludate ions from the resins / soil to the KCl solution. Then, resins were carefully removed from solution and stored in distilled water, while soil extracts were left to settle for half an hour before they were filtered using Whatman No 1 filter paper. Exchange membranes and soil extracts were bulked monthly per plot and analyzed for ammonium, nitrate and phosphates using a Segmented Flow Analyser (SKALAR Analytical BV, Breda, The Netherlands) in Veritec Laboratories, Rotorua, New Zealand.

Carbon balance method

Values of GPP for each plot were determined by measuring or estimating ANPP, APR and TBCA based on Eqn. 6.1. Above-ground net primary productivity (ANPP) was estimated as,

$$\text{ANPP} = F_A + F_W + \Delta C_C + \Delta C_w \qquad (6.2)$$

where F_A is the carbon content of above-ground litterfall, F_W is the C content associated with tree mortality, ΔC_C is the change in C content of live foliage, and ΔCw is C content change in live branches, bark and wood, over a given period of time (Giardina *et al*. 2003). These fluxes can be partitioned as leaf NPP (ΔC_C plus foliage component of F_A) and stems and branch NPP (F_W plus ΔC_W plus the branch and twigs component of F_A).

Above-ground plant respiration, APR, can be estimated as a sum of foliage construction (L_{RC}), foliage maintenance respiration (L_{RM}) and wood construction and maintenance respiration (W_R) from,

$$\text{APR} = L_{RC} + L_{RM} + W_R \qquad (6.3)$$

Total C allocated belowground for root and mycorrhizae construction and maintenance respiration, and C released through root exudates and root turnover (TBCA), was estimated using a carbon mass balance approach (Raich and Nadelhoffer 1989, Giardina and Ryan 2002). The method is based on the idea that all C allocated belowground must be either respired, leached or stored,

$$\text{TBCA} = F_S + F_E - F_A + \Delta C_S + \Delta C_R + \Delta C_L \qquad (6.4)$$

where F_S is the soil respiration C efflux, F_E is the C flux off the system by leaching or erosion, ΔC_S is change in C content in the mineral soil, ΔC_R is the change in C content of root biomass, and ΔC_L is the change in C content in the litter layer.

Above-ground net primary productivity

Above-ground net primary production, which is leaf and wood production plus litterfall, was determined for each of the 10 plots of *Pinus radiata* for the fourth-year of growth ending August 2005. Tree ground-line diameter was measured monthly and height every three months in all 7-9 trees within each plot. Above- and below-ground plant dry mass components in August 2004 were estimated based on allometric equations derived from these tree dimensions (methods described below) at harvest (Table 6.1), while actual values were used for August 2005. Foliage, stem, branch and root mass were calculated from predicted total tree mass in August 2004 (Table 6.1) and actual biomass component proportions from each tree in August 2005. Tissue carbon concentrations determined for each plot were used to transform dry mass to carbon content. The relationship between carbon content (y, g) and biomass (x, g) at the plot level was almost exact ($y = 0.512\ x$, $r^2 = 0.99$, $P < 0.001$).

Litterfall was collected monthly from four plastic containers (dimension 42 × 33 cm, 40 cm depth, 0.139 m^2 horizontal area) within each plot from August 2004 to August 2005.

Samples were oven-dried at 70 ºC and the dry mass recorded. The C content was assumed to be 50% of litterfall (F_A).

Above-ground plant respiration

Above-ground plant respiration (APR) was calculated as the sum of foliage and wood maintenance and construction respiration. Foliage (L_{RC}) and wood (W_R) construction respiration were assumed to be 25% of foliage and wood net-primary productivity respectively (Penning de Vries 1972, 1975, Ryan 1991*a*, 1991*b*). The main component of APR, foliage maintenance respiration (L_{RM}), was estimated as CO_2 efflux at night scaled over the whole growing season using a Q_{10} equal to two, hourly monthly air temperature averages, and interpolated values of the leaf area index (Ryan 1991*a*). Hourly monthly air temperature averages were estimated using monthly daily mean, minimum and maximum temperatures and the following sinusoidal approximation described by Landsberg *et al*. (1986),

$$T(t) = \overline{T} + \left(\frac{T_{max} - T_{min}}{2} \right) \cos \left(\frac{2\,\pi\,(t - t_{max})}{24} \right) \qquad (6.6)$$

where $\overline{T}$ is the average daily temperature, t is the time in hours and t_{max} is the time when $T(t) = T_{max}$. This relationship is often used to scale plant respiration in situations where T_{max} and T_{min} are known (Landsberg 1986).

The rate of respiration at night (R_d) was measured on 7-9 trees on all sites except Golden Downs between 9 pm to 1 am, in late autumn, from 25 April to 7 May 2005. Values of R_d were measured using a portable photosynthesis system (Model 6400, Li-Cor, Lincoln, NE). For each plant, six fascicles were placed inside a 24 cm^2 conifer chamber (Model 640-05, Li-Cor, Lincoln, NE) avoiding shading between needles. Temperature in the cuvette was maintained at 20 ºC while leaf-to-air vapour pressure deficit (D) was always lower than 1 kPa. External CO_2 concentration (C_a) was maintained at 360 μmol mol^{-1} using a CO_2 mixer. Foliage samples were left to stabilize for at least 10 minutes until values of A, C_i and g_s were stable (coefficient of variation ≤ 2%), and then values recorded every 20 seconds for 2 minutes. Total surface area of needles was determined based on fascicle diameters and lengths as described by Turnbull *et al*. (1998). Actual R_d values were similar to those estimated using equations developed in Chapter *Five* (Figure 5.5) and foliage nitrogen concentrations at

harvest. The latter approach was used for all sites as R_d for Golden Downs was not measured (Appendix D).

To scale dark respiration over the growing season total leaf area estimates are required. The leaf area index (LAI, m^2 m^{-2}) was determined as the product of leaf mass and the leaf area to mass ratio (M). Foliage mass in August 2004 was estimated using allometric equations derived in August 2005, while actual values were used in August 2005. Values of M were measured in five samples of five-fascicles each from one-year old and current-year foliage in each plot in August 2005. Monthly values of leaf area were interpolated between August 2004 and August 2005 based on non-destructive measurements of leaf phenology (Appendix D).

Total below-ground carbon allocation

Soil surface CO_2 efflux and mineral soil temperature were measured in all ten plots using a soil respiration chamber (100 mm inner diameter, Model SRC-1, PP Systems, Herts, UK) connected to an infrared gas analyzer (Model EGM-4, PP Systems, Herts, UK) at monthly intervals from August 2004 to August 2005. Ten soil collars made out of polyvinyl chloride (100 mm inner diameter and 50 mm length) were placed in each of 10 plots at the start of the experiment in August 2004. Heterotrophic respiration was estimated based on 3-5 deep polyvinyl chloride collars (100 mm inner diameter and 300 mm length) per plot, which were installed by progressively coring and removing the soil in three 10-cm steps, inserting the deep collar, visually scanning and removing roots from all 10-cm soil horizons, and finally by restoring carefully the soil profile inside the deep collar. Measurements were usually carried out between 10 am and 4 pm. Soil respiration was scaled between successive measurements based on the soil temperature record and site specific soil respiration responses to temperature (Q_{10} = 1.54 – 3.71) derived for each plot (Appendix D).

Carbon lost by leaching (F_E) and changes in soil carbon content (ΔC_S) for the year ending August 2005 were assumed to be nil. Values of ΔC_R were determined as the difference between initial and final root mass multiplied by root carbon concentrations determined for each plot. To determine forest floor carbon (C_L), the organic horizon down to mineral soil was removed from seven 30 × 30 cm frames within each plot in August 2004 and August 2005, samples were oven-dried at 70 ºC and dry mass recorded, and organic matter content determined by loss on ignition. The % C value used to convert organic matter to C was 58%.

Tree harvesting and biomass prediction

Control and fertilized plots of *Pinus radiata* in all five sites were harvested between 15 August and 9 September 2005. Measurement trees within each plot (7-9 depending on mortality) were placed on a tarpaulin and ground line diameter and total height were recorded. Above-ground plant components of each tree were separated by age class (current, older, or dead).

Roots systems were extracted by cutting a cube of soil around each tree about 30 cm deep using a flat spade. The sides of the cube were cut half way between neighbouring trees. The root system and soil were then placed onto a clean tarpaulin. Root systems were shaken to remove soil and placed in a plastic bag. Soil on the tarpaulin was scanned thoroughly for visible roots which were collected and placed inside the plastic bag with the major root system. The soil on the tarpaulin was thoroughly mixed and an approximate 5 kg sample was taken to account for roots unseen but mixed in the soil. The remaining soil was weighed with precision to 50 g, and returned to the excavation site. Five-kilogram soil samples were sealed in plastic bags and frozen at -20°C, until they were washed and remaining roots recovered by flotation. Roots recovered in the sub-sample were extrapolated to the whole soil mass for each tree. Root systems were washed and separated into fine roots (< 2mm), coarse roots (>2mm), and below-ground stem. All biomass samples were oven-dried at 70 °C and dry mass recorded.

Biomass equations were developed by regressing total plant mass (y) against tree ground-line diameter (x) as,

$$\log_e y = b_0 + b_1 \log_e x \qquad (6.7)$$

Slopes ($F_{1,81} = 1.96$, $P = 0.16$) and intercepts ($F_{1,81} = 3.26$, $P = 0.07$) of these log-linear relationships were not influenced by plot ($y = 0.408\ x^{2.168}$, $r^2 = 0.98$, $P < 0.001$). However, individual allometric equations fitted for each plot were preferred (Table 6.1), as the general model appeared to be slightly biased particularly for the control and fertilized plot at Rai Valley.

Table 6.1. Biomass equations ($y = b_0 x^{b1}$) used to predict plant mass (y, g) against tree ground-line diameter (x, mm). Level of significance for regressions for all plots was $P < 0.001$.

	Control			Fertilized		
	b_0	b_1	r^2	b_0	b_1	r^2
Rai Valley	0.487	2.157	0.99	0.380	2.216	0.99
Golden Downs	0.254	2.309	0.99	0.707	2.035	0.99
Tekapo	0.569	2.064	0.99	1.325	1.860	0.98
Catlins	1.038	1.906	0.98	1.944	1.771	0.99
Longwoods	0.391	2.127	0.99	3.343	1.599	0.99

Tissue samples from 5 trees per plot covering the diameter distribution were ground using a Thomas-Wiley Mill to pass a 1 mm sieve. Samples were bulked per plot proportional to tree biomass and analyzed for macro- and micro-nutrient concentrations in Veritec Laboratories, Rotorua, New Zealand. C and N were measured by LECO CNS-2000 (modified Dumas), while Al, B, Ca, Cu, Fe, K, Mg, Mn, Na, P, Zn were measured by HNO_3 : H_2O_2 digestion and ICP-OES.

Data analysis

All analyses were undertaken using SAS (SAS-Institute 1996). Variables were tested for normality and homogeneity of variance and transformations were made as necessary to meet the underlying statistical assumptions of the models used. A two-way analysis of variance (ANOVA) was carried out to test for the main effects of site and fertilisation on carbon allocation and soil chemical characteristics. Pearson correlations were used to explore significant relationships between soil chemical properties and carbon allocation variables. Analysis of covariance was used to test whether slopes and intercepts were significantly different in the relationship between GPP and NPP.

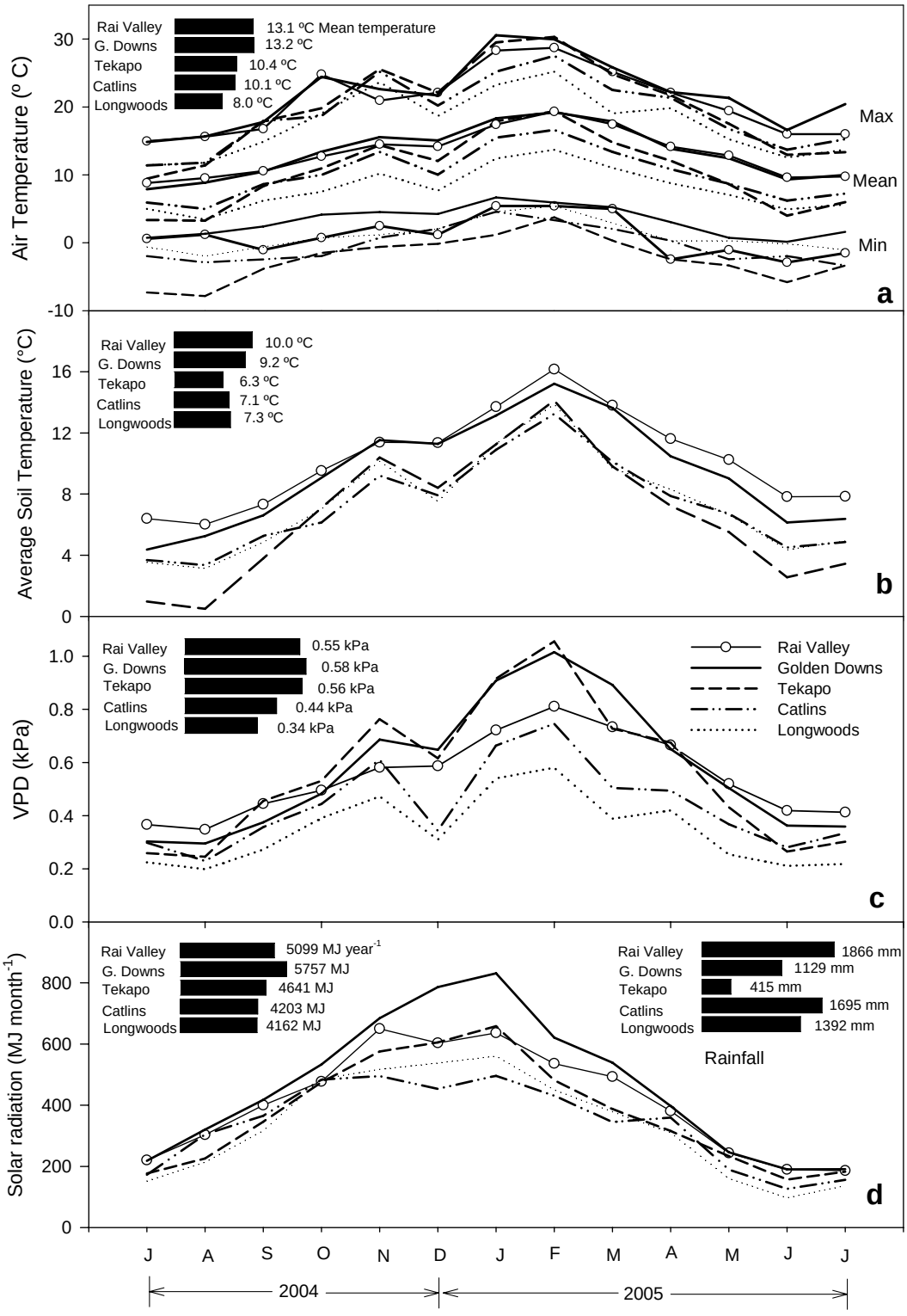


Figure 6.2. Several climatic characteristics in five sites in the South Island of New Zealand for the year ending July 2005: **a** minimum, mean and maximum monthly temperatures, **b** average monthly soil temperatures, **c** mean monthly vapour pressure deficit and **d** solar radiation. Bar graphs inserts correspond to averages or accumulated values for the year ending July 2005.

RESULTS

Climatic and edaphic variation across sites

The climate at sites selected for the study exhibited large differences. The mean annual temperature varied 1.7-fold from about 8-10 °C in Southland (sites 4 and 5) to about 13 °C in the Nelson region (sites 1 and 2). Mean temperatures in Tekapo (site 3) were intermediate compared to other sites (10.4 °C), but this site exhibited the lowest monthly minimums among sites while monthly maximums were similar to those of Golden Downs (2) and Rai Valley (1). Across all sites, average soil temperatures were 1-4 °C lower than the average air temperatures. Rainfall ranged four-fold from 415 mm at Tekapo to 1866 mm at Rai Valley (Figure 6.2).

Selected sites exhibited large differences in soil physical (Table 6.2) and chemical properties (Table 6.3). All soils were Brown except for Longwoods where the soil was Allophanic. Greatest differences in physical properties were found in textural classes, particularly the sand fraction (ranging 7-fold) and the clay fraction (ranging 3-fold) and also in bulk density (ranging more than two-fold).

Table 6.2. Variation in soil physical properties across sites. Data provided by Landcare Research and ENSIS. Specific site values are presented in Appendix D.

Soil physical properties	Mean	Range
Coarse sand (%)	4	1 - 16
Medium sand (%)	4	1 - 8
Fine sand (%)	13	2 - 25
Sand (%)	22	5 – 34
Silt (%)	45	34 – 62
Clay (%)	34	15 – 49
Bulk density (g cm^{-3})	0.86	0.47 - 1.15
Particle density (g cm^{-3})	2.49	2.22 - 2.68
Penetration resistance (Mpa)	0.83	0.68 - 1.23
Total porosity (%, v/v)	66	55 – 79
Macroporosity (%)	19	14 – 24

Soil chemical properties were strongly influenced by site and to lesser extent by fertilization (Table 6.3). Significant variation at the site level was found for all standard soil chemical variables ($r^2 > 0.51$) except exchangeable K and Na and base saturation. Variation across sites was considerable for exchangeable Na (16-fold), exchangeable Ca (7 fold), Olsen

and Bray P (5-8 fold), carbon (6-fold), nitrogen (4-fold), exchangeable Mg (4-fold), CEC (3-fold) and the C:N ratio (2-fold). The main effect of fertilization on these variables was relatively minor compared to the main effect of site as evidenced by partial r^2 (Table 6.3). Fertilization significantly increased exchangeable K (1.9 fold), Olsen P (2.7 fold), Bray P (3.3 fold), Inorganic P (1.9 fold) and Total P (1.2 fold) and slightly decreased pH (2%). Specific plot values for physical and chemical properties are presented in Appendix D.

Table 6.3. Variation in soil chemical properties and volumetric water contents in control and fertilized mini-plots of *Pinus radiata* in five sites in the South Island of New Zealand. Standard chemical properties (0-100 mm) were measured from soil samples taken at harvest (August-September 2005) by Landcare Research and ENSIS. Resin and soil extract exchangeable N and P and θ were measured at monthly intervals during the fourth year after planting. Exchangeable N and P using soil extracts (10 g fresh soil in 50 ml 2M KCl) are expressed as grams per square meter to a soil depth of 30 cm. Significance of main effects of site and fertilization treatments are presented as partial r^2 and *P*-range: ns, non significant at $P \geq 0.05$; *, significant at $P < 0.05$; **, significant at $P < 0.01$; ***, significant at $P < 0.001$. Specific plot values are presented in Appendix D.

	Control		Fertilized		Analysis of variance	
	mean	range	mean	range	Site	Fert.
Standard Chemical properties						
Carbon (%)	9.6	4 - 27	9.2	4 - 24	0.99***	ns
Total N (%)	0.39	0.23 - 0.85	0.38	0.23 - 0.8	0.99***	ns
C:N	22	14 - 31	22	15 - 30	0.97***	ns
pH	4.6	4.2 - 5.1	4.5	4 - 5.1	0.91**	0.06*
CEC (cmol kg^{-1})	23	17 - 43	24	17 - 46	0.99***	ns
Exch. Ca (cmol kg^{-1})	4.6	1 - 9.3	3.3	1.2 - 6.6	0.89**	ns
Exch. Mg (cmol kg^{-1})	2.0	0.9 - 3.9	1.9	1 - 4.1	0.97**	ns
Exch. K (cmol kg^{-1})	0.51	0.32 - 0.75	0.95	0.73 - 1.22	ns	0.66*
Exch. Na (cmol kg^{-1})	0.19	0 - 0.31	0.32	0.06 - 0.68	ns	ns
Sum bases (cmol kg^{-1})	7.3	2 - 14	6.5	3 - 13	0.96**	ns
Base saturation (%)	31	14 - 46	27	19 - 37	ns	ns
Olsen P (μg g^{-1})	11	3 - 31	30	10 - 65	0.67*	0.27*
Bray P (μg g^{-1})	21	4 - 49	69	28 - 109	0.51*	0.44**
Inorganic P (μg g^{-1})	120	42 - 299	230	148 - 409	0.73**	0.25**
Organic P (μg g^{-1})	479	280 - 601	478	273 - 657	0.98**	ns
Total P (μg g^{-1})	599	322 - 898	708	435 - 1065	0.92**	0.06*
Exchangeable N and P (ion exchange membranes)						
NO_3^--N (μg $cm^{-2}month^{-1}$)	2.0	1 - 2.9	12.5	4.9 - 18.3	ns	0.70*
NH_4^+-N (μg $cm^{-2}month^{-1}$)	2.1	1.6 - 3.1	3.4	1.5 - 5.9	ns	ns
NH_4^+-N / NO_3^--N	1.1	0.6 - 1.5	0.3	0.2 - 0.3	ns	0.75**
Total N (μg $cm^{-2}month^{-1}$)	4.1	2.6 - 5.5	15.9	6.4 - 23.1	ns	0.62*
Elem. P (μg $cm^{-2}month^{-1}$)	0.6	0.2 - 2.1	0.7	0.3 - 2.2	0.98***	ns
Exchangeable N and P (soil extracts, 2M KCl)						
NO_3^--N (g m^{-2})	1.0	0.6 - 1.5	1.3	1 - 1.9	0.81**	0.14*
NH_4^+-N (g m^{-2})	3.8	3.3 - 4	4.1	3.3 - 4.7	ns	ns
NH_4^+-N / NO_3^--N	4.1	2.3 - 5.8	3.3	1.7 - 4.1	0.79*	ns
Total N (g m^{-2})	4.8	3.8 - 5.5	5.5	4.6 - 6.2	ns	ns
Elem. P (g m^{-2})	0.1	0.1 - 0.2	0.1	0.1 - 0.2	0.97**	ns
Water availability						
θ (m^3 m^{-3})	0.22	0.14 - 0.26	0.21	0.13 - 0.25	0.98***	ns
Rooting depth (mm)	483	330 - 778	429	386 - 462	ns	ns
Water balance (mm)	105	74 - 183	91	61 - 116	ns	ns

In contrast to standard soil chemical properties, fertilization drastically changed soil exchangeable N as measured by ion exchange membranes and soil extracts (Table 6.3). The main effect of fertilization was to substantially increase the amount of available NO_3^--N with only small and not uncommonly negative effects on NH_4^+-N availability. As a consequence, the NH_4^+-N : NO_3^--N ratio consistently decreased with fertilization except for Tekapo, where fertilization did not have a major effect. Total available N measured by ion exchange membranes increased almost six-fold with fertilization, whereas total N measured by soil extracts increased only slightly with fertilization. Seasonal patterns of soil exchangeable N and P are presented and discussed in Appendix D.

The soil C: N ratio was positively correlated to the NH_4^+-N : NO_3^--N ratio in control plots, and fertilization significantly decreased the NH_4^+-N : NO_3^--N ratio by substantially increasing available NO_3^--N with only minor effects on available NH_4^+-N. These findings were consistent using two independent techniques (ion exchange membranes and soil extracts) as shown in Figure 6.3.

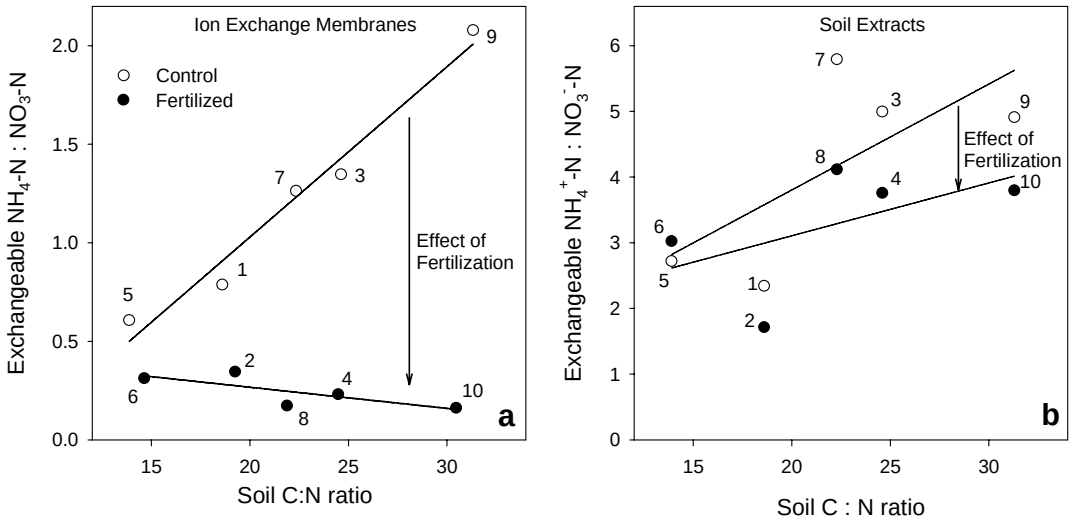


Figure 6.3. The relationship between the soil C:N ratio and soil exchangeable NH_4^+-N to NO_3^--N ratio as measured by (a) ion exchange membranes and (b) soil extracts in control and fertilized plots of *Pinus radiata* in five sites in the South island of New Zealand. Plot numbers are indicated besides each symbol: 1-2 Rai Valley, 3-4 Golden Downs, 5-6 Tekapo, 7-8 Catlins, 9-10 Longwoods. Values of the soil C:N ratio were provided by Landcare and ENSIS. For ion exchange membranes (a): y = -0.6998 + 0.0864 C:N, r^2 = 0.97, P = 0.002 (control); y = 0.4816 – 0.0107 C:N, r^2 = 0.60, P = 0.13 (fertilized). For soil extracts (b) : y = 0.5789 + 0.1612 C:N, r^2 = 0.48, P = 0.20 (control); y = 1.4982 – 0.0804 C:N, r^2 = 0.30, P = 0.34 (fertilized).

Average volumetric water content (θ) was significantly influenced by site but not fertilization treatment (Table 6.3). Values of θ were significantly lower in Tekapo (0.13)

compared to other sites (0.22-0.26). Seasonal fluctuations in volumetric water content are presented in Figure 6.4.

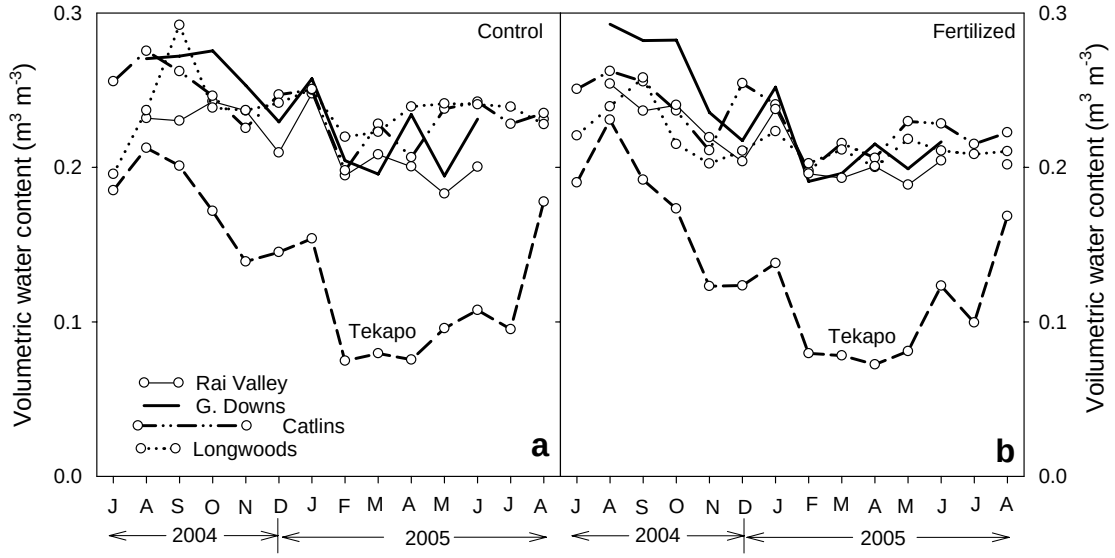


Figure 6.4. Seasonal fluctuations in volumetric water content in control (a) and fertilized (b) plots of *Pinus radiata* at five sites in the South Island of New Zealand.

Carbon assimilation and allocation across sites

Major fractions of gross primary productivity (GPP) are presented in Table 6.4. Gross (GPP) and net C fluxes (NPP, NEP) were not significantly influenced by site or fertilization (Table 6.4). Although not significant, the main effects of site (1.7-2.1) were far greater than the main effects of fertilization (1.1-1.2 fold) on these C fluxes. Carbon use efficiency (NPP:GPP) was not significantly different between sites and fertilization treatments, but it is worth noting that fertilization slightly increased this value from 0.53 to 0.56. The non-significance of fertilization on NPP:GPP was also confirmed by analysis of covariance. The relationship between NPP and GPP was highly significant (NPP = 0.54 GPP, $r^2 = 0.71$, $P < 0.001$) and slopes ($F_{1,6} = 0.29$, $P = 0.61$) and intercepts ($F_{1,6} = 0.35$, $P = 0.57$) of these linear relationships were not influenced by fertilization treatment (Figure 6.5). Hence, carbon-use efficiency (the slope of the NPP : GPP relationship) was not significantly influenced by nutrition. Neither site nor fertilization significantly affected the NEP:GPP fraction (0.35).

Table 6.4. Major fractions of gross primary productivity (GPP) in control and fertilized plots of *Pinus radiata* at five sites in the South Island of New Zealand. Major GPP fractions are: above-ground net primary productivity (ANPP), above-ground plant respiration (APR) and total belowground carbon allocation (TBCA). Main effects of sites (S) and fertilization treatments (F) were assessed by analysis of variance. Significant differences are presented as partial r^2 and significance level: ns, non significant; *, significant at $P < 0.05$; **, significant at $P < 0.01$; ***, significant at $P < 0.001$. Values are presented as means and range with $n = 5$. Components of ANPP are: F_A, C content of above-ground litterfall; ΔC_C, C content change of live foliage; ΔCw, C content change in live branches, bark and wood. Components of APR are: L_{RC}, foliage construction respiration; L_{RM}, foliage maintenance respiration; W_R, wood construction and maintenance respiration. Components of TBCA are: F_S, soil respiration C efflux; ΔC_R, C content change of root biomass; ΔC_L, C content change in the litter layer. Values of F_S are the sum of autotrophic (R_a) and heterotrophic (R_h) respiration.

Carbon Allocation	Control		Fertilized		Anova	
(g C m^{-2} $year^{-1}$)	mean	range	mean	Range	Site	Fert.
ANPP	**1101**	**723 - 1430**	**1169**	**747 - 1281**	ns	ns
Litterfall, F_A	113	63 - 178	173	141 - 214	ns	0.47*
Live foliage C change, ΔC_C	283	235 - 385	260	211 - 337	ns	ns
Wood C change, ΔCw	704	393 - 935	735	396 - 883	ns	ns
APR	**1488**	**725 - 2532**	**1698**	**1005 - 2249**	0.88*	ns
Fol. Const. Resp., L_{RC}	98	80 - 123	107	87 - 126	ns	ns
Fol. Maint. Resp., L_{RM}	1213	545 - 2200	1405	684 - 1933	0.88*	ns
Total Wood Resp., W_R	177	100 - 234	185	100 - 222	ns	ns
TBCA	**1533**	**1074 - 1798**	**1761**	**1121 - 2283**	ns	ns
Soil C efflux, F_S	1236	654 - 1713	1309	752 - 1724	0.92*	ns
Litterfall, $-F_A$	-113	-178 - -63	-173	-214 - -141	ns	0.47*
Root C change, ΔC_R	268	193 - 350	307	200 - 428	ns	ns
Litter layer C change, ΔC_L	142	-86 - 304	319	148 - 522	ns	ns
GPP	**4122**	**3206 - 5654**	**4628**	**3402 - 5518**	ns	ns
ANPP:GPP	0.27	0.23 - 0.32	0.25	0.22 - 0.29	ns	ns
APR:GPP	0.35	0.23 - 0.45	0.37	0.23 - 0.45	0.96***	ns
TBCA:GPP	0.38	0.32 - 0.55	0.38	0.32 - 0.48	0.94**	ns
NPP	**2166**	**1650 - 2784**	**2583**	**1703 - 3174**	ns	ns
NPP:GPP	0.53	0.49 - 0.56	0.56	0.49 - 0.68	ns	ns
NEP	**1400**	**770 - 1980**	**1622**	**1120 - 1910**	ns	ns
NEP:GPP	0.34	0.24 - 0.44	0.35	0.29 - 0.41	ns	ns
Heterothrophic Resp., R_h : F_S	0.62	0.45 - 0.78	0.73	0.59 - 0.78	ns	ns

Carbon allocation patterns significantly differed between sites. At the site level, absolute values of ANPP, APR and TBCA ranged 1.8, 2.8 and 1.9 fold respectively. Only absolute values of APR were significantly influenced by site. The main effects of fertilization on absolute values of ANPP, APR and TBCA were not significant and relatively minor (1.06-1.15) compared to the main effect of site (1.8-2.8 fold). Across sites and fertilization treatments, the fractions of GPP represented by ANPP, APR and TBCA were 26%, 36% and 38%, but the APR : GPP and TBCA : GPP ratios were significantly influenced by site. Values of ANPP:GPP exhibited a narrower range (1.4-fold) than the TBCA:GPP (1.6-fold) and the APR:GPP (2-fold) fractions. Foliage maintenance respiration (L_{RM}) accounted for about 80% of APR and this component was significantly influenced by site but not fertilization treatment. Among all components of TBCA, soil respiration represented most of TBCA (74%-79%) and

was the only component of TBCA significantly influenced by site. The proportion of soil respiration (F_S) represented by heterotrophic respiration (R_h) was neither influenced by site nor fertilization, but it is worth noting that fertilization increased this proportion from 0.62 to 0.73 with a concomitant decrease in autotrophic respiration ($R_a = F_S - R_h$). Of all components of ANPP, APR and TBCA only litterfall was significantly influenced by fertilization treatment.

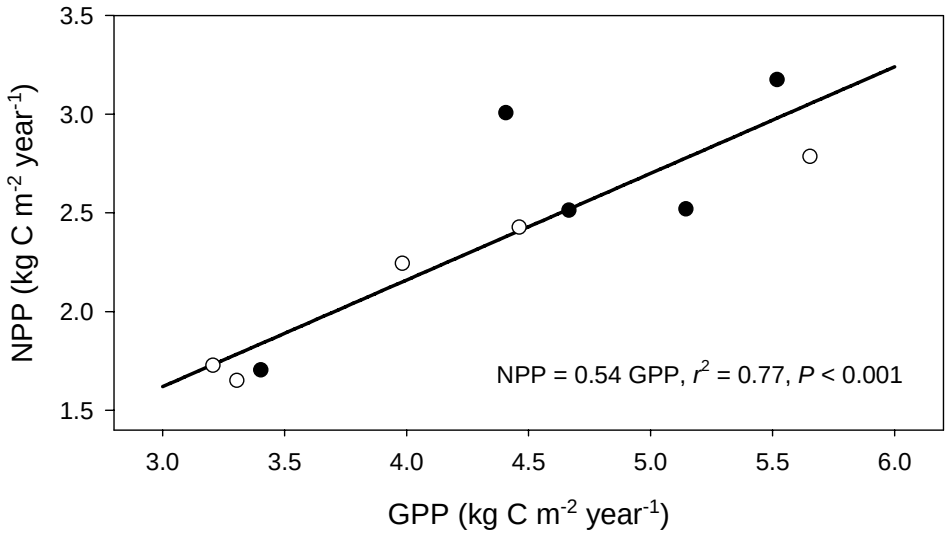


Figure 6.5. The relationship between net- (NPP) and gross-primary productivity (GPP) in control (open symbols) and fertilized (closed symbols) mini-plots of *Pinus radiata* at five sites in the South Island of New Zealand. The slope of the NPP : GPP relationship (0.54) corresponds to the average carbon use efficiency.

Potential edaphic determinants of carbon allocation

All components of GPP: above-ground net primary productivity, ANPP (Pearson r = 0.82, P <0.01); above-ground plant respiration, APR (r = 0.81, P < 0.01) and total below-ground carbon allocation, TBCA (r = 0.64, P < 0.01) scaled positively and significantly with GPP (Figure 6.6). The main component of GPP was TBCA (0.32-0.55) followed closely by APR (0.23-0.45), while ANPP represented the smallest and less variable fraction of GPP (0.23-0.29). At Tekapo and Rai Valley, where control and fertilized plots had similar productivity, carbon allocation below-ground was remarkably stable about 32 % and also very similar between the control and the fertilized plot. At Longwoods, where the control and the fertilized plots exhibited the greatest difference in productivity, the TBCA:GPP ratios were the highest among sites, and also this ratio was 7% higher in the control (0.55) compared to

the fertilized plot (0.48). This was largely explained because soil respiration, the major component of TBCA, was the highest at Longwoods compared to other sites (Appendix D).

Significant Pearson correlations among carbon budget variables and soil chemical properties for the plots under study are presented in Figure 6.6. Absolute values of GPP, ANPP, APR and TBCA were uncorrelated to soil chemical properties and volumetric water content. Also absolute values of GPP were uncorrelated to the ANPP:GPP, APR:GPP and TBCA:GPP fractions, suggesting that crop size did not confound carbon allocation among different GPP components. The fractions of GPP represented by TBCA and APR but not ANPP were well correlated with soil C, N and the C:N ratio but not with total or extractable phosphorus, exchangeable N, exchangeable P and θ.

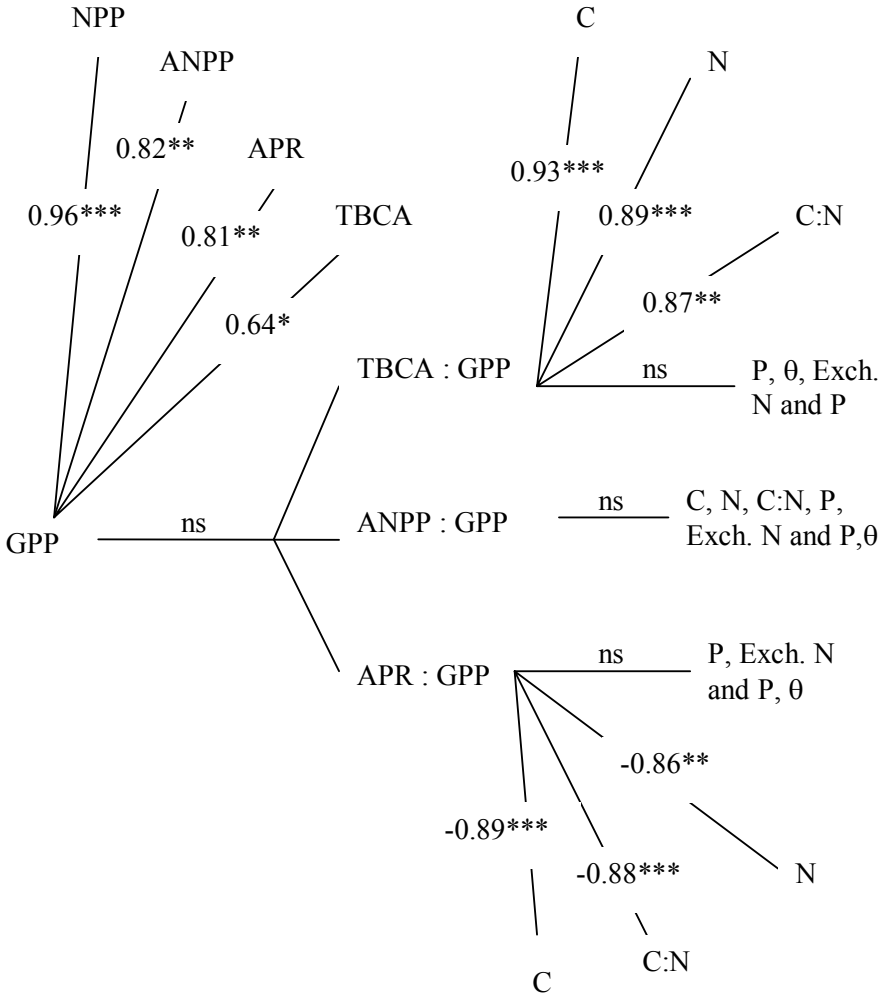


Figure 6.6. Pearson correlations among carbon budget variables and soil chemical properties (and volumetric water content) in control and fertilized mini-plots of *Pinus radiata* at five sites in the South Island of New Zealand. Carbon budget variables are gross- (GPP) and net primary productivity (NPP), above-ground NPP (ANPP), above-ground plant respiration (APR) and total below-ground carbon allocation. Soil variables are soil carbon (C), nitrogen (N), C:N ratio (C:N), total and extractable phosphorus (P) and average volumetric water content (θ). Average exchangeable N and P measured by ion exchange membranes and soil extracts were uncorrelated to GPP fractions.

Closer examination of significant correlations between GPP fractions and soil C, N and C:N ratio, revealed that the two Longwoods plots had excessive leverage on the relationships between TBCA:GPP and APR:GPP against the soil C and N (Figure 6.7; only TBCA:GPP shown). Longwoods exhibited soil C (24-27%) and N (0.80-0.85%) that were far greater than soil C (4.1-7.0 %) and N (0.23-0.34%) in other sites. When Longwoods was taken out of the dataset, values of TBCA:GPP and APR:GPP were uncorrelated to the soil C and N. In contrast, the soil C:N was considerably better distributed, and Longwoods had lower leverage on the relationships between TBCA:GPP and APR:GPP against soil C:N compared to soil C and N. Therefore the soil C:N ratio was selected as the only candidate to describe observed patterns of carbon allocation, with the additional advantage that it was well correlated with the NH_4^+-N : NO_3^--N ratio (Figure 6.3). Because the relationships that follow are based on five sites, they should be considered hypothetical explanations to the way the soil C:N ratio drives carbon allocation.

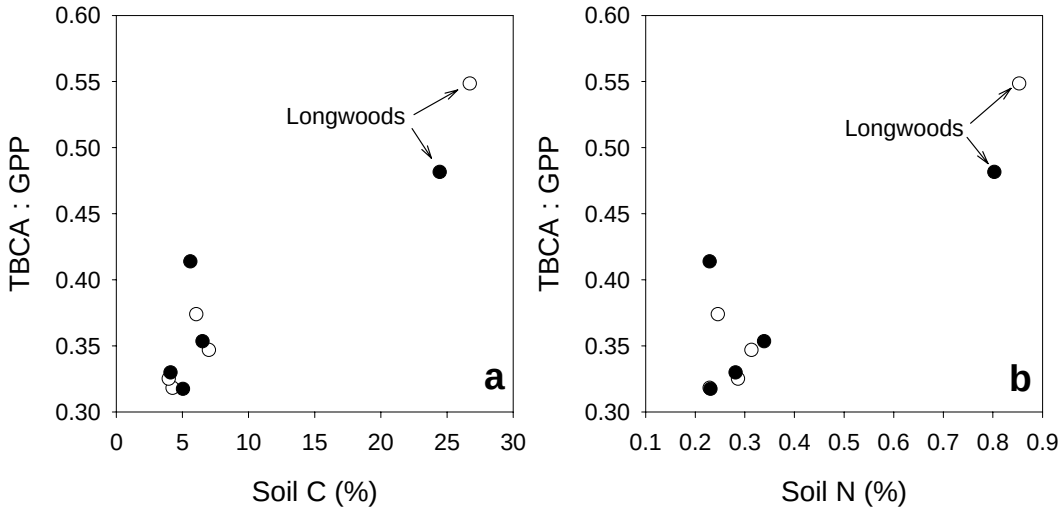


Figure 6.7. High leverage of Longwoods on the relationship between TBCA:GPP to soil C and N in control (open symbols) and fertilized (closed symbols) mini-plots of *Pinus radiata* at five sites in the South Island of New Zealand.

Further exploration on the form of the relationships between GPP fractions against the soil C : N ratio are presented in Figure 6.8. The selected model showed that carbon allocation belowground increased as the soil C : N ratio increased at the expense of above-ground plant respiration with no effect on above-ground net primary productivity. Carbon allocation to roots was about 0.32 at low C: N ratios (C : N below 17), and then progressively increased to a maximum of about 0.60 at soil C : N ratios of about 32 (Figure 6.8).

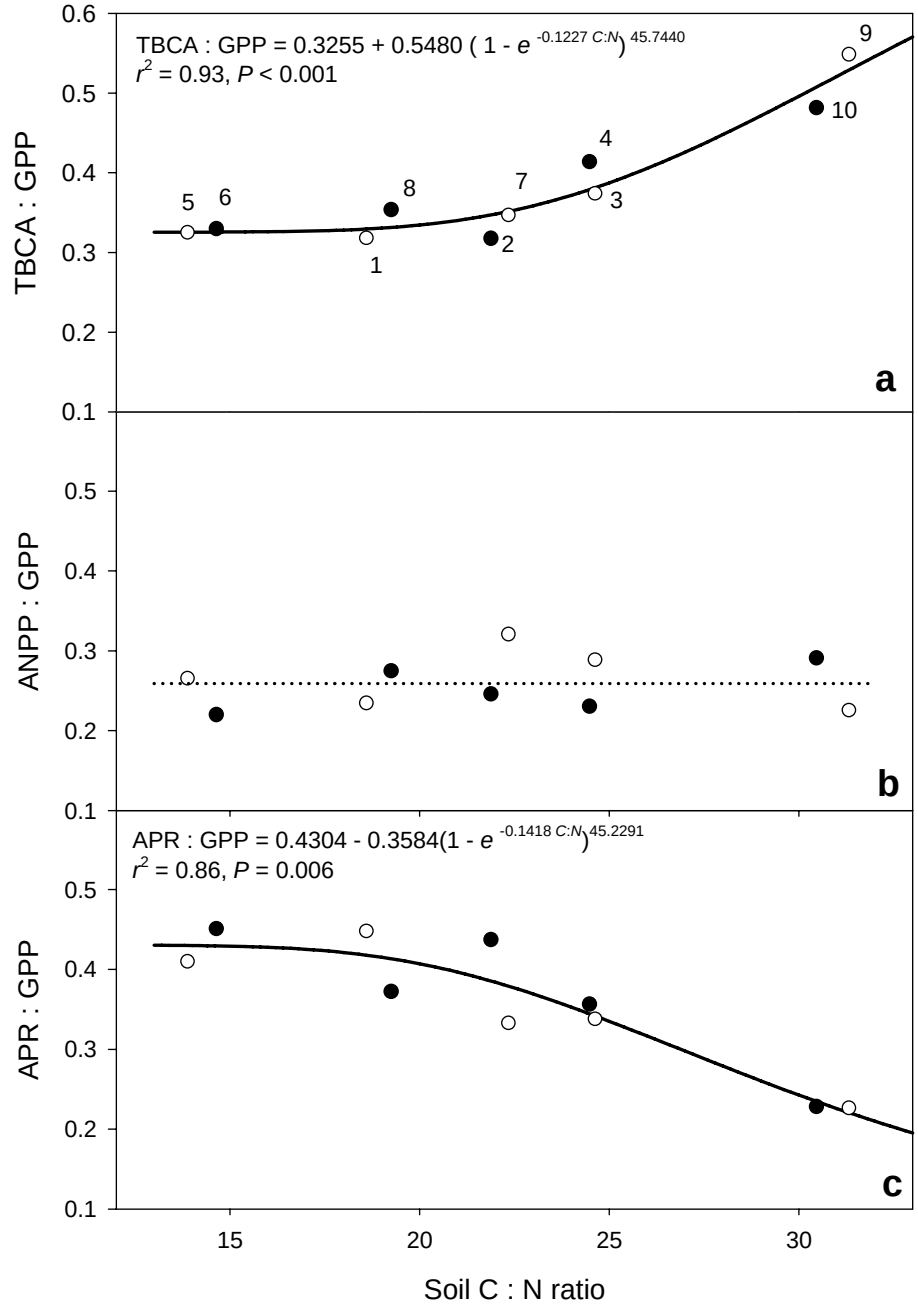


Figure 6.8. Relationships between soil C:N ratio and the ratio of (a) TBCA (b) ANPP and (c) APR to GPP. Solid lines represent best fits for pooled control (open symbols) and fertilized (closed symbols) plots. Plot numbers are indicated in (a) as: 1-2, Rai Valley; 3-4, Golden Downs; 5-6, Tekapo; 7-8, Catlins; 9-10, Longwoods.

Water availability did not change the partitioning of GPP to TBCA or APR. Two contrasting sites, Tekapo (dry) and Rai Valley (wet), exhibited no differences in productivity between the control and the fertilized plot but drastically different volumetric water contents,

and both exhibited the lowest carbon allocation belowground of all sites (0.32-0.33), indicating that water deficit did not change the partitioning of GPP to TBCA. Both sites also showed similar high partitioning of GPP to APR (0.41-0.45). Further evidence was found after correcting for the soil C:N ratio (using equations from Figure 6.8 a,c) and then plotting residuals against the volumetric water content (Figure 6.9).

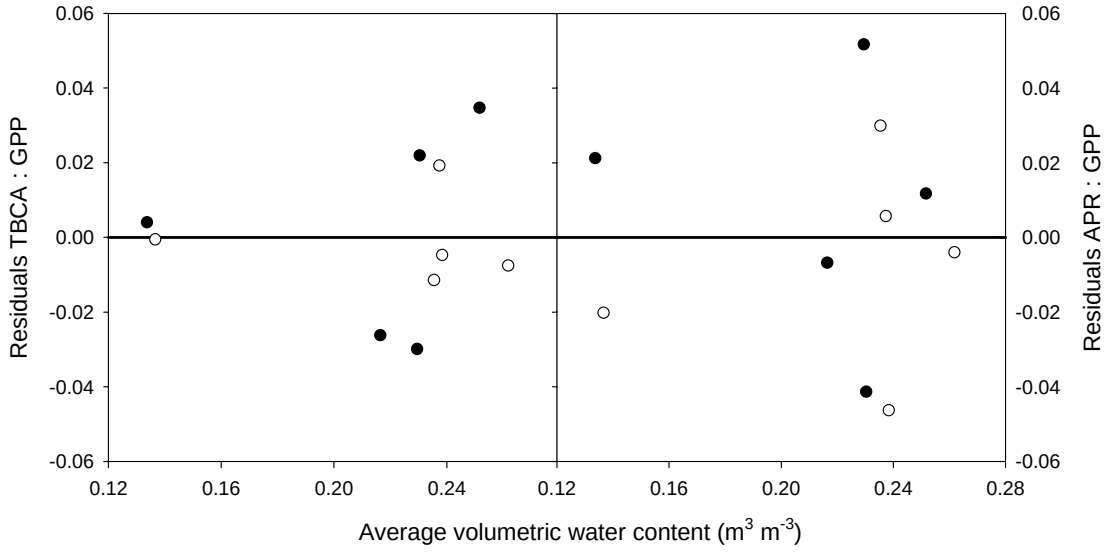


Figure 6.9. Residuals of TBCA : GPP and APR : GPP after correcting for the soil C:N ratio against average volumetric water content across sites and fertilization treatments. Residuals for each plot were calculated using equations presented in Figure 6.8 (a) and (c).

DISCUSSION

Experimenting in-miniature has proved to be a successful method for assessing soil characteristics influencing tree productivity (Watt *et al*. 2005, Davis *et al*. 2007). Using this approach, tree productivity and foliage nutrient concentrations have been shown to be well correlated to total soil N and total and organic P. In this study, a subset of five sites was selected from the 33 sites previously described by Watt *et al*. (2005) and Davis *et al*. (2007), showing both a marked effect of the soil C:N ratio but not P on carbon allocation and a strong relationship between the soil C:N ratio and the NH_4^+-N to NO_3^--N ratio.

Variation in the NH_4^+-N : NO_3^--N ratio across sites and fertilization treatments partially explained differences in productivity Fertilization of all sites increased nitrogen availability in the soil and increased the proportion of NO_3^--N compared to NH_4^+-N. In control plots the NH_4^+-N : NO_3^--N ratio was generally greater for those sites in which the

growth response to fertilization was greater (e.g. Longwoods) and viceversa (e.g. Tekapo). This suggests that productivity across the studied sites may have been controlled by both the NH_4^+-N : NO_3^--N ratio and the absolute amount of N supplied in the soil. This has been observed previously on disturbed sites such as old-fields and pastures where *Pinus radiata* exhibited enhanced growth (Skinner and Attiwill 1981) and where nitrification played a major role (Haynes and Goh 1978; Vitousek *et al.* 1989; Parfitt *et al.* 2003). Supporting evidence highlighting the relevance of the NH_4^+-N : NO_3^--N ratio as a determinant of site quality in *Pinus radiata* was also found in a growth cabinet experiment reported in Appendix B.

Differences in productivity across sites and fertilization treatments were caused by both changes in gross (GPP) and net C fluxes (NPP, NEP) and changes in C allocation. This is relevant because it had been argued that ANPP may increase with nutrient supply at the expense of TBCA without major effects on GPP (Keyes and Grier 1981, Beets and Whitehead 1996). Gross and net C fluxes varied 1.7-2.1 fold across sites while 1.1-1.2 fold as a result of fertilization. Average fractions of GPP represented by ANPP, APR and TBCA were 26%, 36% and 38%, but considerable variation of these fractions was observed across sites. Average site values of ANPP:GPP, TBCA:GPP and APR:GPP ranged 1.4, 1.6 and 2 fold, respectively. Therefore both: differences in C fluxes and C allocation explained variation in productivity across sites and fertilization treatments.

The first hypothesis for this study was that the fraction of GPP allocated to TBCA would increase with nutrient deficiencies at the expense of both ANPP and APR. Carbon allocation to roots was by far the main component of the whole carbon budget, ranging from 0.32 to 0.55 (average 0.38) of GPP across sites. Although fertilization did not change the TBCA:GPP fractions, when all data were pooled to cover a wide site quality gradient, the soil C:N ratio was strongly related to the TBCA:GPP fraction. Soil respiration represented the largest proportion of TBCA across all sites and particularly at Longwoods. This is relevant because it would imply that allometric coefficients commonly used in physiological hybrid models (e.g. 3-PG), would underestimate carbon allocation to roots in sites with low nutrient availability. This was also shown in Chapter *Five*.

The second hypothesis for the study was that carbon use efficiency (NPP:GPP) would be highly conservative and not influenced by nutrient or water availability. Carbon use efficiency (NPP:GPP) is an important parameter in many ecosystem physiological models used in assessing the influence of climate change on the carbon budget of ecosystems from community to planetary scales (e.g. Ichii *et al*. 2005, Turner *et al*. 2006). Waring *et al*. (1998) in a survey of annual carbon budgets from six evergreen and one deciduous forests in New

Zealand, Australia and United States, found that the total NPP : GPP ratio was conservative and about 0.47 ± 0.04 SD, being also remarkably similar to the 0.45 ± 0.05 SD reported by Landsberg and Waring (1997). Carbon use efficiency (CUE) was also highly conservative in this study and not significantly influenced by fertilization treatment (0.54 ± 0.06 SD). However, values of CUE were slightly higher in fertilized (0.56) compared to control plots (0.53), and a similar pattern was found by Giardina *et al*. (2003) in *Eucalyptus* plantations in Hawaii (0.53 compared to 0.51). It was shown in Chapter *Five* that in the greenhouse and provided that extreme nutrient treatments are applied, carbon use efficiency increased from 0.42 in the low nutrient supply regime to 0.51 in the high-nutrient supply regime. Longwoods was the site with the greatest growth response to fertilization, and this was mainly attributed to a decrease in the NH_4^+-N : NO_3^--N ratio rather than dramatic increases in total N availability. Therefore it seems possible that the selection of a more extreme site such as a sand dune or eroded soil might have exhibited lower carbon use efficiencies. This is something that would be worth testing in future research. Carbon use efficiency was very similar between sites with small growth responses to fertilization but with contrasting water availability (Tekapo, dry; Rai Valley, wet), suggesting that water deficit did not influence this parameter.

The third hypothesis for the study was that at least some standard soil chemical properties would be correlated with observed patterns of carbon allocation. None of the soil chemical properties were correlated to the absolute values of GPP, TBCA, ANPP and APR. However, some soil chemical properties (C, N and C : N), but not others (Bray P, Olsen P and Total P, among others), were strongly correlated with the TBCA:GPP and APR:GPP fractions. Soil C and N were discarded as potential predictors of C allocation as one site exhibited excessive leverage on the resulting relationships. The fraction of GPP partitioned to TBCA was shown to increase with the soil C:N ratio, at the expense of APR without major effects on ANPP. There was also a consistent relationship between soil C:N ratio and the exchangeable NH_4^+-N : NO_3^--N ratio, and fertilization acted by reducing this ratio and increasing overall N supply. The results of the study suggests that soil C:N ratio may be associated with processes driving productivity and carbon allocation in *Pinus radiata*. There is evidence in the literature that net N mineralization correlates positively with forest productivity (Reich *et al*. 1997) and negatively with the soil C:N ratio (e.g. Springob and Kirchmann 2003, Parfitt *et al.* 2005). Hence, we might speculate that forest productivity decreases as the soil C:N ratio increases, which is supported by some empirical evidence in *Pinus radiata* (e.g. Smith *et al*. 2002, this study).

In this study soil chemical properties did not relate to ANPP both in absolute or relative terms. Stape *et al*. (2004*b*) found that ANPP did not correlate with any soil chemical variable within a large range of soil characteristics measured in *Eucalyptus* stands in north-eastern Brazil. Landsberg and Hingston (1996) argue that the lack of relationships between soil chemical properties and above-ground productivity are almost universal in forestry, and this might be explained by the complexity of soil chemistry that means soil chemical measurements do not correspond to the chemical species taken up and utilized by trees. Alternatively, it might be that in order to correlate soil characteristics with productivity a complete carbon budget is required as shown in this study (as ANPP did not correlate with any soil chemical property).

Soil respiration is usually assumed to be equally divided between autotrophic (R_a) and heterotrophic (R_h) respiration, and therefore below-ground NPP is generally assumed to be half of TBCA (Stape *et al*. 2004*a*, Law *et al*. 2000, Newman *et al*. 2006). However values of R_a are known to range from one- to two-thirds of the annual carbon release from soils (Raich and Nadelhoffer 1989), and therefore a better understanding on the soil, plant and environmental controls of soil respiration partitioning is required. In this study, autotrophic respiration ranged from 0.22 to 0.55 (average 0.31) whereas heterotrophic respiration from 0.45 to 0.78 (average 0.69). Also, R_h tended to increase with fertilization at the expense of R_a in four out of five sites. This suggests that for the sites under study, equally dividing soil respiration between R_a and R_h would have resulted in biased estimates of NPP (GPP - R_a) and carbon use efficiency.

In conclusion, patterns of carbon allocation were examined in control and fertilized mini-plots of *Pinus radiata* covering a wide climatic and edaphic gradient in the South Island of New Zealand. The study showed that the fraction of the whole-carbon budget allocated belowground progressively increased from about 0.32 at soil C:N ratios below 17 to about 0.60 at soil C:N ratios over 32, being this fraction independent of water availability. The study also showed that the soil C:N ratio strongly correlated to the NH_4^+-N : NO_3^--N ratio, providing a mechanistic explanation to the way that the soil C:N ratio may drive productivity. Because of the unusual nature of the trial and the reduced number of sites studied, further confirmation would be required considering a wider range of forest structures, soil and climatic conditions.

CHAPTER SEVEN

REPRESENTING NUTRITION AND GENOTYPE IN HYBRID AND PHYSIOLOGICAL PRODUCTIVITY MODELS

This chapter is mainly concerned with discussing means to represent nutrition and genotype-nutrition interactions in physiological and hybrid models. Two existing hybrid models (i.e. Landsberg and Waring, 1997, Whitehead *et al*. 2002) were selected as a framework for the discussion. These models were chosen because they represent different mechanistic approaches to primary productivity and both have been successfully used to predict the outcome of physiological processes in *Pinus radiata* plantations in New Zealand. Yet the physiological processes discussed and their implications to process-modelling may well extend to other currently used ecosystem hybrid or physiological models.

THE RADIATION-USE EFFICIENCY MODEL

Primary productivity is determined from light interception, conversion efficiency and the process of allocation of carbohydrates to different functions and components within the plant (Stenberg *et al*. 1994). Monteith (1977) found a strong positive relationship between crop productivity and absorbed photosynthetically active radiation (400 to 700 nm) in Britain, providing a simple yet powerful framework for the development of models based on the radiation-use efficiency concept. Landsberg and Waring (1997) developed a simple linear model (known as 3-PG: Physiological Principles in Predicting Growth) to represent the relationship between photosynthetically active solar radiation absorbed by forest canopies (Q_a) and net primary production of dry mass by forests (P_N),

$$P_N = C_E \, \varepsilon \, \Sigma \, Q_a \min \{F_\theta, F_D\} \, F_T \, F_N \, F_A \qquad (7.1)$$

where ε is the canopy quantum efficiency (default: 0.055 mol CO_2 mol^{-1} quanta) and F_i are the modifying factors reducing the effectiveness of a unit of Q_a as a result of soil water deficit (θ), the vapor pressure deficit of the air (D), temperature (T), fertility (N) and age (A) (Landsberg and Hingston 1996, Landsberg and Waring 1997). The modifiers are dimensionless with values between zero (no growth) and unity (no environmental constraints). Because both soil water and air vapour pressure deficit affect stomatal conductance, only the most limiting of these two

factors, F_θ or F_D, is included in the calculation. The resulting value of Q_a may be interpreted as utilizable radiation by plants. The model uses the ratio of net (P_N) to gross (P_G) primary productivity (Carbon use efficiency, C_E) which has been shown to be conservative for forests (0.45 ± 0.05) to estimate P_N from P_G (Landsberg and Waring 1997).

Carbon allocation to different tree components in this model is determined based on allocation coefficients derived from allometric equations relating the mass of different tree components. The model accounts for plant nutrition through a biomass partitioning mechanism and a user-defined fertility parameter (F_N), allocating more C to roots with lower values of F_N (Landsberg and Waring, 1997). This empirical approach has been used as a consequence of an incomplete understanding of the mechanisms that govern carbon allocation and nutrient uptake, mobilization and retranslocation (Landsberg 1986, Landsberg and Waring 1997, Coops *et al*. 1998).

Potential improvements for the radiation use-efficiency model (Landsberg and Waring, 1997) are discussed in terms of: a fertility modifier, carbon use efficiency (P_N:P_G), carbon allocation to roots and genotype-nutrition interaction representation.

Comparisons on the influence of fertility on productivity are difficult because environmental determinants such as rainfall, solar radiation, temperature and vapour pressure deficit may confound the interpretation of nutrient availability on productivity (Mead 1984). Hence some low nutrient availability sites may exhibit greater productivity than high nutrient availability sites. This confounding effect might be removed by fitting the fertility parameter (F_N) to known values of gross-primary productivity and climatic and water balance data. This approach was followed to fit values of F_N to control and fertilized mini-plots of *Pinus radiata* at five sites in the South Island of New Zealand (Appendix F). The hypothetical relationship between the fertility parameter (F_N), soil N and the C:N ratio is presented in Figure 7.1. Bearing in mind that this relationship was fitted for a reduced number of sites and that Longwoods exhibited a high leverage for soil N, the relationship seems to provide a mechanistic explanation to the way fertility drives productivity i.e. values of F_N decreased with soil C:N ratio and increased with soil N. There is strong evidence on the positive relationship between ANPP and net N mineralization (Reich *et al*. 1997, Newman *et al*. 2006), the negative relationship between net N mineralization and the soil C:N ratio (McLaren and Cameron 1996) and the negative relationship between gross nitrification rates and the soil C:N ratio (Bengtsson *et al*. 2003). By transitivity it might be expected that fertility and the fertility modifier increase with soil N and decrease with the soil C:N ratio.

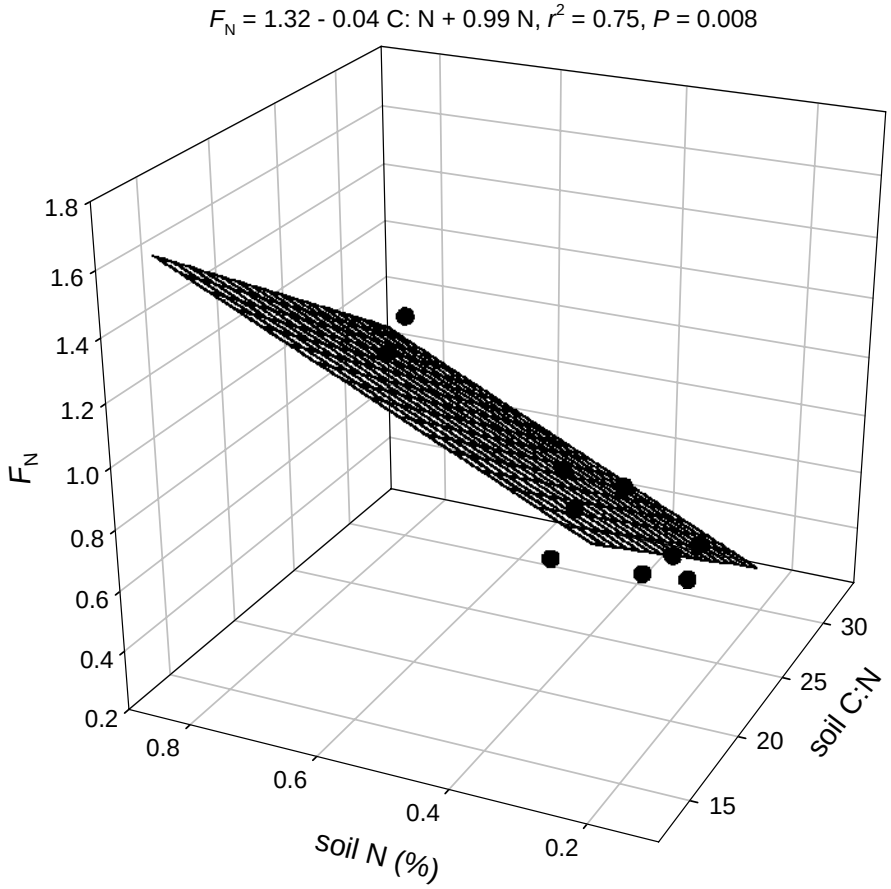


Figure 7.1. The hypothetical relationship between forest productivity, soil N and the soil C:N ratio. The fertility ratio (F_N), a unitless parameter between 0 and 1, was fitted to actual values of GPP obtained in the field (Chapter *Six*) using algorithms from 3-PG (Appendix F).

If confirmed, the relationship presented in Figure 7.1 might prove useful to represent nutrition in hybrid models. However caution should be exercised for sites in which mineral nutrients other than nitrogen are limiting productivity. Stape *et al*. (2004 *a*,*b* , 2006) suggested that using paired-plots with and without fertilization can be used to parameterize the fertility modifier required to calibrate hybrid models such as 3-PG and ProMod (Landsberg 2003, Battaglia and Sands 1997). They related the fertilization response of *Eucalyptus* plantations in Brazil with soil exchangeable K, total P and cation exchange capacity with medium accuracy (r^2 = 0.56, $P < 0.001$). Kimmins and Scoullar (1984) pointed out that the current state of knowledge of plant nutrition does not allow the high resolution often used in today's tree growth models, and that adopting a simpler approach to process descriptions avoiding time resolutions less than one year should be preferred. This broader approach has been followed here. On the other hand, Kimmins and Scoullar (1984) pointed out that narrow scope, high

resolution, short term iteration models might play an important role for understanding in nutritional research. A higher resolution nutrient balance model is discussed within the context of the net carbon canopy exchange model by Whitehead *et al*. (2002).

The radiation use efficiency model implicitly assumes multiplicative (independent) effects of the soil water deficit and the nutritional modifier, because from probability theory if two events (A and B) are independent, then $P(A \cap B) = P(A)\ P(B)$. This seems to be a reasonable modeling assumption that can be illustrated by comparing two sites contrasting in water availability such as Tekapo (dry) and Rai Valley (wet). Both sites exhibited small (none) growth responses to fertilization but Tekapo was drastically limited by water and exhibited a much lower overall productivity (5.4 cf. 3.4 kg C m^{-2} $year^{-1}$). Although water stress arguably reduces nutrient availability in absolute terms, that reduction in relative terms is completely accounted for by the soil water deficit modifier (F_θ), and therefore does not need to be re-accounted for in the nutritional modifier (F_N). Nambiar (1990), pooling data from several studies, showed linear responses of basal area growth to N uptake in irrigated and non-irrigated plots of *Pinus radiata* near Canberra, Australia. Irrigated and non-irrigated trajectories were almost parallel to each other, suggesting that the effect of nutrient and water supply might be considered independent (additive rather than interactive). Assuming independence of processes in relative terms over long time spans (i.e. 1 year) greatly simplifies the representation of nutrition in the radiation-use efficiency model, avoiding the need to disentangle fertility and water stresses on shorter time scales.

In 3-PG it is currently assumed that carbon use efficiency (P_N:P_G) is constant at about 0.45 and not affected by nutrition. Chapter *Five* showed that carbon use efficiency significantly increased with fertility from 0.43 to 0.52 in the greenhouse, whereas in the field study in Chapter *Six* carbon-use efficiency was within this range but was not significantly influenced by site or fertilization (although it increased from 0.53 to 0.56 with fertilization). However, growing conditions were far better controlled in the greenhouse compared to the field. Also, productivity in the field was strongly controlled by both: the ratio between inorganic forms of nitrogen and total nitrogen available, and therefore it is possible that treatments were not as extreme as those achieved in the greenhouse. The fact that carbon use-efficiency increased with nutrient supply consistently in four clones in the greenhouse, suggests that this trend should be observed in the field provided that extreme environments are measured. This is a result that can be easily incorporated in the radiation use efficiency model, and that may account for differences of up to 20% in net primary productivity between fertile and unfertile sites assuming

that carbon-use efficiency increases by 10%. Yet, problems remain in assessing fertility and finding in the field the extremes observed in the greenhouse.

Allocation theory predicts that nutrient deficiencies would shift photosynthate away from leaves and stems, where carbon is used for light capture, to belowground processes, where carbon is used to support fine and coarse root growth and respiration, exudates and to sustain mycorrhizae (Raich and Nadelhoffer 1989, Cannel and Dewar 1994). Similarly, in Chapter *Five* it was found that the fraction of the total carbon budget allocated below ground decreased with nutrient supply, and this is something currently represented but not validated in the radiation-use efficiency model. The findings in this thesis extend previous work in two ways: by demonstrating that reductions in TBCA were triggered mainly by N rather than P supply (Chapter *Five*) and by developing a relationship between TBCA : GPP and the soil C:N ratio (Chapter *Six*). Although this last relationship is based on a limited number of sites, it provides a preliminary approximation to carbon allocation belowground starting at 33% of GPP at soil C:N ratios below 17, climbing steadily for soil C:N ratios over 20, to a likely maximum of about 60% of GPP for C:N ratios over 30 (TBCA : GPP = 0.3255 + 0.5480 + 0.5480 $(1 - e^{-0.1227\ C:N})^{45.7440}$).

Results from Chapter *Five* and *Six* suggest that allocation and allometry are not equivalent, but that allometric coefficients might be corrected easily. In the greenhouse (Chapter *Five*), trees were cultivated in silica sand where root turnover was probably very small and heterotrophic respiration was mostly suppressed. Total below-ground carbon allocation was similarly partitioned between root growth and root plus mycorrhizal respiration, but in the low nutrient supply regime soil respiration represented about 60% of total carbon allocated belowground, compared to 49% at higher N and P addition regimes. Only weak evidence of this partitioning was obtained in the field (Chapter *Six*), but in Longwoods, the least fertile site, fertilization increased the root growth fraction from 11% to 20% of TBCA. All these results suggests that allometric coefficients, as used in the radiation-use efficiency model, might still be the best surrogates for partitioning carbon allocation belowground for all but the most severely nutrient deficient environments.

In Chapters *Two* and *Three*, photosynthesis of a fast- and a slow growing clone were compared, but no evidence was found revealing that differences in growth performance can be attributed to differences in photosynthetic performance. Similarly in Chapter *Four* we looked at nitrogen remobilization efficiency and we found that there were differences among genotypes, but such differences were completely explained by the size of the nitrogen pool in the previous

year to that in which remobilization took place, suggesting that nitrogen remobilization efficiency was similar among genotypes. In Chapter *Five* we examined patterns of carbon allocation in the greenhouse, and we found that carbon allocation above-ground was about 2-4% greater in faster-growing genotypes, and these differences might compound over time to account for differences in plant mass growth. Therefore fast- growing genotypes might be represented in the radiation use efficiency model by increasing allocation aboveground and hence the leaf area but not their photosynthetic or internal nutrient cycling performance.

NET CANOPY CARBON EXCHANGE MODEL

The net canopy carbon exchange model described by Whitehead *et al*. (2002) combines the coupled photosynthesis-stomatal conductance model for individual leaves described by Leuning (1995), with a daily water balance model integrating transpiration from the tree canopy, evaporation from the understorey, and soil and drainage from the root zone (Whitehead and Kelliher 1991). The canopy is divided into five horizontal layers and irradiance, photosynthesis, stomatal conductance and transpiration are integrated for each layer. The model is driven by daily weather data comprising solar irradiance, minimum and maximum air temperature, and rainfall.

Potential improvements for this model are discussed in terms of: adding relationships between photosynthetic model parameters and P_a, using nutrient ratios to discriminate nitrogen ($N_a/P_a \leq 23$ mol mol^{-1}) from phosphorus deficiencies ($N_a/P_a > 23$ mol mol^{-1}), including corrections for transfer conductance (g_m), adding a nutrient balance model to account for seasonal fluctuations in foliage nitrogen (N_a) and phosphorus (P_a) concentrations, expanding the model to account for carbon use efficiency (NPP:GPP) and developing an algorithm to determine carbon allocation to roots.

Coupled photosynthesis – stomatal conductance model

The coupled photosynthesis-stomatal conductance model for single leaves was proposed by Leuning (1995), and comprises the biochemical model of leaf photosynthesis described by Farquhar *et al*. (1980), the model of electron transport response to irradiance by Farquhar and Wong (1984), and the model of stomatal conductance response to air vapour pressure deficit

developed by Leuning (1995). The biochemical model of leaf photosynthesis by Farquhar *et al*. (1980) describes the rate of photosynthesis (A) as,

$$A = \min \left\{ A_c \, , \, A_q \right\} - R_d \tag{7.5}$$

where A_c and A_q are the photosynthetic rates limited by Rubisco carboxylation and by electron transport rate respectively, and min {} indicates the minimum of these two rates. R_d is the rate of daytime respiration resulting from processes other than photorespiration. The photosynthetic rate limited by Rubisco carboxylation (A_c) is given by,

$$A_c = V_{c\max} \frac{C_i - \Gamma^*}{C_i + K_c \, (1 + O_i \, / \, K_o)} \tag{7.6}$$

where V_{cmax} is the maximum rate of Rubisco carboxylation under saturating RuBP and CO_2, C_i and O_i are the intercellular CO_2 and O_2 concentrations, Γ^* is the CO_2 compensation concentration in the absence of day respiration, and K_c and K_o are the Michaelis constants for CO_2 and O_2, respectively. The photosynthetic rate limited by RuBP regeneration driven by electron transport (A_q) is given by,

$$A_q = \frac{J \, (C_i - \Gamma^*)}{4 \, (Ci + 2\Gamma^*)} \tag{7.7}$$

where J is the rate of electron transport at a given irradiance Q. Farquhar and Wong (1984) described the response of J to Q by a non-rectangular hyperbola as,

$$\theta J^2 - (\alpha Q + J_{\max}) \, J + \alpha Q J_{\max} = 0 \tag{7.8}$$

where θ is the convexity of the non-rectangular hyperbola, α is the quantum yield of electron transport, and J_{max} is the maximal electron transport rate driving regeneration of RuBP. Photosynthetic model parameters are strongly influenced by temperature as described by Bernachi *et al*. (2002). Leuning (1995) described the response of stomatal conductance to air vapour pressure deficit as,

$$g_s = g_0 + \frac{aA}{(C_s - \Gamma^*)(1 + D_s / D_0)} \quad (7.9)$$

where g_s is stomatal conductance to CO_2 transfer, g_0 is the residual stomatal conductance in the dark, C_s is the CO_2 concentration at the leaf surface, D_s is the air saturation deficit at the leaf surface, and D_0 and a are empirical parameters.

Results from Chapter *Two* and *Three* may contribute to better estimates of carbon assimilation using the coupled model of leaf photosynthesis - stomatal conductance (Leuning 1995) under nitrogen and phosphorus limitations, by using nutrient ratios to discriminate nitrogen ($N_a/P_a < 23$ mol mol^{-1}) from phosphorus deficiencies ($N_a/P_a > 23$ mol mol^{-1}), by adding relationships between photosynthetic model parameters V_{cmax} and J_{max} to P_a, and by correcting the estimation of photosynthetic parameters V_{cmax} and J_{max} by accounting for transfer conductance (g_m).

Strong linear relationships between photosynthetic parameters and foliar nitrogen (r^2 = 0.78-0.82) were found in Chapter *Two*, that closely matched those reported by Walcroft *et al.* (1997) for *Pinus radiata* under similar experimental conditions ($V_{cmax} = 0.573\ N_a - 1.806$ cf. $V_{cmax} = 0.520\ N_a - 3.784$; and $J_{max} = 0.742\ N_a + 2.668$ cf. $J_{max} = 0.731\ N_a + 3.574$, respectively). Also values of V_{cmax} and J_{max} were well correlated to foliage phosphorus ($V_{cmax} = 11.363\ P_a + 4.933$, $r^2 = 0.59$, $P < 0.001$; $J_{max} = 17.559\ P_a + 7.210$, $r^2 = 0.75$, $P < 0.001$), and this modification can be readily incorporated into the coupled leaf photosynthesis – stomatal conductance model (Leuning 1995) to account for phosphorus limitations.

Nutrient ratios have been extensively used to address optimum nutrition and explain particular nutrient limitations (e.g. Ingestad 1971, 1979, Ingestad and Lund 1986). Hence, using nutrient ratios to separate nutrient limitations may provide useful means of modeling nutrient-limited photosynthesis at the scale of leaves, plants and ecosystems. In Chapter *Two*, a threshold N_a / P_a ratio of 23 (mole basis) distinguished nitrogen ($N_a / P_a \leq 23$) from phosphorus ($N_a/P_a > 23$) deficiencies. Using this approach, significant relationships between V_{cmax} and J_{max} and N_a and P_a were found, and it was shown that effects of nitrogen and phosphorus supply on photosynthesis were statistically independent. Thus, applied to physiological models, nutrient ratios may provide the means to firstly discriminate nitrogen from phosphorus deficiencies; and secondly, to estimate values of V_{cmax} and J_{max}. These modified functions can then be used to

parameterize the coupled model of leaf photosynthesis - stomatal conductance described by Leuning (1995).

Photosynthetic parameters V_{cmax} and J_{max} are usually determined by fitting the Farquhar *et al*. (1980) model to the *in vivo* response of net assimilation to intercellular CO_2 concentration (A / C_i curves). The internal transfer conductance (g_m) was once considered sufficiently large so that CO_2 concentration in the intercellular air spaces (C_i) could be assumed similar to the CO_2 concentration in the chloroplasts (C_c) for the purposes of fitting the Farquhar *et al*. (1980) model. However, transfer conductance is now known to be finite imposing similar limitations to photosynthesis as those imposed by stomata (Harley *et al*. 1992, Loreto *et al*. 1992, von Caemmerer 2000, Warren *et al*. 2003). In Chapter *Three*, it was shown that values of g_m scaled with nutrient supply, and approximately with the rate of photosynthesis at saturating irradiance and ambient CO_2 ($g_m = 0.020\ A_{sat}$, $r^2 = 0.25$, $P < 0.001$) and with stomatal conductance ($g_m = 1.16\ g_s$, $r^2 = 0.14$, $P = 0.02$). This caused values of V_{cmax} and J_{max} calculated on a C_c basis to be on average 15.4 % and 3.1 % greater than those on a C_i basis, which translated into different slopes of the J_{max} / V_{cmax} relationship (C_c basis: $J_{max} = 2.11\ V_{cmax}$, $r^2 = 0.88$, $P < 0.001$; C_i basis: $J_{max} = 2.43\ V_{cmax}$, $r^2 = 0.86$, $P < 0.001$). For the purposes of modeling, g_m could be calculated based on equations previously outlined, and incorporated into the coupled leaf photosynthesis - stomatal conductance model described by Leuning (1995) by replacing equation 7.10 for C_i into equations 7.6 and 7.7,

$$C_c = C_i - \frac{A}{g_m} \qquad (7.10)$$

where C_c is the CO_2 concentration in the chloroplast, C_i is the intercellular CO_2 concentration, A is the photosynthesis rate and g_m is transfer conductance.

The non-rectangular hyperbola model used to describe the response of electron transport rate to irradiance (Farquhar and Wong 1984) is completely determined by three parameters: maximal electron transport rate (J_{max}), convexity of the non-rectangular hyperbola (θ) and the quantum yield of electron transport. J_{max} was shown to scale with nitrogen and phosphorus supply in Chapter *Two* and *Three*. Yet a question remains whether values of θ and α are differentially affected by nutrient supply. Some partial evidence to answer this question is provided in Appendix A using chlorophyll fluorescence techniques. It was found that values of θ and α were not influenced by nutrient supply being on average (± 1 SE) 0.851 ± 0.005 (no

units, value between 0 and 1) and 0.258 ± 0.001 mol electrons mol quanta^{-1}, respectively. This is also a result that simplifies parameterization of the Farquhar and Wong (1984) model to account for nitrogen and phosphorus deficiencies in *Pinus radiata*.

The Leuning (1995) model describing the response of stomatal conductance to air vapour pressure deficit can be also analyzed from the perspective of nitrogen and phosphorus deficiencies. It can be seen from equation 7.9 that at a given air vapor pressure deficit; values of g_s are proportional to the rates of photosynthesis (A). In Chapter *Two* and *Three*, stomatal limitations scaled with nitrogen and phosphorus supply as a fixed proportion of the light-saturated photosynthesis rate (13-19 %) independent of the nutrient supply regime. Therefore in Eqn.7.9 the effects of nutrient supply on the g_s to D relationship are entirely mediated through alterations to the photosynthetic rates.

Nutrient balance model

The net carbon uptake model (Whitehead *et al*. 2002, 2004*b*, Whitehead and Walcroft 2005) uses the vertical distribution of nitrogen concentration within the canopy to drive photosynthesis. This is because photosynthetic rates are linearly related to foliar nitrogen concentrations when N is limiting, and hence values of key physiological parameters within the canopy can be specified by the distribution of leaf nitrogen in a multilayer model (Leuning 1995). However, seasonal fluctuations in foliage nitrogen concentrations are not accounted for in the net carbon uptake model and therefore a nutrient balance approach may improve estimates by describing seasonal fluctuations in foliage nutrient concentrations. Some of the results in this thesis may assist to conceptualize such model for *Pinus radiata*.

A nutrient balance model may account for nutrient uptake, storage and remobilization at the tree level. The nutrient balance approach might be similar to that used to model water, except that plant water storage is short-lived (days) compared to nutrient storage which represents a large fraction (more than 50%) of nutrients that would be subsequently remobilized to support new growth and over longer time scales (months). One might start by assuming that foliage is the only source for nutrient remobilization as the contributions of woody and root components are marginal (Nambiar 1987). This is corroborated by a key finding in Chapter *Four* that 87% of all N remobilized during spring-summer was provided by the foliage.

A procedure to scale nutrient uptake from a yearly to a monthly scale seems feasible. Nutrient uptake on a yearly step can be calculated as the difference in nutrient content at two

times using destructive sampling and allometric equations. Total nutrient uptake might be downscaled to a monthly step using, for instance, the monthly values of exchangeable ions measured by ion exchange membranes presented in Chapter *Six*. Because ion exchange membranes integrate nutrient availability over a given period, it seems practical to assume that seasonal patterns of nutrient availability match those of nutrient uptake. An implicit assumption of this statement is that nutrient uptake is luxurious in nature and driven by nutrient availability.

A procedure to account for nutrient remobilization may also be feasible to implement. Chapter *Four* showed that nitrogen remobilization efficiency was higher in plants growing in the high nutrient supply regime (65%) compared to those growing at lower N and P addition rates (42-48%). The enhanced capacity for nitrogen remobilization was mainly explained by larger fascicles and greater allocation to foliage in the high nutrient supply regime. In Chapter *Four* it was also suggested that nitrogen remobilization seemed to be independent of uptake and exclusively driven by sink strength. In Chapter *Six*, current-year fascicle elongation was used to scale leaf area, and similarly could be used to scale sink strength and allocate remobilization over time.

The relative independence of uptake and remobilization makes it easier to assume that uptake might be considered luxurious in nature with the main aim to build up reserves that would be remobilized in the future to support new growth. However, once the source of nutrient remobilization has been exhausted, and provided that there are several flushes of growth, it seems likely that uptake replenishes storage that could be immediately used to support new growth. An example of the likely output of a nutrient balance model is presented in Figure 7.2. This output was constructed from data drawn from the control plot in Rai Valley described in Chapter *Six*. These results are qualitative in nature and have not been validated but reflect the potential of using a nutrient balance approach to better represent nutrition in physiological models. Note that the model correctly predicts that foliage nitrogen concentrations fluctuate with season and decrease with age as found for *Pinus radiata* by Nambiar and Fife (1987).

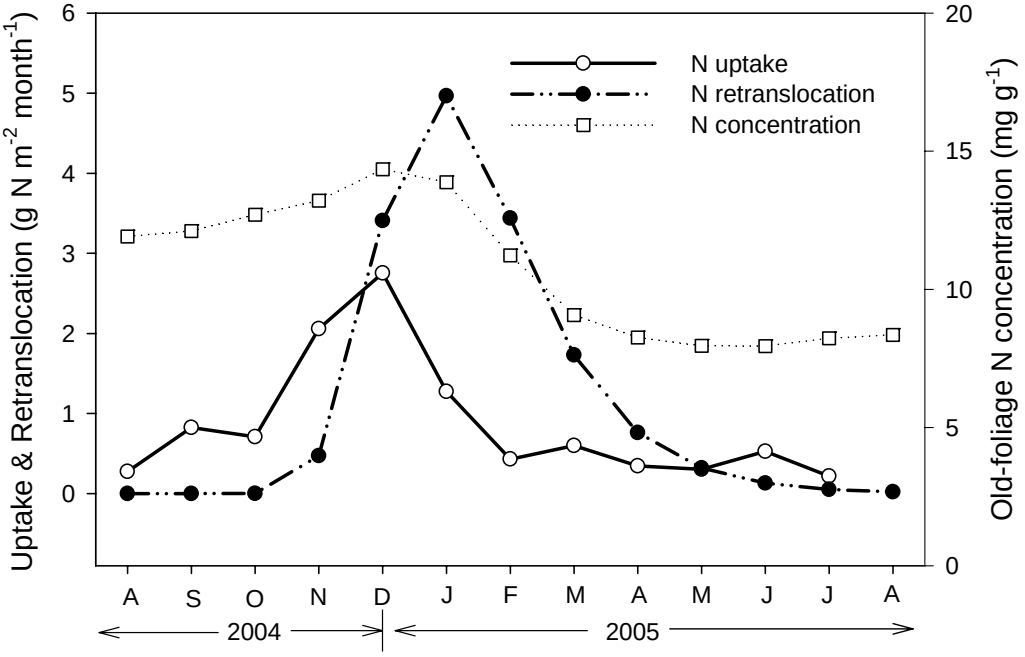


Figure 7.2. A nutrient balance model may account for nutrient uptake and retranslocation and with only a few assumptions may represent seasonal variations in foliage nutrient concentrations. In this figure nitrogen uptake and remobilization were estimated for the control plot in Rai Valley (Chapter *Six*). N uptake was calculated as the difference between N content between August 2004 and August 2005, and scaled monthly using results from ion exchange membranes. N retranslocation was assumed to be 65% of the N pool at August 2004 based on findings from Chapter *Four*. N retranslocation was scaled based on non-destructive measurements of leaf phenology. Fluctuations in N concentrations in one-year and older foliage are an exclusive consequence of N uptake and retranslocation and growth. Old-foliage was assumed the only source for N retranslocation.

The canopy net carbon exchange model by Whitehead *et al*. (2002) could be extended to predict NPP and carbon allocation to different tree components. Basically, the canopy net carbon exchange model provides an estimate of total carbon assimilation but not net primary production. Hence using values of carbon use efficiency found in this study net primary productivity could be determined in this model. As in the radiation use efficiency model, carbon allocation to roots can be estimated based on allometric coefficients (Landsberg *et al*. 1997), or based on the relationship between TBCA and soil C:N ratio presented in this thesis in Chapter *Six*. Genotype could be represented in the Whitehead *et al*. (2002) model by increasing aboveground components at the expense of roots in faster growing genotypes but not their photosynthetic performance.

In conclusion, this chapter aimed to assess means to represent nutrition and genotype-fertility interactions in physiological and hybrid forest models. Nutrition may be represented in

physiological models (e.g. Whitehead *et al*. 2002) by using nutrient ratios to discriminate nutrient deficiencies, by assuming that the effects of N and P deficiencies on photosynthesis are statistically independent, and by incorporating relationships between photosynthetic parameters and foliage phosphorus concentrations. Also, canopy multilayer models may improve in biological realism by incorporating a nutrient balance sub-model to account for seasonal and age fluctuations in foliage nutrient concentrations to drive photosynthesis. Nutrition may be represented in the radiation-use efficiency model (hybrid) by acknowledging that carbon use efficiency might slightly increase with fertility, that a fertility modifier might be fitted to whole carbon budgets and correlated to soil chemical properties, and that relative carbon allocation belowground might increase as the soil C:N ratio increases. Finally, faster-growing genotypes may be represented in both physiological and hybrid models by increasing carbon allocation above ground without affecting their photosynthetic performance.

CHAPTER EIGHT
CONCLUDING REMARKS

The overall aim of the study was to assess means to represent plant and soil nutrition of *Pinus radiata* in tree productivity physiological and hybrid models. The expression of tree growth and genotype in relation to nutrition were assumed to operate through three fundamental processes: photosynthesis, carbon allocation and plant nutrient cycling. These processes were studied using a combination of greenhouse and field experimentation. This thesis showed that nutrient-limited photosynthesis, carbon allocation and internal nutrient cycling could all be represented in such models. The discussion that follows emphasizes how thesis objectives were achieved, key findings and future directions where new research might be directed.

The fundamental process of photosynthesis has been extensively studied, and advances have been particularly rapid since the appearance of portable gas-exchange measurement systems. This is not surprising as all life on earth and the survival of the human species is dependent upon this process. Farquhar *et al*. (1980) proposed a biochemical model of C_3 leaf photosynthesis that is widely used in ecophysiological research and to scale carbon assimilation from leaves to canopies. In this model, CO_2 assimilation is mainly limited by the maximal rate of ribulose-1,5-*bis*phosphate (RuBP) carboxylase-oxygenase (Rubisco) carboxylation (V_{cmax}) and by the maximal electron transport rate driving regeneration of RuBP (J_{max}). A key finding in Chapter *Two* was that the effects of nitrogen and phosphorus supply on photosynthesis were statistically independent, that nutrient ratios can be used to separate nitrogen from phosphorus deficiencies, and that photosynthetic parameters V_{cmax} and J_{max} scaled with foliage nitrogen and phosphorus concentrations. These findings could be readily incorporated into the coupled photosynthesis-stomatal conductance model described by Leuning (1995), widely used to scale carbon assimilation over temporal and spatial scales (Chapter *Seven*).

Most estimates of photosynthetic parameters V_{cmax} and J_{max} are derived from the response of net assimilation to internal CO_2 concentration (A / C_i curves), with the implicit assumption that CO_2 concentration in the intercellular air spaces (C_i) is similar to the CO_2 concentration in the chloroplasts (C_c). However, values of C_c have been shown to be lower than those of C_i and measured by a term called transfer conductance (g_m). This difference leads to underestimates of the photosynthetic parameters V_{cmax}, J_{max} when calculated on a C_i rather than C_c basis (Harley *et al*. 1992, Loreto *et al*. 1992, von Caemmerer 2000, Long and Bernacchi 2003). A key finding in Chapter *Three* was that transfer conductance (g_m) scaled with nitrogen and phosphorus supply as a fixed proportion (0.16) of the light-saturated photosynthetic rate

(A_{sat}) posing a remarkably stable C_c - C_i gradient (48 ± 2 μmol mol^{-1}) which was independent of N or P deficiencies. Photosynthetic parameters V_{cmax} and J_{max} calculated on a C_c basis were on average 15.4 % and 3.1 % greater than those on a C_i basis, which translated into different slopes of the J_{max} / V_{cmax} relationship (C_c basis: J_{max} = 2.11 V_{cmax}, r^2 = 0.88, P < 0.001; C_i basis: J_{max} = 2.43 V_{cmax}, r^2 = 0.86, P < 0.001). Transfer conductance could be readily incorporated into the coupled photosynthesis-stomatal conductance model described by Leuning (1995), and hence physiological models, by using one of the following relationships: g_m = 0.020 A_{sat} (r^2 = 0.25) or g_m = 1.16 g_s (r^2 = 0.14) reported in Chapter *Three* and discussed in Chapter *Seven*.

The first objective of this thesis was to assess stomatal, mesophyll and biochemical limitations to photosynthesis as posed by nutrient deficiencies, and it was achieved in Chapters *Two* and *Three* using the biochemical model of C_3 leaf photosynthesis described by Farquhar *et al*. (1980). In absolute terms, limitations posed by g_s and g_m increased with single or joined additions of nitrogen and phosphorus supply; but in relative terms, both limitations scaled as a fixed proportion of A_{sat} (< 20% each). Biochemical limitations (V_{cmax} and J_{max}) were the predominant component of photosynthesis (> 60%) scaling independently with nitrogen and phosphorus supply.

Seasonal changes in foliage nutrient concentrations have been observed in *Pinus radiata* and attributed to nutrient remobilization from old to new tissues to buffer the asynchrony of soil nutrient resources. Nutrient remobilization may account for 50% or more of nitrogen and phosphorus requirements of this species, and hence plays a key role in the nutrient economy of trees. The second objective of this thesis was to estimate internal nutrient remobilization responses to nutrient deficiencies, being achieved in Chapter *Four*. A key finding in this chapter was that trees remobilized 65% of the nitrogen pool from the previous year in the high nutrient supply regime compared to 42-48% obtained at lower nitrogen and phosphorus addition rates, emphasizing the need for a balanced nutrition for trees to realize their maximum growth and carbon sequestration potential. Most N remobilization occurred during spring-summer (77%), coincidently with the largest proportion of needle development (80%), suggesting that N remobilization was driven by sink-strength. Old-foliage was by far the main source for internal cycling, providing 87% of all N remobilized during spring-summer, decreasing to an overall 64% by the end of the second year, with the difference (36%) being provided by stems and mainly roots. It seems quite surprising that plants remobilized more, in absolute and relative terms, in the high-nutrient supply regime even when plants were abundantly supplied with nutrients suggesting that uptake and remobilization are independent processes. In Chapter *Seven* it was speculated that findings from Chapter *Four* might be logically integrated into a nutrient

balance model which could account for seasonal changes in foliage nutrient concentration, which in turn may drive photosynthetic parameters in the coupled photosynthesis-stomatal conductance model described by Leuning (1995). Such a model could be constructed by assuming that uptake and remobilization are independent processes, that uptake is luxurious in nature with the main aim to build up reserves that could be remobilized in the future, that remobilization is driven by sink strength, and that leaf expansion can be used as a surrogate of sink strength.

Carbon allocation to roots is one of the least understood processes slowing the development of physiological models. Among the components of the C budget belowground, fine root biomass and production has been investigated the most, using sequential coring, root ingrowth cores and minirhizotrons, among others (Ostonen *et al*. 2005, Giardina and Ryan 2002). However these methods are extremely labor intensive, have implied assumptions that are difficult to test, have statistical problems and results do not always agree (Nadelhoffer 2000, Ostonen *et al*. 2005). Also, fine root estimates do not account for root respiration and exudates, heterotrophic respiration and mycorrhizal respiration and turnover (Giardina and Ryan 2002). The carbon balance approach (Raich and Nadelhoffer 1989, Giardina and Ryan 2002) overcomes many of the difficulties and assumptions of previous methods. The third objective for this thesis was to compare tree growth and carbon allocation patterns across nutrient availability gradients both in the greenhouse and the field, being accomplished in Chapters *Five* and *Six*, respectively. A key finding in Chapter *Five* was that relative below-ground carbon allocation increased mostly with nitrogen but also with phosphorus deficiencies at the expense of aboveground primary productivity without major effects on aboveground plant respiration. Carbon-use efficiency (NPP : GPP) significantly increased mainly with nitrogen but also with phosphorus supply, which provides new understanding on ways that nutrition regulates productivity.

Carbon allocation to roots was also measured in control and fertilized mini-plots at five sites with contrasting climate and soil conditions in the South Island of New Zealand. Although the limited number of sites restrict the generalization of findings, relative carbon allocation to roots increased with the soil C:N ratio, which in turn correlated well with the ratio of soil exchangeable fractions of inorganic nitrogen (NH_4^+-N : NO_3^--N). Both relationships, if confirmed, provide a link between carbon allocation and soil mineralization, which are feasible to be implemented in physiological and hybrid models. In Chapter *Seven*, a fertility modifier (f_N) was fitted to actual GPP values obtained in the field (Chapter *Six*), climatic and water balance data using algorithms from 3-PG. Fitted values of f_N were correlated to soil chemical

properties yielding the following relationship: $F_N = 1.32 – 0.04$ C:N + 0.99 N (%) ($r^2 = 0.75$, $P = 0.008$). If confirmed, this relationship may prove useful to estimate the fertility modifier of *Pinus radiata* over wider geographic areas.

The expression of genotype on tree growth was hypothesized to operate through the same fundamental processes as nutrition: photosynthesis, carbon allocation and plant nutrient cycling. Genotypes did not differ in photosynthetic performance per unit leaf area despite exhibiting differences in growth performance in Chapters *Two* and *Three*. Similarly genotypes did not differ in internal nutrient remobilization efficiency in Chapter *Four*. However genotypes did show differences in carbon allocation and such differences were associated with differences in growth performance. Faster-growing genotypes allocated about 2-4% more to above-ground components, presumably increasing leaf area that compounds canopy photosynthesis and increases overall carbon assimilation over time. All these results contribute to give answer to the fourth objective of this thesis i.e. to assess key physiological processes that may explain differences in genotypic growth performance in relation to nutrition. Also, genotype × nutrition interactions were seldom significant, and when significant, they were far less significant than the main effects of nutrient treatment and genotype. This suggests that at least from a hybrid modelling perspective; genotype × nutrition interactions can be safely ignored. Therefore faster-growing genotypes may be represented in physiological and hybrid models by increasing carbon allocation above-ground and hence leaf area, but not by modifying their photosynthetic or nutrient remobilization performance as shown in Chapters *Five* and *Seven*.

The fifth objective of this thesis was to discuss means by which photosynthesis, internal nutrient cycling and carbon allocation patterns may be integrated in physiological and hybrid models to represent nutrition and genotype-fertility interactions, and this objective was achieved in Chapter *Seven* by examination of one hybrid (Landsberg and Waring 1997) and one physiological model (Whitehead *et al.* 2002). Nutrition might be represented in the radiation-use efficiency model (Landsberg and Waring 1997) by recognizing that carbon use efficiency slightly increases with nutrient availability, and if confirmed, by including a preliminary relationship between carbon allocation to roots against the soil C: N ratio, and a preliminary relationship between the fertility modifier of 3-PG (F_N) and soil N and soil C:N ratio. The representation of nutrition in the net canopy carbon exchange model (Whitehead *et al.* 2002) might be extended by using nutrient ratios to discriminate nutrient deficiencies, adding relationships between photosynthetic parameters and foliage P, adding a nutrient balance model to account for seasonal fluctuations in foliage nutrient concentrations, accounting for carbon use

efficiency to determine NPP, and using a preliminary relationship between carbon allocation to roots and the soil C:N ratio.

In order to better represent plant and soil nutrition in process-based models further research is needed. Further research required conforms generally to those areas previously identified by Landsberg (2003) i.e. carbon assimilation and allocation, nutrient availability in soils, nutrient uptake and internal cycling within the plant. However, this thesis revealed some specific areas where new research is urgently needed.

In terms of photosynthesis research, it seems necessary to improve our understanding of the use of nutrient ratios to discriminate among nutrient deficiencies, to explore the influences of nutrient deficiencies other than nitrogen and phosphorus on photosynthetic parameters of the Farquhar *et al*. (1980) model and transfer conductance, to find the set of conditions under which triose-phosphate export (T_p) limitations occur, and how these T_p limitations are affected by nutrient availability.

Carbon allocation has been observed to proportionally increase in nutrient deficient environments, but the mechanisms explaining such phenomena are largely unknown. Here there seems to be a good opportunity to combine carbon allocation and photosynthesis research to gain insight into the mechanisms of carbon allocation. For instance, greenhouse experiments manipulating carbohydrate accumulation (e.g. using different pot sizes) and starvation (e.g. low irradiance) may provide some relationships and feedback mechanisms between carbon assimilation and allocation that may be useful in physiological models. Other challenges in carbon allocation in the field comprise the study on the responses of auto- and heterotrophic soil respiration to temperature, water balance and nutrient availability; the cost-benefit analysis of mycorrhizae; and the effect of organic and inorganic sources of nitrogen on carbon assimilation and partitioning, among others.

In terms of soil nutrient availability, nutrient uptake and internal cycling by plants the task seems also immense. Soil nutrient availability, being strongly dependent on soil and climatic conditions, and also changing one to three orders of magnitude seasonally, seems to be unlikely to be represented by universal relationships. However in Chapter *Six*, it was shown that the soil C : N ratio was well correlated with the NH_4^+ : NO_3^- ratio measured using cation and anion exchange membranes. This is an example of how combining techniques may help us to understand why and how some soil variables correlate to soil processes. In Chapter *Seven*, it was argued that a nutrient balance model that integrates nutrient uptake and internal cycling may provide a means to explore key areas where new research should concentrate.

In terms of representing genotypes in process-based models, more research would be required to unravel the physiological reasons explaining greater growth performance in certain genotypes. Throughout this study, it has been shown that faster-growing genotypes allocated about 2-4% more to above ground components, but the photosynthetic and nutrient internal cycling performance was equivalent among genotypes. In this study, genotype × nutrition interactions were sometimes significant but always far less significant than the main effects of nutrient treatment and genotype. These results coincide with Carson *et al*. (2004) who argued that genotype × fertility interactions in *Pinus radiata* are seldom significant, suggesting that selecting genotypes for better growth performance in poor fertility sites would not be substantially better than selecting for growth on all sites irrespective of nutrient availability. In this thesis, the comparison of genotypes was performed considering different nutrient supply regimes, hence representing genotype in physiological models would greatly benefit from comparing the physiology of genotypes across different combinations of water and nutrient availability.

In terms of representing nutrition in physiological models, two approaches seem to be promising: a once-for-all (top-down) and a step-by-step (down-up) approach. In the once-for-all approach, a fertility modifier (F_N) is fitted to actual estimates of the whole carbon budget, climatic and water balance data, using algorithms such as those described for 3-PG, and then fitted values of F_N are correlated to soil chemical properties. This approach would provide an index of fertility from 0 to 1 that seems to be independent of climate and water availability, and is therefore potentially useful in the radiation-use efficiency model as a fertility modifier as described in Chapter *Seven*. The advantage of this approach would be that soil characteristics are unlikely to change rapidly, as opposed to soil nutrient availability, but at the expense that an empirical rather than mechanistic explanation is obtained. In contrast, in the step-by-step approach, a nutrient balance model that accounts for nutrient uptake and internal cycling run on a monthly time step would provide foliage nutrient concentrations that could be used to drive the coupled photosynthesis - stomatal conductance model described by Leuning (1995), which in turn might be used to scale carbon assimilation from leaves to canopies using a model such as the one described by Whitehead *et al*. (2002). The advantage of this approach would be that a more mechanistic explanation of plant and soil nutrition would be obtained, and used to find gaps where new research should be developed, but at the likely expense that predictions would be poorer and models more difficult to parameterise than for the once-for-all approach.

In order to build reliable hybrid forest models in the future, an increasing amount of soil and physiological variables should be measured. Permanent sample plots, traditionally used to

build mensurational forest models, may now be used to record additional variables allowing the construction of hybrid models. Standard soil chemical properties such as the soil C, N and total and extractable P are unlikely to change much over the plot measurement period. Therefore soil properties should be ideally determined for the whole set of plots. These soil properties are likely to be correlated to productivity when whole-carbon budgets are determined.

More intensive soil and physiological measurements should be carried out in a reduced number of permanent sample plots covering the gradient in soil and environmental conditions. Monthly measurements of soil exchangeable NH_4^+-N, NO_3^- -N and exchangeable P in the intensively-measured subset of permanent sample plots, such as those presented in Chapter *Six*, might be used to calibrate a nutrient balance model that could be used over the whole set of permanent sample plots. Measurements of monthly foliage nutrient concentrations at selected sites might be also useful to validate estimates provided by the nutrient balance model. Similarly monthly measurements of soil respiration and litterfall in the intensively-measured subset of permanent sample plots might provide estimates of below ground carbon allocation that may correlate well with soil chemical properties. A twin-plot approach might also be useful to represent nutrition in tree-productivity hybrid models. Locating closely together a control and a fertilized plot (twins) in the intensively-measured sites, may provide reliable estimates of the effect of fertilization and its correlation to soil properties, that could be used to extrapolate to the whole set of permanent sample plots. The soil and physiological measurements outlined above may provide managers with some pointers about what might be collected in permanent sample plots to improve the representation of tree and soil nutrition in hybrid productivity models.

Research directions outlined above might help to enhance models in the future, but in the meantime, results from this thesis may provide a preliminary framework to represent plant and soil nutrition in physiological or hybrid models.

REFERENCES

Aerts, R. and F.S. Chapin 2000. The mineral nutrition of wild plants revisited: A re-evaluation of processes and patterns. Advances in Ecological Research. 30:1-67.

Ågren, G.I. and T. Ingestad 1987. Root:shoot ratio as a balance between nitrogen productivity and photosynthesis. Plant, Cell and Environment. 10:579-586.

Albaugh, T.J., L. Allen, P.M. Dougherty, L.W. Kress and J.S. King 1998. Leaf area and above- and belowground growth responses of Loblolly Pine to nutrient and water limitations. Forest Science. 44:317-328.

Amthor, J.S. 2000. The McCree-de Wit-Penning de Vries-Thornley respiration paradigms: 30 years later. Annals of Botany. 86:1-20.

Atkin, O.K., J.R. Evans, M.C. Ball, H. Lambers and T.L. Pons 2000. Leaf respiration of Snow Gum in the light and dark. Interactions between temperature and irradiance. Plant Physiology. 122:915-923.

Azcón-Bieto, J. 1983. Inhibition of photosynthesis by carbohydrates in wheat leaves. Plant Physiology. 73:681-686.

Baldocchi, D.D. and P.C. Harley 1995. Scaling carbon dioxide and water vapour exchange from leaf to canopy in a deciduous forest. II. Model testing and application. Plant Cell and Environment. 18:1157-1173.

Battaglia, M. and P. Sands 1997. Modelling site productivity of *Eucalyptus globulus* in response to climatic and site factors. Australian Journal of Plant Physiology. 24:831-850.

Battaglia, M., P. Sands, D. White and D. Mummery 2004. CABALA: a linked carbon, water and nitrogen model of forest growth for silvicultural decision support. Forest Ecology and Management. 193:251-282.

Beets, P.N., G.R. Oliver, M.O. Kimberley, S.H. Pearce and B. Rodgers 2004. Genetic and soil factors associated with variation in visual magnesium deficiency symptoms in *Pinus radiata*. Forest Ecology and Management. 189:263-279.

Beets, P.N. and D. Whitehead 1996. Carbon partitioning in *Pinus radiata* stands in relation to foliage nitrogen status. Tree Physiology. 16:131-138.

Bengtsson, G., P. Bengtson and K.F. Månsson 2003. Gross nitrogen mineralization-, immobilization-, and nitrification rates as a function of soil C/N ratio and microbial activity. Soil Biology and Biochemistry. 35:143-154.

Benson, M.L., J.J. Landsberg and C.J. Borough 1992. The biology of forest growth experiment: an introduction. Forest Ecology and Management. 52:1-16.

Bernacchi, C.J., A.R. Portis, H. Nakano, S. Von Caemmerer and S.P. Long 2002. Temperature response of mesophyll conductance. Implications for the determination of Rubisco enzyme kinetics and for limitations to photosynthesis in vivo. Plant Physiology. 130:1992-1998.

Bernacchi, C.J., E.L. Singsaas, C. Pimentel, A.R. Portis Jr and S.P. Long 2001. Improved temperature response functions for models of Rubisco-limited photosynthesis. Plant, Cell and Environment. 24:253-259.

Blakemore, L.C., P.L. Searle and B.K. Daly 1987. Methods for chemical analysis of soils. NZ Soil Bureau Scientific Report. 80:21-45.

Bloom, A.J., F.S. Chapin and H.A. Mooney 1985. Resource Limitation in Plants--An Economic Analogy. Annual Review of Ecology and Systematics. 16:363-392.

Boomsma, D.B. and I.R. Hunter 1990. Effects of water, nutrients and their interactions on tree growth, and plantation forest management practices in Australasia: A review. Forest Ecology and Management. 30:455-476.

Bothwell, K.S., C.E. Prescott and M.D. Jones 2001. Factors contributing to the superior growth and N nutrition of 11-year-old lodgepole pine compared with Sitka spruce on a N-poor cedar-hemlock cutover. Canadian Journal of Forest Research. 31:1272-1279.

Bown, H.E., M.S. Watt, P.W. Clinton, E.G. Mason and B. Richardson 2007. Partititioning concurrent influences of nitrogen and phosphorus supply on photosynthetic model parameters of *Pinus radiata*. Tree Physiology. 27:335-344.

Bremner, J.M. 1965. Inorganic forms of nitrogen. *In* Methods of soil analysis: Part 2, Chemical and Microbiological properties Ed. D.D.E. C.A. Black, J.L. White, L.E. Ensminger and F.E. Clark. American Society of Agronomy, Madison, Wisconsin, USA., pp. 1179-1237.

Brooks, A. and G.D. Farquhar 1985. Effect of temperature on the CO_2/O_2 specificity of ribulose-1,5-bisphosphate carboxylase/oxygenase and the rate of respiration in the light. Planta. 165:397-406.

Burdon, R.D. 1976. Foliar macronutrient concentrations and foliage retention in Radiata Pine clones on four sites. New Zealand Journal of Forestry Science. 5:250-259.

Cannell, M.G.R. and R.C. Dewar 1994. Carbon allocation in trees: a review of concepts for modeling. Advances in Ecological Research. 25 59-104.

Carson, S.D., M.F. Skinner, A.T. Lowe and M.O. Kimberley 2004. Performance differences in *Pinus radiata* progeny with differing site nutrient availability. Canadian Journal of Forest Research. 34:2410-2423.

Carswell, F.E., P. Millard, G.N.D. Rogers and D. Whitehead 2003. Influence of nitrogen and phosphorus supply on foliage growth and internal recycling of nitrogen in conifer seedlings. Functional Plant Biology. 30:49-55.

Chapin, F.S. 1980. The mineral-nutrition of wild plants. Annual Review of Ecology and Systematics. 11:233-260.

Conroy, J.P. 1992. Influence of elevated atmospheric CO_2 concentrations on plant nutrition. Australian Journal of Botany. 40:445-456.

Conroy, J.P., P.J. Milham, M.L. Reed and E.W. Barlow 1990. Increases in phosphorus requirements for CO_2-enriched Pine species. Plant Physiology. 92:977-982.

Conroy, J.P., R.M. Smillie, M. Kuppers, D.I. Bevege and E.W. Barlow 1986. Chlorophyll a fluorescence and photosynthetic and growth responses of *Pinus radiata* to phosphorus deficiency, drought, stress, and high CO_2. Plant Physiology. 81:423-429.

Coops, N.C., R.H. Waring and J.J. Landsberg 1998. Assessing forest productivity in Australia and New Zealand using a physiologically-based model driven with averaged monthly weather data and satellite-derived estimates of canopy photosynthetic capacity. Forest Ecology and Management. 104:113-127.

Cotterill, P.P. and E.K. Nambiar 1981. Seedling physiology of three Radiata pine families with parents of contrasting growth. Australian Forest Research. 11:13-22.

Cown, D.J. 1997. New Zealand forest management and wood quality trends. *In* CTIA/IUFRO International Wood Quality Workshop: Timber management toward wood quality and end-product value Eds. S.Y. Zhang, R. Gosselin and G. Chauret, Quebec City, Canada, pp. 39-54.

Davis, M.R., G. Coker, R.L. Parfitt, R. Simcock, P.W. Clinton, L.G. Garrett and M.S. Watt 2007. Relationships between soil and foliar nutrients in young densely planted mini-plots of *Pinus radiata* and *Cupressus lusitanica*. Forest Ecology and Management. 240:122-130.

De Lucia, E.H., D. Whitehead and M.J. Clearwater 2003. The relative limitation of photosynthesis by mesophyll conductance in co-occurring species in a temperate rainforest dominated by the conifer *Dacridium cupressinum*. Functional Plant Biology. 30:1197-1204.

Ethier, G.J., N.J. Livingston, D.L. Harrison, T.A. Black and J.A. Moran 2006. Low stomatal and internal conductance to CO_2 versus Rubisco deactivation as determinants of the photosynthetic decline of ageing evergreen leaves. Plant Cell and Environment. 29:2168-2184.

Evans, J.R. 1989. Photosynthesis and nitrogen relationships in leaves of C_3 plants. Oecologia. 78:9-19.

Evans, J.R. and S. von Caemmerer 1996. Carbon dioxide diffusion inside leaves. Plant Physiology. 110:339-346.

Farquhar, G.D. and T.D. Sharkey 1982. Stomatal conductance and photosynthesis. Annual Review of Plant Physiology. 33:317-345.

Farquhar, G.D., S. von Caemmerer and J.A. Berry 1980. A biochemical model of photosynthetic CO_2 assimilation in leaves of C_3 species. Planta. 149:78-90.

Farquhar, G.D. and S.C. Wong 1984. An empirical model of stomatal conductance. Australian Journal of Plant Physiology. 11:191-210.

Field, C. and H.A. Mooney 1986. The photosynthesis - nitrogen relationship in wild plants. *In* The Economy of Plant Form and Function Ed. T.J. Givnish. Cambridge University Press, Cambridge, pp. 25-55.

Fife, D.N. and E.K. Nambiar 1982. Accumulation and retranslocation of mineral nutrients in developing needles in relation to seasonal growth of young radiata pine trees. Annals of Botany. 50:817-829.

Fife, D.N. and E.K. Nambiar 1984. Movement of nutrients in Radiata pine needles in relation to the growth of shoots. Annals of Botany. 54:303-314.

Fife, D.N. and E.K. Nambiar 1995. Effect of nitrogen on growth and water relations of Radiata pine families. Plant and Soil. 168:279-285.

Forrest, W.G. and J.D. Ovington 1971. Variation in dry weight and mineral nutrient content of *Pinus radiata* progeny. Silvae Genetica. 20:174-179.

Frak, E., X. Le Roux, P. Millard, S. Guillaumie and R. Wendler 2005. Nitrogen availability, local light regime and leaf rank effects on the amount and sources of N allocated within the foliage of young walnut (*Juglans nigra* x *regia*) trees. Tree Physiology. 26:43-49.

Garcia, O. 1984. New class of growth models for even-aged stands: *Pinus radiata* in Golden Downs forest. New Zealand Journal of Forestry Science. 14:65-88.

Genty, B., J. Briantais and N.R. Baker 1989. The relationship between the quantum yield of photosynthetic electron transport and quenching of chlorophyll fluorescence. Biochimica et Biophysica Acta. 990:87-92.

Giardina, C.P. and M.G. Ryan 2002. Total belowground carbon allocation in a fast-growing *Eucalyptus* plantation estimated using a carbon balance approach. Ecosystems. 5:487-499.

Giardina, C.P., M.G. Ryan, D. Binkley and J.H. Fownes 2003. Primary production and carbon allocation in relation to nutrient supply in a tropical experimental forest. Global Change Biology. 9:1438-1450.

Gillon, J.S. and D. Yakir 2000. Internal conductance to CO_2 diffusion and $C^{18}OO$ discrimination in C_3 leaves. Plant Physiology. 123:201-213.

Grace, J.C. 1987. Theoretical ratio between "one-sided" and total surface area for pine needles. New Zealand Journal of Forestry Science. 17:292-296.

Grassi, G. and F. Magnani 2005. Stomatal, mesophyll conductance and biochemical limitations to photosynthesis as affected by drought and leaf ontogeny in ash and oak trees. Plant Cell and Environment. 28:834-849.

Grassi, G., P. Meier, R. Cromer, D. Tompkins and P.G. Jarvis 2002. Photosynthetic parameters in seedlings of *Eucalyptus grandis* as affected by rate of nitrogen supply. Plant, Cell and Environment. 25:1677-1688.

Griffin, K.L., D.T. Tissue, M.H. Turnbull and D. Whitehead 2000. The onset of photosynthetic acclimation to elevated CO_2 partial pressure in field-grown *Pinus radiata* D.Don after 4 years. Plant, Cell and Environment. 23:1089-1098.

Hall, S.R., V.H. Smith, D.A. Lytle and M.A. Leibold 2005. Constraints on primary producer N:P stoichiometry along N:P supply ratio gradients. Ecology. 86:1894-1904.

Hamilton, J.G., E.H. DeLucia, K. George, S.L. Naidu, A.C. Finzi and W.H. Schlesinger 2002. Forest carbon balance under elevated CO_2. Oecologia. 131:250-260.

Hanba, Y.T., H. Kogami and I. Terashima 2002. The effect of growth irradiance on leaf anatomy and photosynthesis in *Acer* species differing in light demand. Plant Cell and Environment. 25:1021-1030.

Harley, P.C., F. Loreto, G. Di Marco and T.D. Sharkey 1992. Theoretical considerations when estimating the mesophyll conductance to CO_2 flux by analysis of the response of photosynthesis to CO_2. Plant Physiology. 98:1429-1436.

Harley, P.C. and T.D. Sharkey 1991. An improved model of C_3 photosynthesis at high CO_2: Reversed O_2 sensitivity explained by lack of glycerate reentry into the chloroplast. Photosynthesis Research. 27:169-178.

Hawkins, B.J., S.B.R. Kiiskila and G. Henry 1999. Biomass and nutrient allocation in Douglas-fir and amabilis fir seedlings: influence of growth rate and temperature. Tree Physiology. 19:59-63.

Haynes, B.E. and S.T. Gower 1995. Belowground carbon allocation in unfertilized and fertilized red pine plantations in northern Wisconsin. Tree Physiology. 15:317-325.

Haynes, R.J. and K.M. Goh 1978. Ammonium and nitrate nutrition of plants. Biological Reviews. 53:465-510.

Helmisaari, H.S. 1992. Nutrient retranslocation within the foliage of *Pinus sylvestris*. Tree Physiology. 10:45-58.

Hobbie, E.A. 2006. Carbon allocation to ectomycorrhizal fungi correlates with belowground allocation in culture studies. Ecology. 87:563-569.

Hogan, K.P., D. Whitehead, J. Kallarackal, J.G. Buwalda, J. Meekings and G.N.D. Rogers 1996. Photosynthetic activity of leaves of *Pinus radiata* and *Nothofagus fusca* after 1 year of growth at elevated CO_2. Australian Journal of Plant Physiology. 23:623-630.

Hunter, I.R. and J.D. Graham 1982. Growth response of phosphorus-deficient *Pinus radiata* to various rates of superphosphate fertilizer. New Zealand Journal of Forestry Science. 12:49-61.

Hunter, I.R. and J.D. Graham 1983. Three-year response of *Pinus radiata* to several types and rates of phosphorus fertilizer on soils of contrasting phosphorus retention. New Zealand Journal of Forestry Science. 13:229-238.

Hunter, I.R., J.D. Graham, J.M. Prince and G.M. Nicholson 1986. What site factors determine the 4-year basal area response of *Pinus radiata* to nitrogen fertilizer? New Zealand Journal of Forestry Science. 16:30-40.

Hunter, I.R., B.E. Rodgers, A. Dunningham, J.M. Prince and A.J. Thorn 1991. An atlas of Radiata Pine nutrition in New Zealand. *In* New Zealand Forest Research Institute Bulletin No 165, Rotorua, p. 24.

Hunter, I.R. and W. Smith 1996. Principles of forest fertilization - illustrated by New Zealand experience. Fertilizer Research. 43:21-29.

Ichii, K., H. Hashimoto, R. Nemani and M. White 2005. Modelling the interannual variability and trends in gross and net primary productivity of tropical forests from 1982 to 1999. Global and Planetary Change. 48:274-286.

Ingestad, T. 1971. A definition of optimum nutrient requirements in Birch Seedlings. II. Physiologia Plantarum. 24:118-125.

Ingestad, T. 1979. Mineral nutrient requirements of *Pinus silvestris* and *Picea abies* seedlings. Physiologia Plantarum. 45:373-380.

Ingestad, T. 1982. Relative addition rate and external concentration; driving variables used in plant nutrition research. Plant, Cell and Environment:443-453.

Ingestad, T. and A. Lund 1986. New concepts on soil fertility and plant nutrition as illustrated by research on forest trees and stands. Geoderma. 40:237-252.

Ingestad, T. and A.B. Lund 1986. Theory and techniques for steady state mineral nutrition and growth of plants. Scandinavian Journal of Forest Research. 1:439-453.

Jarvis, P.G. 1995. Scaling processes and problems. Plant Cell and Environment. 18:1079-1089.

Johnsen, K., L. Samuelson, R. Teskey, S. McNulty and T. Fox 2001. Process models as tools in forestry research and management. Forest Science. 47:2-8.

Johnson, J.D. 1984. A rapid technique for estimating total surface area of pine needles. Forest Science. 30:913-921.

Kellomäki, S. and K.Y. Wang 1996. Photosynthetic responses to needle water potentials in Scots pine after a four-year exposure to elevated CO_2 and temperature. Tree Physiology. 16:765-772.

Keyes, M.R. and C.C. Grier 1981. Above- and below-ground net production in 40-year-old Douglas fir stands on low and high productivity sites. Canadian Journal of Forest Research. 11:599-605.

Kimmins, J.P., P.G. Comeau and W. Kurz 1990. Modelling the interactions between moisture and nutrients in the control of forest growth. Forest Ecology and Management. 30:361-379.

Kimmins, J.P. and K.A. Scoullar 1984. The role of modelling in tree nutrition research and site nutrient management. *In* Nutrition of plantation forests Eds. G.D. Bowen and E.K. Nambiar. Academic Press, London. 463-487. 516 pp.

Knecht, M.F. and A. Göransonn 2004. Terrestrial plants require nutrients in similar proportions. Tree physiology. 24:447-460.

Knight, P.J. 1978. Foliar concentrations of ten mineral nutrients in nine *Pinus radiata* clones during a 15-month period. New Zealand Journal of Forestry Science. 8:351-368.

Landsberg, J. 2003. Modelling forest ecosystems: State of the art, challenges, and future directions. Canadian Journal of Forest Research. 33:385-397.

Landsberg, J.J. 1986. Experimental approaches to the study of the effect of nutrients and water on carbon assimilation by trees. Tree Physiology. 2:427-444.

Landsberg, J.J. 1986. Physiological ecology of forest production. Academic Press, London. 198 p.

Landsberg, J.J. and S.T. Gower 1997. Applications of physiological ecology to forest management. Academic Press, San Diego. 354 p.

Landsberg, J.J. and F.J. Hingston 1996. Evaluating a simple radiation/dry matter conversion model using data from *Eucalyptus globulus* plantations in Western Australia. Tree Physiology. 16:801-808.

Landsberg, J.J., M.R. Kaufmann, D. Binkley, J. Isebrands and P.G. Jarvis 1991. Evaluating progress toward closed forest models based on fluxes of carbon, water and nutrients. Tree Physiology. 9:1-15.

Landsberg, J.J. and R.H. Waring 1997. A generalized model of forest productivity using simplified concepts of radiation-use efficiency, carbon balance and partitioning. Forest Ecology and Management. 95:209-228.

Law, B.E., R.H. Waring, P.M. Anthoni and J.D. Aber 2000. Measurements of gross and net ecosystem productivity and water vapour exchange of a *Pinus ponderosa* ecosystem, and an evaluation of two generalized models. Global Change Biology. 6:155-168.

Leuning, R. 1995. A critical appraisal of a combined stomatal-photosynthesis model for C_3 plants. Plant, Cell and Environment. 18:339-355.

Leuning, R., F.M. Kelliher, D.G.G. De Pury and E.D. Schulze 1995. Leaf nitrogen, photosynthesis, conductance and transpiration scaling from leaves to canopies. Plant, Cell and Environment. 18:1183-1200.

Lewis, D.L., K.L. Griffin, R.B. Thomas and B.R. Strain 1994. Phosphorus supply affects the photosynthetic capacity of loblolly pine grown in elevated carbon dioxide. Tree Physiology. 14:1229-1244.

Lewis, N.B. and I.S. Ferguson 1993. Management of radiata pine. Inkata Press, Melbourne. 404 p.

Long, S.P. and C.J. Bernacchi 2003. Gas exchange measurements, what can they tell us about the underlying limitations to photosynthesis? Procedures and sources of error. Journal of Experimental Botany. 54:2393-2401.

Loreto, F., P.C. Harley, G. Di Marco and T.D. Sharkey 1992. Estimation of mesophyll conductance to CO_2 flux by three different methods. Plant Physiology. 98:1437-1443.

Loustau, D., M. Brahim, J.P. Gaudillere and E. Dreyer 1999. Photosynthetic responses to phosphorus nutrition in two-year-old maritime pine seedlings. Tree Physiology. 19:707-715.

Lowell, K.E. 1986. A growth model for fertilized Radiata Pine in Golden Downs forests, New Zealand. Australian Forest Research. 16:261-271.

MacLaren, J.P. 1993. Radiata Pine Grower's Manual. New Zealand Forest Research Institute Limited. FRI Bulletin No 184. 140 p.

Madgwick, H.A.I. 1994. *Pinus radiata* : biomass, form and growth. H.A.I. Madgwick, Rotorua, N.Z. 428 p.

Mäkelä, A. 1986. Implications of the pipe model theory on dry matter partitioning and height growth in trees. Journal of Theoretical Biology. 123:103-120.

Mäkelä, A. 2003. Process-based modelling of tree and stand growth: towards a hierarchical treatment of multiscale processes. Canadian Journal of Forest Research. 33:398-409.

Mäkelä, A., J.J. Landsberg, A.R. Ek, T.E. Burk, M. Ter-Mikaelian, G.I. Agren, C.D. Oliver and P. Puttonen 2000. Process-based models for forest ecosystem management: current state of the art and challenges for practical implementation. Tree Physiology. 20:289-298.

Manter, D.K. and J. Kerrigan 2004. A/C_i curve analysis across a range of woody plant species: influence of regression analysis parameters and mesophyll conductance. Journal of Experimental Botany. 55:2581-2588.

Marschner, H. 1995. Mineral nutrition of higher plants. Academic Press, London. 889 p.

Mason, E.G. and P.G. Milne 1999. Effects of weed control, fertilization and soil cultivation on the growth of *Pinus radiata* at midrotation in Canterbury, New Zealand. Canadian Journal of Forest Research. 29:985-992.

Mason, E.G., R.W. Rose and L.S. Rosner 2007. Time vs light: A potentially useable light sum hybrid model to represent the juvenile growth of Douglas-fir subject to varying levels of competition. Canadian Journal of Forest Research. 37: 795-805.

McDowell, N.G., N.J. Balster and J.D. Marshall 2001. Belowground carbon allocation of Rocky Mountain Douglas-fir. Canadian Journal of Forest Research. 31:1425-1436.

McLaren, R.G. and K.C. Cameron 1996. Soil Science: Sustainable Production and Environmental Protection. Oxford University Press, Victoria, Australia. 304 p.

McMurtrie, R.E., M.L. Benson, S. Linder, S.W. Running, T. Talsma, W.J.B. Crane and B.J. Myers 1990*b*. Water / nutrient interactions affecting the productivity of stands of *Pinus radiata*. Forest Ecology and Management. 30:415-423.

McMurtrie, R.E., H.L. Gholz, S. Linder and S.T. Gower 1994. Climatic factors controlling the productivity of pine stands: a model based analysis. *In* Ecological Bulletins No 43: Environmental constraints on the structure and productivity of pine forest ecosystems: a comparative analysis Eds. H.L. Gholz, S. Linder and R.E. McMurtrie, Copenhagen.

McMurtrie, R.E. and J.J. Landsberg 1992. Using a simulation model to evaluate the effects of water and nutrients on the growth and carbon partitioning of *Pinus radiata*. Forest Ecology and Management. 52:243-260.

McMurtrie, R.E., R. Leuning, W.A. Thomson and A.M. Wheeler 1992. A model of canopy photosynthesis and water use incorporating a mechanistic formulation of leaf CO_2 exchange. Forest Ecology and Management. 52:261-278.

McMurtrie, R.E., D.A. Rook and F.M. Kelliher 1990*a*. Modelling the yield of *Pinus radiata* on a site limited by water and nitrogen. Forest Ecology and Management. 30:381-413.

McMurtrie, R.E. and L. Wolf 1983. Above- and below-ground growth of forest stands: a carbon budget model. Annals of Botany. 52:437-448.

Mead, D.J. 1984. Diagnosis of nutrient deficiencies in plantations. *In* Nutrition of plantation forests Eds. G.D. Bowen and E.K. Nambiar. Academic Press, London. 259-291. 516 pp.

Mead, D.J. 2005*a*. Fertilising. *In* 2005 Forestry Handbook Ed. M. Colley. New Zealand Institute of Forestry (Inc.), 2005., Christchurch, N.Z, pp. 110-112.

Mead, D.J. 2005*b*. Opportunities for improving plantation productivity. How much? How quickly? How realistic? Biomass and Bioenergy. 28:249-266.

Mead, D.J. and R.L. Gadgil 1978. Fertilizer use in established radiata pine stands in New Zealand. New Zealand Journal of Forestry Science. 8:105-134.

Mead, D.J. and C.M. Preston 1994. Distribution and retranslocation of ^{15}N in lodgepole pine over eight growing seasons. Tree Physiology. 14:389-402.

Millard, P. and M.F. Proe 1992. Storage and internal cycling of nitrogen in relation to seasonal growth of Sitka spruce. Tree Physiology. 10:33-43.

Millard, P. and M.F. Proe 1993. Nitrogen uptake, partitioning and internal cycling in *Picea sitchensis* (Bong.) Carr. as influenced by nitrogen supply. New Phytologist. 125:113-119.

Miller, B.D. and B.J. Hawkins 2003. Nitrogen uptake and utilization by slow- and fast-growing families of interior spruce under contrasting fertility regimes. Canadian Journal of Forest Research. 33:959-966.

Miller, B.D. and B.J. Hawkins 2007. Ammonium and nitrate uptake, nitrogen productivity and biomass allocation in interior spruce families with contrasting growth rates and mineral nutrient preconditioning. Tree Physiology. 27:901-909.

Miyazawa, S.I. and I. Terashima 2001. Slow development of leaf photosynthesis in an evergreen broad-leaved tree, *Castanopsis sieboldii*: relationships between leaf anatomical characteristics and photosynthetic rate. Plant Cell and Environment. 24:279-291.

Monteith, J.L. 1977. Climate and the efficiency of crop production in Britain. Philosophical Transactions of The Royal Society of London Series B-Biological Sciences. 281:277-294.

Mooney, H.A. and S.L. Gulmon 1982. Constraints on leaf structure and function in reference to herbivory. Bioscience. 32:199-206.

Murray, M.B., R.I. Smith, A. Friend and P.G. Jarvis 2000. Effect of elevated [CO_2] and varying nutrient application rates on physiology and biomass accumulation of Sitka spruce (*Picea sitchensis*). Tree Physiology. 20:421-434.

Nadelhoffer, K.J. 2000. The potential effects of nitrogen deposition on fine-root production in forest ecosystems. New Phytologist. 147:131-139.

Nadelhoffer, K.J., J.D. Aber and J.M. Melillo 1985. Fine roots, net primary production, and soil nitrogen availability: a new hypothesis. Ecology. 66:1377-1390.

Nadelhoffer, K.J. and J.W. Raich 1992. Fine root production estimates and belowground carbon allocation in forest ecosystems. Ecology. 73:1139-1147.

Nambiar, E.K. 1984. Plantation forests: their scope and a perspective on plant nutrition. *In* Nutrition of plantation forests Eds. G.D. Bowen and E.K. Nambiar. Academic Press, London. 1-15. 516 pp.

Nambiar, E.K. 1990. Interplay between nutrients, water, root growth and productivity in young plantations. Forest Ecology and Management. 30:213-232.

Nambiar, E.K. 1990. Management of forests under nutrient and water stress. Water, Air and Soil Pollution. 54:209-230.

Nambiar, E.K. and G.D. Bowen 1986. Uptake, distribution and retranslocation of nitrogen by *Pinus radiata* from ^{15}N-labelled fertilizer applied to podzolized sandy soil. Forest Ecology and Management. 15:269-284.

Nambiar, E.K. and D.N. Fife 1987. Growth and nutrient retranslocation in needles of Radiata pine in relation to nitrogen supply. Annals of Botany. 60:147-156.

Nambiar, E.K. and D.N. Fife 1991. Nutrient retranslocation in temperate conifers. Tree Physiology. 9:185-207.

Nambiar, E.K.S. 1987. Do nutrients retranslocate from fine roots? Canadian Journal of Forest Research. 17:913-918.

Nasholm, T. and A.J.S. McDonald 1990. Dependence of amino acid composition upon nitrogen availability in birch (*Betula pendula*). Physiologia Plantarum. 80:507-514.

New Zealand Ministry for the Environment 2006. Projected balance of emissions units during the first commitment period of the Kyoto Protocol. Ministry for the Environment Technical Report, p. 64.

Newman, G.S., M.A. Arthur and R.N. Muller 2006. Above- and belowground net primary production in a temperate mixed deciduous forest. Ecosystems. 9:317-329.

Nordborg, F., U. Nilsson and G. Olander 2003. Effects of different soil treatments on growth and net nitrogen uptake of newly planted *Picea abies* (L.) Karst. seedlings. Forest Ecology and Management. 180:571-582.

NZFOA 2007. Facts and Figures 2006/2007. New Zealand Forest Owners Association, Wellington, p. 28.

Oleksyn, J., P.B. Reich, R. Zytkowiak, P. Karolewski and M.G. Tjoelker 2003. Nutrient conservation increases with latitude of origin in European *Pinus sylvestris* populations. Oecologia. 136:220-235.

Ostonen, I., K. Lohmus and K. Pajuste 2005. Fine root biomass, production and its proportion of NPP in a fertile middle-aged Norway spruce forest: comparison of soil core and ingrowth core methods. Forest Ecology and Management. 212:264-277.

Parfitt, R.L., D.J. Ross, D.A. Coomes, S.J. Richardson, M.C. Smale and R.A. Dahlgren 2005. N and P in New Zealand soil chronosequences and relationships with foliar N and P. Biogeochemistry. 75:305-328.

Parfitt, R.L., N.A. Scott, D.J. Ross, G.J. Salt and K.R. Tate 2003. Land-use change effects on soil C and N transformations in soils of high N status: comparisons under indigenous forest, pasture and pine plantation. Biogeochemistry. 66:203-221.

Peisker, M. and H. Apel 2001. Inhibition by light of CO_2 evolution from dark respiration: Comparison of two gas exchange methods. Photosynthesis Research. 70 291-298.

Penning de Vries, F.W.T. 1972. Respiration and growth. *In* Crop processes in controlled environments Ed. K.E.C. A.R. Rees, D.W. Hand and R.G. Hurd. Academic Press, London, pp. 327-347.

Penning de Vries, F.W.T. 1975. Use of assimilates in higher plants. *In* Photosynthesis and productivity in different environments Ed. J.P. Cooper. Cambridge University Press, Cambridge, pp. 459-480.

Piel, C., E. Frak, X. Le Roux and B. Genty 2002. Effect of local irradiance on CO_2 transfer conductance of mesophyll in walnut. Journal of Experimental Botany. 53:2423-2430.

Pons, T.L. and R.A.M. Welschen 2002. Overestimation of respiration rates in commercially available clamp-on leaf chambers. Complications with measurement of net photosynthesis. Plant Cell and Environment. 25:1367-1372.

Proe, M.F., A.J. Midwood and J. Craig 2000. Use of stable isotopes to quantify nitrogen, potassium and magnesium dynamics in young Scots pine (*Pinus sylvestris*). New Phytologist. 146:461-469.

Proe, M.F. and P. Millard 1994. Relationship between nutrient supply, nitrogen partitioning and growth in young Sitka spruce (*Picea sitchensis*). Tree Physiology. 14:75-88.

Proe, M.F. and P. Millard 1995. Effect of P supply upon seasonal growth and internal cycling of P in sitka sprice (*Picea sitchensis* (Bong) Carr) seedlings. Plant and Soil. 168:313-317.

Proe, M.F., H.M. Rauscher and J. Yarie 1994. Computer simulation models and expert systems for predicting productivity decline. *In* Impacts of forest harvesting on long-term site productivity Eds. W.J. Dyck, D.W. Cole and N.B. Comerford. Chapman & Hall, London, pp. vii, 371.

Raich, J.W. and K.J. Nadelhoffer 1989. Belowground carbon allocation in forest ecosystems: global trends. Ecology. 70:1346-1354.

Raison, R.J. and B.J. Myers 1992. The Biology of Forest Growth experiment: linking water and nitrogen availability to the growth of *Pinus radiata*. Forest Ecology and Management. 52:279-308.

Read, D.J. 1991. The role of the mycorrhizal symbiosis in the nutrition of plant communities. *In* Ecophysiology of ectomycorrhizae of forest trees Ed. T.M.W. Foundation, Falun, Sweden, pp. 27-53.

Reich, P.B., D.F. Grigal, J.D. Aber and S.T. Gower 1997. Nitrogen mineralization and productivity in 50 hardwood and conifer stands on diverse soils. Ecology. 78:335-347.

Reich, P.B. and A.W. Schoettle 1988. Role of phosphorus and nitrogen in photosynthetic and whole plant carbon gain and nutrient use efficiency in eastern white pine. Oecologia. 77:25-33.

Ripullone, F., G. Grassi, M. Lauteri and M. Borghetti 2003. Photosynthesis-nitrogen relationships: interpretation of different patterns between *Pseudotsuga menziesii* and *Populus x euroamericana* in a mini-stand experiment. Tree Physiology. 23:137-144.

Ryan, M.G. 1991*a*. Effects of climate change on plant respiration. Ecological Applications. 1:157-167.

Ryan, M.G. 1991*b*. A simple method for estimating gross carbon budgets for vegetation in forest ecosystems. Tree Physiology. 9:255-266.

Ryan, M.G., S.T. Gower, R.M. Hubbard, R.H. Waring, H.L. Gholz, W.P. Cropper and S.W. Running 1995. Woody tissue maintenance respiration of four conifers in contrasting climates. Oecologia. 101:133-140.

Ryan, M.G., R.M. Hubbard, S. Pongracic, R.J. Raison and R.E. McMurtrie 1996. Foliage, fine-root, woody-tissue and stand respiration in *Pinus radiata* in relation to nitrogen status. Tree Physiology. 16:333-343.

Ryan, M.G., M.B. Lavigne and S.T. Gower 1997. Annual carbon cost of autotrophic respiration in boreal forest ecosystems in relation to species and climate. Journal of Geophysical Research 102:28871-28883.

Sage, R.F. and R.W. Pearcy 1987. The nitrogen use efficiency of C_3 and C_4 plants. Plant Physiology. 84:959-963.

Salifu, K.F. and V.R. Timmer 2001. Nutrient retranslocation response of *Picea mariana* seedlings to nitrogen supply. Soil Science Society of America Journal. 65:905-913.

Salifu, K.F. and V.R. Timmer 2003. Nitrogen retranslocation response of young *Picea mariana* to nitrogen-15 supply. Soil Science Society of America Journal. 67:309-317.

Sands, R. and D.R. Mulligan 1990. Water and nutrient dynamics and tree growth. Forest Ecology and Management. 30:91-111.

Schreiber, U., W. Bilger and C. Neubauer 1994. Chrorophyll fluorescence as a nonintrusive indicator for rapid assessment of *in vivo* photosynthesis. *In* Ecophysiology of photosynthesis Eds. E.D. Schulze and M.M. Caldwell. Springer Verlag, Berlin, pp. 49-70.

Sharkey, T.D. 1985. Photosynthesis in intact leaves of C_3 plants: physics, physiology and rate limitations. The Botanical Review. 51:54-105.

Sheriff, D.W., E.K.S. Nambiar and D.N. Fife 1986. Relationship between nutrient status, carbon assimilation and water use efficiency in *Pinus radiata* (D. Don) needles. Tree Physiology. 2:73-88.

Singsaas, E.L., D.R. Ort and E.H. De Lucia 2003. Elevated CO_2 effects on mesophyll conductance and its consequences for interpreting photosynthetic physiology. Plant, Cell and Environment. 27:41-50.

Skinner, M.F. and P.M. Attiwill 1981. The productivity of pine plantations in relation to previous land use. Plant and Soil. 60:161-176.

Smith, C.T., A.T. Lowe, M.F. Skinner, P.N. Beets, S.H. Schoenholtz and S. Fang 2002. Response of radiata pine forests to residue management and fertilization across a fertility gradient in New Zealand. Forest Ecology and Management. 138:203-223.

Soderstrom, B. 1991. The fungal partner in the mycorrhizal symbiosis. *In* Ecophysiology of ectomycorrhizae of forest trees Ed. T.M.W. Foundation, Falun, Sweden, pp. 5-26.

Sorensson, C.T., D.J. Cown, B.J. Ridoutt and X. Tian 1997. The significance of wood quality in tree breeding: A case study of Radiata Pine in New Zealand. *In* CTIA/IUFRO International Wood Quality Workshop: Timber management toward wood quality and end-product value Eds. S.Y. Zhang, R. Gosselin and G. Chauret, Quebec City, Canada, pp. IV-35-44.

Sorensson, C.T. and C.J. Shelbourne 2005. Clonal forestry. *In* 2005 Forestry Handbook Ed. M. Colley. New Zealand Institute of Forestry (Inc.), 2005., Christchurch, N.Z, pp. 92-96.

Springob, G. and H. Kirchmann 2003. Bulk soil C to N ratio as a simple measure of net N mineralization from stabilized soil organic matter in sandy arable soils. Soil Biology and Biochemistry. 35:629-632.

Stape, J.L. 2002. Production ecology of clonal *Eucalyptus* plantations In northeastern Brazil. *In* Department of Forest Sciences. Colorado State University, Fort Collins, Colorado, p. 233.

Stape, J.L., D. Binkley, W.S. Jacob and E.N. Takahashi 2006. A twin-plot approach to determine nutrient limitation and potential productivity in Eucalyptus plantations at landscape scales in Brazil. Forest Ecology and Management. 223:358-362.

Stape, J.L., D. Binkley and M.G. Ryan 2004*b*. Eucalyptus production and the supply, use and efficiency of use of water, light and nitrogen across a geographic gradient in Brazil. Forest Ecology and Management. 193:17-31.

Stape, J.L., M.G. Ryan and D. Binkley 2004*a*. Testing the utility of the 3-PG model for growth of *Eucalyptus grandis x urophylla* with natural and manipulated supplies of water and nutrients. Forest Ecology and Management. 193:219-234.

Stenberg, P., T. Kuuluvainen, S. Kellomaki, J.C. Grace, E.J. Jokela and H.L. Gholz 1994. Crown structure, light interception and productivity of pine trees and stands. *In* Ecological Bulletins No 43: Environmental constraints on the structure and productivity of pine forest ecosystems: a comparative analysis Eds. H.L. Gholz, S. Linder and R.E. McMurtrie, Copenhagen.

Stockfors, J. and S. Linder 1998. Effect of nitrogen on the seasonal course of growth and maintenance respiration in stems of Norway spruce trees. Tree physiology. 18:155-166.

Sylvia, D.M. and A.G. Jarstfer 1997. Distribution of mycorrhiza on competing pines and weeds in a southern pine plantation. Soil Science Society of America Journal. 61:139-144.

Takashima, T., K. Hikosaka and T. Hirose 2004. Photosynthesis or persistence: nitrogen allocation in leaves of evergreen and deciduous *Quercus* species. Plant Cell and Environment. 27:1047-1054.

Terashima, I. and K. Ono 2002. Effects of $HgCl_2$ on CO_2 dependence of leaf photosynthesis: Evidence indicating involvement of aquaporins in CO_2 diffusion across the plasma membrane. Plant Cell Physiology. 43:70-78.

Thornley, J.H.M. 1969. A model to describe the partitioning of photosynthate during vegetative plant growth. Annals of Botany. 33:419-430.

Thornley, J.H.M. 1972. A balanced quantitative model fro root : shoot ratios in vegetative plants. Annals of Botany. 36:431-441.

Thornley, J.H.M. 1991. A transport-resistance model of forest growth and partitioning. Annals of Botany. 68:221-226.

Turnbull, M.H., R. Murthy and K.L. Griffin 2002. The relative impacts of daytime and night-time warming on photosynthetic capacity in *Populus deltoides*. Plant, Cell and Environment. 25:1729-1737.

Turnbull, M.H., D.T. Tissue, K.L. Griffin, G.N.D. Rogers and D. Whitehead 1998. Photosynthetic acclimation to long-term exposure to elevated CO_2 concentration in *Pinus radiata* D.Don is related to age of needles. Plant, Cell and Environment. 21:1019-1028.

Turner, D.P., W.D. Ritts, W.B. Cohen, S.T. Gower, S.W. Running, M. Zhao, M.H. Costa, A.A. Kirschbaum, J. Ham, M., S.R. Saleska and D.E. Ahl 2006. Evaluation of MODIS NPP and GPP products across multiple biomes. Remote Sensing and Environment. 102:282-292.

Turner, J. and M.J. Lambert 1986. Nutrition and nutritional relationships of *Pinus radiata*. Annual Review of Ecology and Systematics. 17:325-350.

Tyerman, S.D., C.M. Niemietz and H. Bramley 2002. Plant aquaporins: multifunctional water and solute channels with expanding roles. Plant Cell and Environment. 25:173-194.

Valentine, H.T. 1985. Tree-growth models: derivations employing the Pipe-model theory. Journal of Theoretical Biology. 117:579-585.

Valentine, H.T. and A. Mäkelä 2005. Bridging process-based and empirical apporaches to modeling tree growth. Tree Physiology. 25:769-779.

Villar, R., A.A. Held and J. Merino 1995. Dark leaf respiration in light and darkness of an evergreen and a deciduous plant species. Plant Physiology. 107:421-427.

Vincent, T.G. and J.S. Dunstan 1989. Register of commercial seedlots issued by the New Zealand Forest Service. Forest Research Institute Report. 44

Vitousek, P.M., P.A. Matson and K. Van Cleve 1989. Nitrogen availability and nitrification during succesion: Primary, secondary, and old-field seres. Plant and Soil. 115:229-239.

von Caemmerer, S. 2000. Biochemical models of leaf photosynthesis. CSIRO Publishing, Collingwood,Victoria, Australia. 165 p.

von Caemmerer, S. and J.R. Evans 1991. Determination of the average partial pressure of CO_2 in chloroplasts from leaves of several C_3 plants. Australian Journal of Plant Physiology. 18:287-305.

von Caemmerer, S. and G.D. Farquhar 1981. Some relationships between the biochemistry of photosynthesis and the gas exchange of leaves. Planta. 153:376-387.

Walcroft, A.S., D. Whitehead, W.B. Silvester and F.M. Kelliher 1997. The response of photosynthetic model parameters to temperature and nitrogen concentration in *Pinus radiata* D. Don. Plant Cell and Environment. 20:1338-1348.

Waring, R.H., J.J. Landsberg and J. Williams 1998. Net primary production of forests: a constant fraction of gross primary production? Tree Physiology. 1998:129-134.

Warren, C.R. 2006. Why does photosynthesis decrease with needle age in *Pinus pinaster*? Trees. 20:157-164.

Warren, C.R. and M.A. Adams 2002. Phosphorus affects growth and partitioning of nitrogen to Rubisco in *Pinus pinaster*. Tree Physiology. 22:11-19.

Warren, C.R. and M.A. Adams 2006. Internal conductance does not scale with photosynthetic capacity: implications for carbon isotope discrimination and the economics of water and nitrogen use in photosynthesis. Plant Cell and Environment. 29:192-201.

Warren, C.R., E. Dreyer and M.A. Adams 2003*a*. Photosynthesis-Rubisco relationships in foliage of *Pinus sylvestris* in response to nitrogen supply and the proposed role of Rubisco and amino acids as nitrogen stores. Trees. 17:359-366.

Warren, C.R., G.J. Ethier, N.J. Livingston, N.J. Grant, D.H. Turpin, D.L. Harrison and T.A. Black 2003*b*. Transfer conductance in second growth Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) canopies. Plant, Cell and Environment. 26:1215-1227.

Warren, C.R., N.J. Livingston and D.H. Turpin 2004. Water stress decreases the transfer conductance of Douglas-fir (*Pseudotsuga menziesii*) seedlings. Tree Physiology. 24:971-979.

Watt, M.S., G. Coker, P.W. Clinton, M.R. Davis, R. Parfitt, R. Simcock, L. Garret, T.W. Payn, B. Richardson and A. Dunningham 2005. Defining sustainability of plantation forests through identification of site quality indicators influencing productivity- a national view for New Zealand. Forest Ecology and Management. 216:51-63.

Webber, B. 1978. Potential increase in nutrient requirements of *Pinus radiata* under intensified management. New Zealand Journal of Forestry Science. 8:146-159.

Whitehead, D., G.M.J. Hall, A.S. Walcroft, K.J. Brown, J.J. Landsberg, D.T. Tissue, M.H. Turnbull, K.L. Griffin, W.S.F. Schuster, F.E. Carswell, C.M. Trotter, I.L. James and D.A. Norton 2002. Analysis of the growth of rimu (*Dacrydium cupressinum*) in South Westland, New Zealand, using process-based simulation models. International Journal of Biometeorology. 46:66-75.

Whitehead, D. and F.M. Kelliher 1991. Modeling the water balance of a small *Pinus radiata* catchment. Tree Physiology. 9:17-33.

Whitehead, D., J.R. Leathwick and A.S. Walcroft 2001. Modeling annual carbon uptake for the indigenous forests of New Zealand. Forest Science. 47:9-20.

Whitehead, D. and A.S. Walcroft 2005. Forest and shrubland canopy carbon uptake in relation to foliage nitrogen concentration and leaf area index: a modelling analysis. Annals of Forest Science. 62:525-535.

Whitehead, D., A.S. Walcroft, K.L. Griffin, D.T. Tissue, M.H. Turnbull, V.C. Engel, K.J. Brown and W.S.F. Schuster 2004*a*. Scaling carbon uptake from leaves to canopies: Insights from two forests with contrasting properties. *In* Forests at the Land-Atmosphere Interface Ed. J.G. M. Mencuccini, J. Moncrieff and K.G. McNaughton. CAB International, pp. 231-254.

Whitehead, D., A.S. Walcroft, N.A. Scott, J.A. Towsend, C.M. Trotter and G.N.D. Rogers 2004*b*. Characteristics of photosynthesis and stomatal conductance in the shrubland species manuka (*Leptospermum scoparium*) and kanuka (*Kunzea ericoides*) for the estimation of annual carbon uptake. Tree Physiology. 24:795-804.

Will, G.M. 1965. Increased phosphorus uptake by Radiata Pine in Riverhead Forest following superphosphate applications. New Zealand Journal of Forestry. 10:33-42.

Will, G.M. 1978. Nutrient Deficiencies in Pinus-Radiata in New-Zealand. New Zealand Journal of Forestry Science. 8:4-14.

Will, G.M. 1985. Nutrient deficiencies and fertiliser use in New Zealand exotic forests. Forest Research Institute Bulletin No 97, Rotorua, New Zealand, p. 53.

Williams, J. and D. Eamus 1997. Plant ecophysiology: linking pattern and process - a Review. Australian Journal of Botany. 1997:351-357.

Woollons, R.C., A.G.D. Whyte and D.J. Mead 1988. Long-term growth responses in *Pinus radiata* fertilizer experiments. New Zealand Journal of Forestry Science. 18:199-209.

Wright, I.J., P.B. Reich, M. Westoby, D.D. Ackerly, Z. Baruch, F. Bongers, J. Cavender-Bares, T. Chapin, J.H.C. Cornelissen, M. Diemer, J. Flexas, E. Garnier, P.K. Groom, J. Gulias, K. Hikosaka, B.B. Lamont, T. Lee, W. Lee, C. Lusk, J.J. Midgley, M. Navas, Ü. Niinemets, J. Oleksyn, N. Osada, H. Poorter, P. Poot, L. Prior, V.I. Pyankov, C. Roumet, S.C. Thomas, M.G. Tjoelker, E.J. Veneklaas and R. Villar 2004. The worlwide leaf economics spectrum. Nature. 428:821-827.

Wullschleger, S.D. 1993. Biochemical limitations to carbon assimilation in C_3 plants - A retrospective analysis of the A/C_i curves from 109 species. Journal of Experimental Botany. 44:907-920.

Zerihun, A. and K.D. Montagu 2004. Belowground to aboveground biomass ratio and vertical root distribution responses of mature Pinus radiata stands to phosphorus fertilization at planting Canadian Journal of Forest Research. 34 1883-1894.

APPENDIX A

CHLOROPHYLL FLUORESCENCE RESPONSES OF *PINUS RADIATA* CLONES TO NITROGEN AND PHOSPHORUS SUPPLY

Summary Chlorophyll fluorescence responses were measured in five clones of *Pinus radiata* D. Don cultivated in a glasshouse in a factorial combination of nitrogen and phosphorus supply. Plant growth in stem diameter, height and leaf area scaled with nutrient supply and were also influenced by clone. Fascicle size (mass) conformed to plant growth increasing with nutrient supply being larger in the fast- compared with the slow-growing clone. Dark (F_v/F_m) and light-adapted (Φ_{PSII}) photochemical efficiency of PSII were highest at high N, high P supply compared to lower N and P rates. In contrast, Stern-Volmer non-photochemical quenching (N_q) which relates to the proportion of energy dissipated as heat, increased as plants became more N and P deficient. Chlorophyll fluorescence variables did not differ between clones. Plants were mainly limited by N at month 6 probably because they were still relying on internal P reserves from planted cuttings. Accordingly, chlorophyll fluorescence variables were correlated with foliage N but not with foliage P. However, there were positive (but not always significant) linear relationships between photosynthetic rates at ambient CO_2 concentration and saturation irradiance (A_{sat}), F_v/F_m and Φ_{PSII} and both foliar nitrogen (N_a) and phosphorus (P_a) concentration on an area basis independent of clones at months 9 and 18 when a ratio N_a/P_a equal to 23 mol mol^{-1} was used to partition N from P deficiencies. These findings suggest that in genotypes with contrasting growth performance the response of F_v/F_m and Φ_{PSII} to nutrient limitation is equivalent. Light-use efficiency may be reduced in poor-fertility environments as a consequence of decreased photochemical activity, increased heat dissipation capacity and a reduction in leaf area and fascicle size. The study also suggests that genotypes may be represented in productivity process-based models by allocating more leaf area to those genotypes growing faster, but not by modifying their photochemical efficiency or photosynthetic performance.

Keywords: chlorophyll fluorescence, quantum efficiency of PSII, electron transport, genotype, nutrient limitation.

INTRODUCTION

Photochemical efficiency of PSII and phenology can profoundly influence productivity, and the extent to which nutritional supply and genotype might affect these processes was studied in two clones of radiata pine.

The efficiency by which light is harvested by photosystem II (PS II) reaction centers of the electron transport system can be assessed using chlorophyll fluorescence techniques (Genty et al. 1989). PSII is highly sensitive and easily damaged by environmental stresses (Ball et al. 1994, Krause and Weis 1991, Maxwell and Johnson 2000) such as extreme temperatures, light, nutrient and water limitations (Bolhar-Nordenkampf and Oquist 1993). Because chlorophyll fluorescence can be measured easily and rapidly, it allows study of physiological responses at scales relevant to population ecology (Ball et al. 1994). For instance, chlorophyll fluorescence has been used to assess frost hardiness in *Pinus halepensis* (Puertolas et al. 2005), to compare healthy and virus-infected plants of *Brassica juncea* (Guo et al. 2005), to assess chilling-dependent photoinhibition of *Eucalyptus nitens* (Close and Beadle 2003), to examine sink limitation effects on photosynthetic performance of *Abies balsamea* (Lavigne et al. 2001) and to discriminate photoprotection from photodamage in *Actinidia deliciosa* (Greer 1995).

Chlorophyll is located in the pigment-protein complexes embedded in the thylakoid membrane where excitation energy is funnelled into the reaction centres (P_{680} = PSII, P_{700} = PSI) and converted into chemical energy (NADPH and ATP) which is required to drive the photosynthetic carbon reduction (Calvin) cycle (Schreiber et al. 1994, Seibert 1995, Blankenship 2002). Light energy absorbed by chlorophyll molecules can be used to drive photosynthesis (photochemistry), dissipated as heat or re-emitted as chlorophyll fluorescence (Krause and Weis 1991). As these processes are mutually exclusive, changes in the efficiency of photochemistry and heat dissipation can be determined by measuring chlorophyll fluorescence (Maxwell and Johnson 2000). At room temperature, variable fluorescence originates almost exclusively from PSII and therefore fluorescence changes reflect primarily the state of PSII (Schreiber et al. 1994). The ratio of variable to maximum chlorophyll fluorescence (F_v/F_m) is a relative measure of the maximum efficiency of excitation energy captured when all PS II centres are open with typical values of about 0.80-0.83 for a wide variety of dark-adapted non-stressed C_3 plants (Ball et al 1994, Krause and Weis 1991). In light-adapted leaves, the quantum efficiency of open PSII centres (Φ_{PSII}) represents the efficiency with which excitation energy captured by the light-harvesting antennae is passed on to PSII centres and used in photochemistry (Demmig-Adams et al. 1995). Values of Φ_{PSII} can be also reduced by non-photochemical processes mainly through the dissipation of excitation

energy as heat before it reaches the PSII reaction centres (Krause and Weis 1991). Xantophyll cycle-dependent energy dissipation has been shown to be the predominant mechanism of the acclimation of leaves to high irradiance (Demmig-Adams et al. 1995).

Nitrogen and phosphorus are the most likely nutrients limiting primary producers in terrestrial (Aerts and Chapin 2000) and aquatic ecosystems (Hall et al. 2005) and *Pinus radiata* D. Don being widely planted in the southern hemisphere (Lewis and Ferguson 1993) has been shown to be growth limited by soil nitrogen and phosphorus in New Zealand (Watt et al. 2005). The overall quantum yield of canopies (radiation-use efficiency) is a key parameter in productivity process based models, and photochemical efficiency (Φ_{PSII}) has been shown to be strongly and linearly correlated with the photosynthetic quantum yield (Φ_{CO2}) under experimental conditions (Genty et al. 1989). Therefore chlorophyll fluorescence methods may provide valuable means to better understand the influence of nutrition in radiation-use efficiency. This is relevant as nutrition is said to be the factor represented with less confidence in process-based models (Landsberg et al. 1991).

The aim of the study was to assess chlorophyll fluorescence responses of *Pinus radiata* to combined nitrogen and phosphorus supply, and to test whether clones with contrasting growth patterns may exhibit differences in photochemical efficiency of PSII and phenology. A third objective of the study was to examine the extent to which chlorophyll fluorescence may assist us to represent nutrient limitations in productivity-process-based models.

MATERIALS AND METHODS

Plant material

Plant material was selected from a glasshouse experiment laid out using a factorial design with five genotypes, two levels of nitrogen (N_0=1.43 mM and N_1=7.14 mM) and two levels of phosphorus (P_0=0.084 mM and P_1=0.420 mM) supply. Genotypes were selected to represent a gradient in growth performance within a set of 400 genotypes planted in the Purokohukohu Experimental Basin (Beets et al. 2004). The experimental design comprised four nutrient treatments, three blocks, five genotypes and between 8 to 10 replicates per treatment (182 plants).

One-year old *Pinus radiata* cuttings from all five clones (Clones A, B, C, D and E in descending order of growth performance) were raised under standard ENSIS (formerly New Zealand Forest Research Institute) nursery conditions and cultivated in 4.25 dm^3 and 42 dm^3 containers filled with silica sand during the first and second year of growth respectively.

Nutrient treatments were randomly allocated to the plants, constrained so that the genotype by nutrition factorial was balanced, and applied for 24 months until plants were harvested in July 2006. Plants were supplied with 0.5-1 dm^3 of nutrient solution with increasing frequency from once to twice a week to account for increasing demands with plant size. Plants were watered daily in excess demand to that required by the largest plants. Nutrients other than nitrogen and phosphorus were provided in optimum proportions in relation to nitrogen as defined by Ingestad (1971, 1979). Plants were grown in a thermostatically controlled glasshouse where temperature fluctuated between 6 ºC and 38 °C (19 ± 5 °C) during the day, and 1 °C to 26 ºC (15 ± 4 °C) during the night depending on weather conditions. Average vapour pressure deficit (± 1 SD) was 0.8 ± 0.8 kPa (range 0-4.6 kPa) during the day and 0.16 ± 0.18 kPa (range 0-1.2 kPa) during the night. Roots of all plants were artificially inoculated with spores of *Rhizopogon rubescens* Tul. and confirmed as mycorrhizal either by visual inspection of roots or by the presence of fruiting bodies.

Chlorophyll fluorescence measurements

Chorophyll fluorescence responses of *Pinus radiata* were measured at months 6, 9 and 18 of the experiment. All genotypes were measured at month 6 while only genotypes B and E were measured at month 9 and 18. At month 6 (182 plants), a pulse-amplitude modulated fluorometer (Mini-PAM-2000, Heinz-Walz, Effeltrich, Germany) equipped with dark leaf-clips (Model DLC-8, Heinz-Walz) and a leaf-clip holder (Model 2030-B, Heinz-Walz) were used to measure the maximum quantum yield (F_v/F_m) and quantum efficiency of PSII (Φ_{PSII}) at progressing levels of irradiance (rapid-light curves), respectively.

At month 9, plants from clones B and E (68 plants) were moved to a growth cabinet the day before measurements were undertaken. Plants were dark adapted (temperature fluctuated between 20-25 ˚C while leaf-to-air vapor pressure deficit ranged from 1-1.5 kPa) for 30 minutes and maximum quantum yield followed by rapid-light curves were triggered, in order to determine Stern-Volmer non-photochemical quenching (NPQ). At month 18, plants from clones B and E (48 plants) were concurrently measured for gas exchange and chlorophyll fluorescence using a portable photosynthesis system (Model 6400, Li-Cor, Lincoln, NE) equipped with an integrated chlorophyll fluorescence detector (LI-6400-40 leaf chamber, Li-Cor Inc.). Plants measured at month 18 were shifted from the glasshouse to a thermostatically controlled room maintained at 20 °C the day before measurements were undertaken. Temperature was maintained at 20˚C while VPD was maintained, with the exception of three plants, below 1 kPa. Plants were left to equilibrate for 10 min at 360 µmol

mol^{-1} CO_2 concentration and saturating irradiance (1500 μmol m^{-2} s^{-1}), before measuring the photosynthetic rate (A_{sat}).

Maximum quantum yield of PSII (F_v/F_m) was determined as $(F_m\text{-}F_o)/F_m$, where F_o is the minimum fluorescence and F_m is the maximum fluorescence of the dark adapted leaf after a light-saturating pulse of about 8000 μmol m^{-2} s^{-1} and 800 ms duration. Rapid light-response curves were measured using the light-curve programme of the Mini-PAM, where actinic light intensity was increased in eight steps every 30 s during 4 min. The leaf-clip holder was wrapped with dark cloth to avoid interference of external irradiance with the actinic light provided by the instrument. The measured irradiance was corrected by a factor of 0.8, to account for the distance between the plane of the leaf and the quantum sensor in the Mini-PAM (Rascher et al. 2000). Effective quantum yield of PSII in the light (Φ_{PSII}) was calculated as $(F_m' - F) / F_m'$, where F and F_m' are the steady and maximal fluorescence in the light-adapted sample respectively (Schreiber et al. 1994). Apparent electron transport rates were calculated as $J = \Phi_{PSII} \times Q \times 0.84 \times 0.5$, where 0.5 is the assumed fraction of absorbed photon energy partitioned to PSII (Maxwell and Johnson 2002), and 0.84 is an average light absorption coefficient estimated for 37 C_3 species (Björkman and Demmig 1987). Stern-Volmer non-photochemical quenching (N_q) was calculated at month 9 as F_m/F_m' -1 (Maxwell and Johnson 2000).

The response of apparent electron transport rate (J) to irradiance (Q) was examined at month 6 using the model described by Prioul and Chartier (1977): $\theta J^2 - (\alpha Q + J_{max})J + \alpha Q J_{max}$, where θ is an index of the J/Q curve convexity ($0 \leq \theta \leq 1$), α is the initial slope of the J/Q curve and J_{max} is the fitted asymptotic maximum value of J. The model fitted the data precisely ($r^2 > 0.98$, $P < 0.001$) with little apparent bias. Similarly, the response of Stern-Volmer non-photochemical quenching (N_q) to irradiance (Q) was examined at month 9 using a von Bertalanffy type model: $N_q = a\ (1 - e^{-b\,Q})^{c}$, where a , b and c were fitted parameters.

Electron transport rates estimated using the Mini-Pam and the Li-Cor were different e.g. up to 400 using the Mini-Pam (within ranges given by Bilger et al. 1995) compared to up to 120 using the Li-Cor (which were similar to those calculated using gas exchange measurements). Differences may have arisen because measurements using the Mini-Pam were most likely done under non-steady state conditions. The rate of electron transport based on gas exchange was calculated as: $4(A_{sat} + R_d)$, where A_{sat} is the rate of photosynthesis at ambient CO_2 and saturating irradiance and R_d is the rate of day respiration calculated using the Laisk method (both projected leaf area basis), following von Caemmerer (2000).

Foliage characteristics

Fascicle diameter, length, mass and leaf area were measured in three fascicles per plant following chlorophyll fluorescence measurements and reported at month 6. Leaf area of needles (s) was calculated based on fascicle diameter (d), length (l) and the number of needles per fascicle (n) as: $s = (\pi d + nd)\, l$ (Turnbull et al. 1998). Foliage samples were oven-dried at 70 °C to constant weight and then dry mass recorded. Samples were chemically analyzed by Veritec Laboratories, Rotorua, New Zealand. Tissue N and P concentration were determined using Kjeldahl digestion and colorimetric methods using a Segmented Flow Analyser (SKALAR Analytical BV, Breda, The Netherlands).

Growth measurements

Growth in plant diameter, height and crown diameter are reported at month 6. Estimates of leaf area were determined as the product of leaf mass and the leaf area to mass ratio. Foliage mass at month 6 was estimated from the following equations of foliage dry mass, W, (g) against plant diameter, D, (mm) developed at month 11; $W = 0.3879\ D^{1.8842}$, $r^2 = 0.99$, $P < 0.001$, Clone A; $W = 0.0380\ D^{2.7338}$, $r^2 = 0.98$, $P < 0.001$, Clone B; $W = 0.1621\ D^{2.1943}$, $r^2 = 0.98$, $P < 0.001$, Clone C; $W = 0.0724\ D^{2.5118}$, $r^2 = 0.98$, $P < 0.001$, Clone D; $W = 0.0399\ D^{2.7239}$, $r^2 = 0.99$, $P < 0.001$, Clone E. Results presented at month 6 were consistent with those found at months 9 and 18.

Data analysis

All analyses were made at the plant level with SAS software (1996; SAS Institute, Cary, NC). Variables were tested for normality and homogeneity of variance and transformations were made as necessary to meet the underlying statistical assumptions of the models used. The main and interactive effects of N and P supply and genotype on chlorophyll fluorescence, fascicle size and plant growth variables were examined by analysis of variance and covariance. Tukey's least significant difference test was used to distinguish among individual means where applicable with a confidence level of $P \leq 0.05$. Differences in slopes and intercepts between genotypes in the linear relationships between chlorophyll fluorescence variables and foliage nutrient concentration were tested for significance by analysis of covariance.

RESULTS

Treatment influences on growth

Plant growth in diameter, height and leaf area significantly increased with nutrient supply ($F_{3,38} > 12.1$, $P < 0.001$), while clones, on a growth scale, performed generally as A ≥ B ≥ C ≥ D > E, and genotypes A and E were always significantly different ($F_{4,38} < 3$, $P < 0.032$) (Table 1). Fascicle diameter, length and mass conformed to plant growth scaling with nutrient supply ($F_{3,38} > 25.8$, $P < 0.001$) and being generally greater in those genotypes with greater growth performance ($F_{4,38} > 16.8$, $P < 0.001$) (Table 1). Average mass per fascicle was two-fold greater in Clone A than in Clone E. Plant growth and fascicle size variables in imbalanced treatments N_0P_1 and N_1P_0 were intermediate between balanced treatments N_0P_0 and N_1P_1, suggesting an interactive effect of N and P supply on productivity (Table 1).

Table 1. Growth in plant diameter, height and leaf area, fascicle diameter, fascicle length and mass per fascicle across nutrient treatments and clones at month 6 (n = 182). Nutrient treatments comprised two nitrogen supply regimes (N_0=1.43 and N_1=7.14 mM) and two phosphorus supply regimes (P_0=0.084 and P_1=0.420 mM). Values are presented as means (± 1 SE) for each treatment and clone. Significance of main effects of clones (C) and nutrient treatments (T) or the interaction between clones and treatments (C × T) are shown as F values, P values and P range (ns, non significant; *, significant at $P < 0.05$; ***, significant at $P < 0.001$). Separation of means was determined by a Tukey test. Different letters indicate significant differences at $P < 0.05$.

	n	Diameter (mm)	Height (mm)	Leaf area (m^2)	Fascicle diameter (mm)	Fascicle length (mm)	Fascicle mass (mg)
Treatments							
N_0P_0	46	3.1 ± 0.1 a	94 ± 8 a	0.17 ± 0.01 a	1.32 ± 0.03 a	89 ± 2 a	39 ± 2 a
N_0P_1	46	3.4 ± 0.1 a	138 ± 9 b	0.19 ± 0.01 a	1.35 ± 0.03 a	97 ± 2 b	43 ± 2 a
N_1P_0	44	3.8 ± 0.1 b	127 ± 9 b	0.24 ± 0.01 b	1.59 ± 0.04 b	109 ± 3 c	52 ± 2 b
N_1P_1	46	4.5 ± 0.2 c	182 ± 12 c	0.32 ± 0.02 c	1.65 ± 0.03 b	115 ± 3 c	55 ± 2 b
Clones							
A	39	3.8 ± 0.2 bc	154 ± 14 b	0.27 ± 0.01 c	1.67 ± 0.04 c	117 ± 2 c	60 ± 2 d
B	31	4.1 ± 0.2 c	113 ± 12 a	0.21 ± 0.02 b	1.45± 0.04 b	106 ± 3 b	52 ± 2 c
C	37	3.8 ± 0.1 bc	147 ± 14 b	0.26 ± 0.02 c	1.45 ± 0.03 b	94 ± 3 a	42 ± 2 b
D	38	3.7 ± 0.2 b	144 ± 9 b	0.23 ± 0.02 bc	1.48 ± 0.05 b	104 ± 3 b	49 ±2 c
E	37	3.2 ± 0.1 a	113 ± 8 a	0.17 ± 0.01 a	1.30 ± 0.03 a	91 ± 3 a	32 ± 1 a
Anova							
T		34.9, <0.001 ***	12.07,<0.001 ***	46.6,<0.001 ***	32.9,<0.001 ***	44.3,<0.001 ***	25.8,<0.001 ***
C		8.70,<0.001 ***	2.97, 0.032 *	15.7,<0.001 ***	16.8,<0.001 ***	29.0,<0.001 ***	43.6,<0.001 ***
C × T		1.24, 0.29 ns	1.11, 0.38 ns	0.77,0.68 ns	1.0,0.47 ns	1.45,0.19 ns	1.11, 0.38 ns

Treatment influences on chlorophyll fluorescence variables

Photochemical efficiency of light-adapted leaves (Φ_{PSII}), which measures the proportion of light used in photochemistry (Maxwell and Johnson 2000), decreased with

increasing irradiance (Q), with a steeper decrease and significantly lower Φ_{PSII} values reached at Low N Low P supply than at higher N and P addition rates ($F_{3,38} = 28.4$, $P < 0.001$) (Table 2, Figure 1a). Because apparent electron transport rates (J) are directly proportional to the product of Φ_{PSII} and Q, J values scaled with irradiance (Figure 1b) and reached significantly higher asymptotic J values (J_{max}) at high N high P supply compared to lower N and P addition rates ($F_{3,38} = 63.1$, $P < 0.001$) (Table 2, Figure 2b). However, the initial slope (α) and convexity (θ) of the J/Q response fitted with the model described by Prioul and Chartier (1977), was not significantly influenced by nutrient treatment ($F_{3,38} < 1.94$, $P > 0.13$) or clone ($F_{4,38} < 1.70$, $P > 0.17$) or their interaction ($F_{12,38} < 1.51$, $P > 0.16$) being on average (± 1 SE) 0.258 ± 0.001 mol electrons mol quanta^{-1} and 0.851 ± 0.005 (no units, value between 0 and 1), respectively (data not shown).

Table 2. Apparent maximal rate of electron transport (J_{max}), photochemical efficiency of PSII (Φ_{PSII}) at saturating irradiance (> 2000 µmol photons m^{-2} s^{-1}), maximal photochemical efficiency of PSII (F_v/F_m) of dark-adapted leaves, and the ratio of Φ_{PSII} to F_v/F_m (R) across nutrient treatments and clones at month 6 (n = 182). Nutrient treatments comprised two nitrogen supply regimes (N_0=1.43 and N_1=7.14 mM) and two phosphorus supply regimes (P_0=0.084 and P_1=0.420 mM). Values are presented as means (± 1 SE) for each treatment and clone. Significance of main effects of clones (C) and nutrient treatments (T) or the interaction between clones and treatments (C × T) are shown as F values, P values and P range (ns, non significant; ***, significant at $P < 0.001$). Separation of means was determined by a Tukey test. Different letters indicate significant differences at $P < 0.05$.

	n	J_{max} (µmol m^{-2} s^{-1})	Φ_{PSII}	F_v/F_m	R
Treatments					
N_0P_0	46	266 ± 7 a	313 ± 6 a	804 ± 3 a	389 ± 8 a
N_0P_1	46	289 ± 6 a	329 ± 5 a	814 ± 2 b	404 ± 7 a
N_1P_0	44	349 ± 8 b	374 ± 6 b	827 ± 3 c	453 ± 7 b
N_1P_1	46	399 ± 9 c	402 ± 7 c	837 ± 2 d	480 ± 9 c
Clones					
A	39	330 ± 11 a	354 ± 10 a	818 ± 3 a	425 ± 10 a
B	31	318 ± 14 a	348 ± 9 a	818 ± 4 a	427 ± 12 a
C	37	332 ± 14 a	351 ± 11 a	825 ± 3 a	428 ± 11 a
D	38	322 ± 13 a	358 ± 8 a	820 ± 3 a	434 ± 9 a
E	37	325 ± 10 a	363 ± 9 a	820 ± 3 a	442 ± 10 a
Anova					
T		63.07,<0.001 ***	28.4,<0.001 ***	30.97,<0.001 ***	24.90,<0.001 ***
C		0.47,0.76 ns	0.61,0.65 ns	0.90,0.47 ns	0.73,0.58 ns
C × T		0.47,0.92 ns	0.78,0.67 ns	0.58,0.85 ns	0.82,0.63 ns

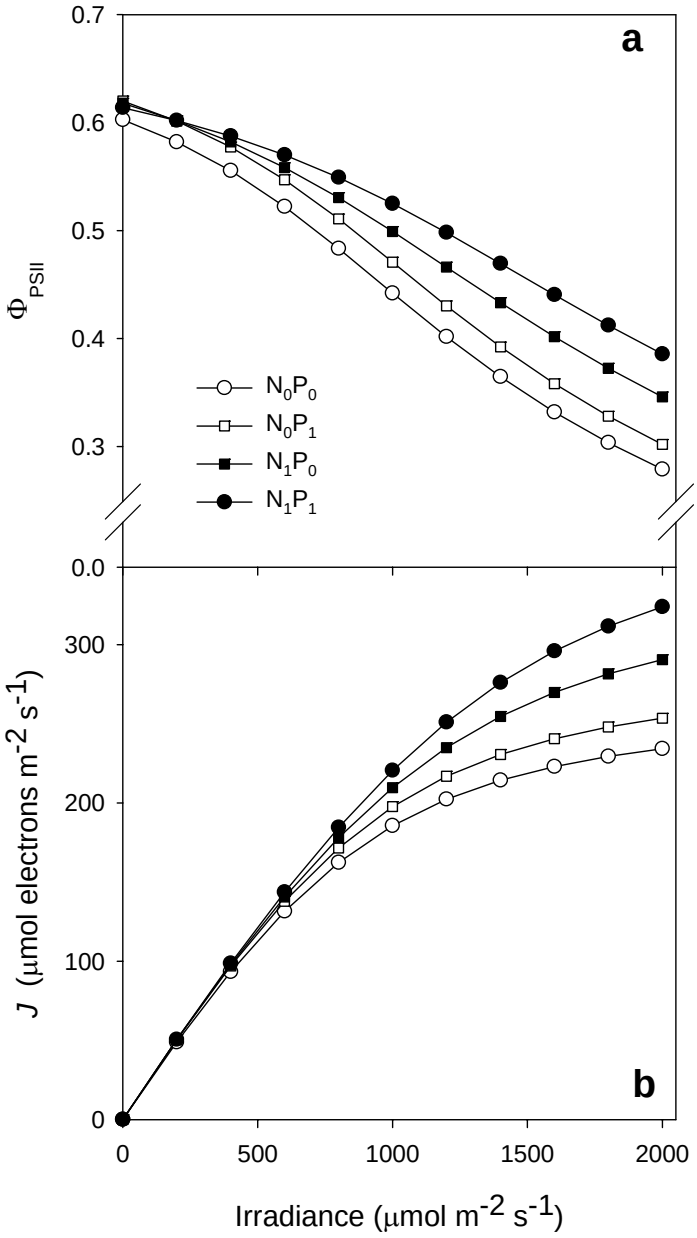


Figure 1. The response of **a** photochemical efficiency of PSII open reaction centres (Φ_{PSII}) and **b** apparent electron transport rate (J) to irradiance (Q). Nutrient treatments comprised two nitrogen supply regimes (N_0=1.43 and N_1=7.14 mM) and two phosphorus supply regimes (P_0=0.084 and P_1=0.420 mM). Symbols: ○ = N_0P_0; □ = N_0P_1; ■ = N_1P_0 and ● = N_1P_1. The Φ_{PSII}/Q and J/Q curves did not differ between clones. The model of Prioul and Chartier (1977) was fitted to the J / Q response: $\theta J^2 - (\alpha Q + J_{max})J + \alpha Q J_{max}$, where θ is an index of the J/Q curve convexity ($0 \leq \theta \leq 1$), α is the initial slope of the J/Q curve and J_{max} is the fitted asymptotic maximum value of J. Values of α and θ were not influenced by nutrient treatment or clone being on average (± 1 SD) 0.258 ± 0.001 mol electrons mol quanta^{-1} and 0.85 ± 0.01 (no units, value between 0 and 1), respectively. Values of J_{max} increased with nutrient supply independent of clone being on average (± 1 SE) 266 ± 7, 289 ± 6, 349 ± 8 and 399 ± 9 μmol electrons m^{-2} s^{-1} for nutrient treatments N_0P_0, N_0P_1, N_1P_0 and N_1P_1 respectively. Because measured J values are calculated as $\Phi_{PSII} \times Q \times 0.84 \times 0.5$, fitted Φ_{PSII} values are presented as J / $Q \times 2.381$.

The maximum photochemical efficiency of PSII (F_v/F_m), measured when all PSII reaction centre are open in dark-adapted leaves, significantly increased with nutrient supply ($F_{3,38}$ = 30.97, P < 0.001) (Table 2, Figure 2a) as a result of a slight (but not significant) reduction in ground fluorescence (F_o) concomitantly with significant increases in maximum fluorescence (F_m) as nutrient supply increased (data not shown). The range in values of F_v/F_m was relatively narrow (0.75 to 0.86) compared to light-saturated Φ_{PSII} values (0.20 to 0.50) and the ratio Φ_{PSII} to F_v/F_m (R) was significantly greater at high N high P supply than at lower N and P addition rates ($F_{3,38}$ = 24.9, P < 0.001) (Table 2), suggesting that not only photochemical efficiency but also the proportion of reaction centre which are open in the light increased with nutrient supply (Table 2). Chlorophyll fluorescence variables did not significantly differ between clones ($F_{4,38}$ < 0.90, P > 0.47) (Table 2).

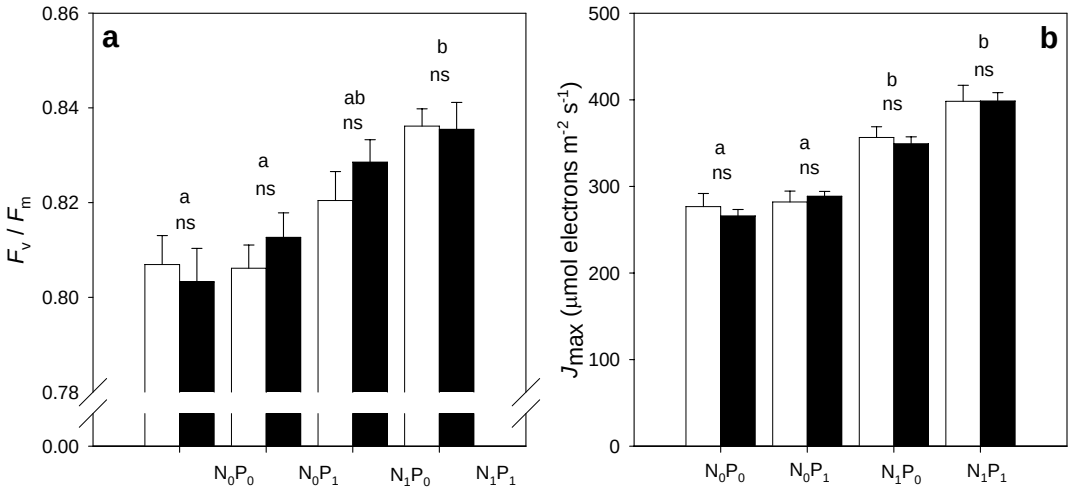


Figure 2. Influence of a factorial combination of nitrogen and phosphorus supply on **a** maximum quantum efficiency of PSII (F_v / F_m), and **b** maximum apparent electron transport rate (J_{max}) of a fast- (Clone A, open bars) and a slow-growing clone (Clone E, closed bars). Treatments comprised a combination of two nitrogen supply regimes (N_0=1.43 and N_1=7.14 mM) and two phosphorus supply regimes (P_0=0.084 and P_1=0.420 mM). Values are presented as means (± 1 SE) for each treatment and clone. Different letters indicate significant differences among treatments at P < 0.05. Differences between clones were non-significant (ns).

Stern-Volmer non-photochemical quenching (N_q), which directly relates to the amount of energy dissipated as heat, significantly increased with increasing irradiance ($F_{3,5}$ = 14-3691, P < 0.007), and the increase was steeper and higher N_q values were reached (but not significantly so, $F_{3,60}$ = 1.19, P = 0.32) at low N low P supply compared to higher N and P addition rates (Figure 3). A von Bertalanffy type model fitted the data well (r^2 > 0.98, P < 0.001) with little apparent bias (68 plants fitted individually from clones B and E at month 9). We observed that maximum N_q values (parameter a) tended (but not significantly) to decrease with nutrient supply ($F_{3,60}$ = 1.19, P = 0.32), that parameter b was significantly higher in treatment N_1P_0 compared to other treatments ($F_{3,60}$ = 7.31, P < 0.001), and that parameter c

was not influenced by nutrient treatment ($F_{3,60} = 0.93$, $P = 0.43$). Model parameters *a*, *b* and *c* did not differ between clones ($F_{1,60} < 1.17$, $P > 0.28$) (Table 3).

Table 3. The relationship between Stern-Volmer non-photochemical quenching (N_q) and irradiance (Q) as explained by a von Bertalanffi type model [$N_q = a\,(1 - e^{-bQ})^c$] across nutrient treatments and clones at month 9 (n = 68). Nutrient treatments comprised two nitrogen supply regimes (N_0=1.43 and N_1=7.14 mM) and two phosphorus supply regimes (P_0=0.084 and P_1=0.420 mM). Values are presented as means (± 1 SE) for each treatment and clone. Significance of main effects of clones (C) and nutrient treatments (T) or the interaction between clones and treatments (C × T) are shown as F values, P values and P range (ns, non significant; **, significant at $P < 0.01$). Separation of means was determined by a Tukey test. Different letters indicate significant differences at $P < 0.05$.

	n	*a*	*b* ×10^{-3}	*c*
Treatments				
N_0P_0	17	3.3 ± 0.4 a	2.16 ± 0.22 a	1.75 ± 0.16 a
N_0P_1	18	2.6 ± 0.1 a	2.45 ± 0.24 a	1.86 ± 0.16 a
N_1P_0	17	2.7 ± 0.2 a	3.49 ± 0.34 b	2.63 ± 0.62 a
N_1P_1	16	2.6 ± 0.3 a	2.11 ± 0.19 a	2.10 ± 0.31 a
Clones				
B	31	3.1 ± 0.3 a	2.62 ± 0.26 a	2.40 ± 0.37 a
E	37	2.6 ± 0.1 a	2.51 ± 0.14 a	1.82 ± 0.10 a
Anova				
T		1.19, 0.32 ns	7.31,<0.001 **	0.93,0.43 ns
C		0.81, 0.37 ns	0.45, 0.50 ns	1.17,0.28 ns
C × T		0.74, 0.53 ns	1.74, 0.17 ns	1.17, 0.33 ns

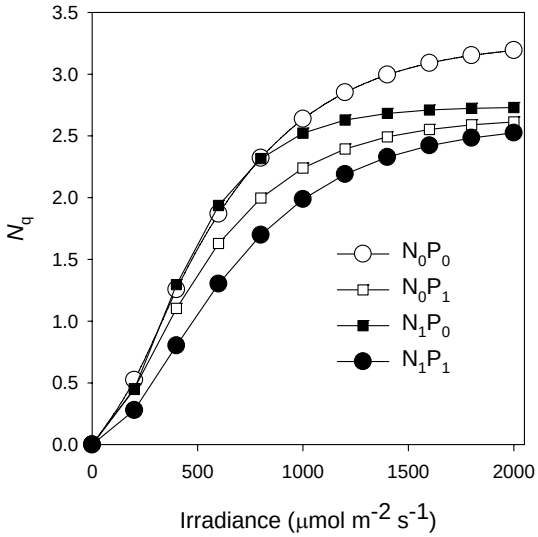


Figure 3. The response of Stern-Volmer non-photochemical quenching (N_q) to irradiance (Q) as influenced by nutrient treatments at month 9. A von Bertalanffy type model [$N_q = a\,(1 - e^{-bQ})^c$] was fitted individually for clones B and E (68 plants). Model fitted the data well ($r^2 > 0.98$, $P < 0.001$) with little apparent bias. Only model parameter *b* was significantly higher in treatment N_1P_0 than in other treatments. Model parameters *a*, *b* and *c* were not influenced by clone. Symbols: ○ = N_0P_0 [$N_q = 3.27\,(1 - e^{-0.00216Q})^{1.74876}$]; □ = N_0P_1 [$N_q = 2.65(1 - e^{-0.00245Q}))^{1.86143}$]; ■ = N_1P_0 [$N_q = 2.74\,(1 - e^{-0.00349Q})^{2.63065}$] and ● = N_1P_1 [$N_q = 2.60\,(1 - e^{-0.00211Q})^{2.09986}$]. Presented equations correspond to the average of 16-18 plants per nutrient treatment.

Treatment influences on foliage nutrient concentrations

Foliage N and P concentration on a mass basis (N_m, P_m) conformed to nutrient treatments across clones and times of analysis (Appendix). At month 6, observed N_m ranged threefold from 5.6 to 19.5 mg g^{-1}, and the gap was maintained at months 9 (4.8-19.5 mg g^{-1}) and 18 (4.8-20.4 mg g^{-1}). In contrast, observed P_m ranged threefold (0.9-3.3 mg g^{-1}) at month 6, with plants becoming progressively more P limited in P limited treatments at month 9 (0.39-2.54 mg g^{-1}) and 18 (0.44-2.04 mg g^{-1}). The ratio of foliage N_m to P_m ranged 5-fold from 2.6 to 13.9 g g^{-1} at month 6, with ranges becoming wider at month 9 (2.0 - 30 g g^{-1}) and 18 (2.3 - 41.2 g g^{-1}). Values of N_m/P_m at month 18 resembled closely those observed at month 9, with a slight trend of N_m/P_m values in treatments N_0P_1 and N_1P_0 to become more extreme (Appendix).

If we consider a ratio of N_m/P_m equal to 10 g g^{-1} (23 molal basis) as separating nitrogen ($N_m/P_m \leq 10$) from phosphorus deficiencies ($N_m/P_m > 10$) (Reich and Schoettle 1988), then plants were predominantly N limited even in the P supply deficient treatments at month 6, while the P limited proportion increased at month 9 and even more at month 18. Results thus suggest that internal P reserves of cuttings at the start of the experiment were gradually depleted in P deficient treatments (and enhanced in P rich treatments) and exhausted between months 6 and 9.

Foliage N and P were generally higher in Clone E than in Clone B at month 6, and this difference became highly significant for foliage N at months 9 ($F_{3,60} = 29.2$, $P < 0.001$) and 18 ($F_{3,39} > 125.6$, $P < 0.001$), while foliage P generally (but not always) followed this pattern. Despite this, N_m/P_m values at months 9 and 18 were remarkably similar between clones, with similar scaling with nutrient treatment and exactly the same (Tukey's) group separation (Table 4). The leaf area to mass ratio (M) significantly increased with nutrient supply at month 6 ($F_{3,38} = 10.64$, $P < 0.001$) with the slowest-growing clone (E) exhibiting larger M values than other clones ($F_{4,38} = 36.97$, $P < 0.001$). Values of M did not generally differ among nutrient treatments at months 9 and 18, but were generally greater in clone E than clone B (Appendix).

After analysing separately the influence of nutrition and clone on the leaf area to mass ratio (M) and foliage N and P on a mass basis (N_m, P_m), we integrate these two measures expressing foliage N and P on an area basis ($N_a = N_m \times M$, $P_a = P_m \times M$) in the following section.

Chlorophyll fluorescence and foliage nutrient concentration

The relationship between chlorophyll fluorescence variables and foliage nitrogen and phosphorus concentrations were examined at months 6, 9 and 18. Values of F_v / F_m and J_{max} exhibited highly significant positive linear relationships with N_a ($F_{1,170} > 40$, $P < 0.001$) but not with P_a ($F_{1,170} < 1.24$, $P > 0.27$) at month 6. Slopes ($F_{4,170} < 0.67$, $P > 0.61$) and intercepts ($F_{4,170} < 0.46$, $P > 0.76$) of these linear relationships did not differ significantly between clones (Figure 4).

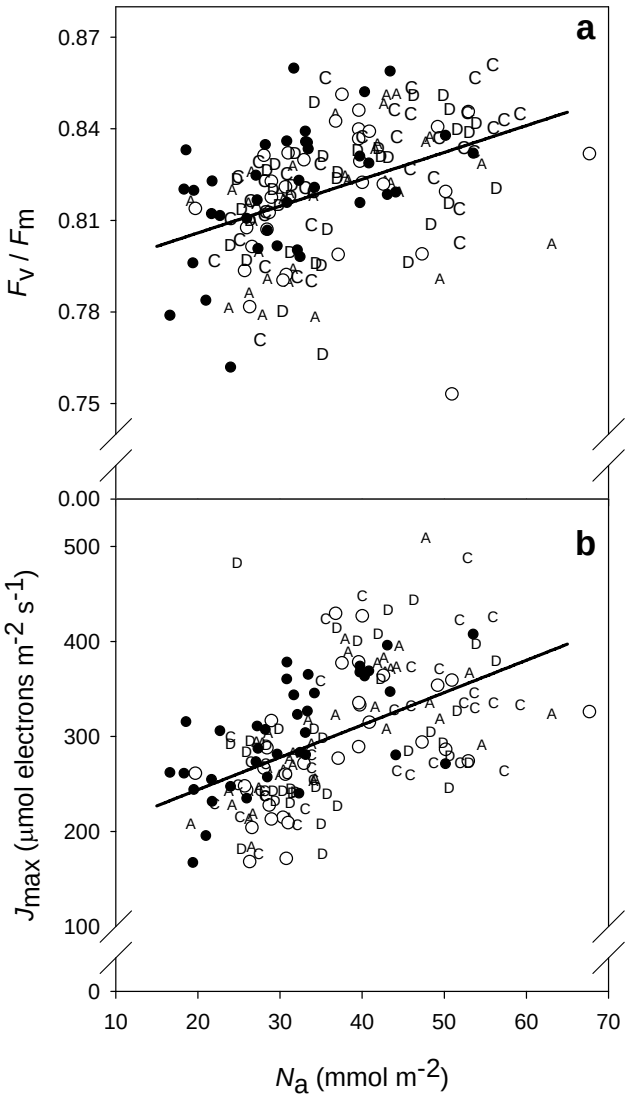


Figure 4. Relationship between **a** maximum quantum efficiency of PSII (F_v / F_m) and **b** maximum apparent electron transport rate (J_{max}) and foliage nitrogen concentration on an area basis (N_a) at **month 6**. The relationships between F_v / F_m, J_{max}, and foliage phosphorus concentration were not significant. Symbols: A = clone A, ○ = clone B, C = clone C, D = clone D and ● = clone E. Slopes and intercepts of the linear relationships between F_v / F_m, J_{max}, and N_a did not differ between clones, and therefore a single line was fitted: F_v / F_m = 0.788304 + 0.000879 N_a, r^2 = 0.19, $P < 0.001$; J_{max} = 175.8722 + 3.4058 N_a, r^2 = 0.27, $P < 0.001$.

The ratio of N_a to P_a was used to partition the population of chlorophyll fluorescence and gas exchange measurements as nitrogen ($N_a/P_a \leq 23$ mol mol^{-1}) or phosphorus ($N_a/P_a > 23$ mol mol^{-1}) deficient at months 9 and 18. This approach was not used at month 6 as most values were below this threshold (166 out of 182), and because observations that passed this threshold were uncorrelated to P_a ($F_{7,15} < 0.78$, $P > 0.62$). Values of the light-saturated rate of photosynthesis (A_{sat}), F_v / F_m and J_{max} were strong and linearly related to N_a ($F_{1,43} > 8$, $P < 0.008$) and P_a ($F_{1,23} > 33$, $P < 0.001$), except for the relationship between F_v / F_m and P_a that was non-significant ($F_{3,23} = 1.76$, $P = 0.18$) at month 9. Slopes and intercepts of the relationship between A_{sat}, F_v / F_m and J_{max}, to either N_a or P_a were not significantly different between clones (Fig. 5 a-f). The relationships of A_{sat}, maximal photochemical efficiency in the light (F'_v / F'_m) and J_{max} to N_a were highly significant ($F_{1,14} > 6.6$, $P < 0.02$) at month 18. Values of A_{sat}, J_{max} and F'_v / F'_m scaled positively with P_a, but in this case only the relationship between F'_v / F'_m and P_a was significant ($F_{1,25} = 6.1$, $P = 0.02$) (Figure 6 a-f). Slopes ($F_{1,14\text{-}25} < 1.73$, $P > 0.21$) and intercepts ($F_{1,14\text{-}25} < 1.35$, $P > 0.26$) of the relationship between A_{sat}, F_v / F_m and J_{max}, to either N_a or P_a were not significantly different between clones (Fig. 6 a-f). These results show that stoichiometry ratios may assist to better explain the influence of nutrient deficiencies on chlorophyll fluorescence and photosynthetic variables, and suggests that the effect of nitrogen and phosphorus supply on the chlorophyll fluorescence response are statistically independent and unaffected by genotype.

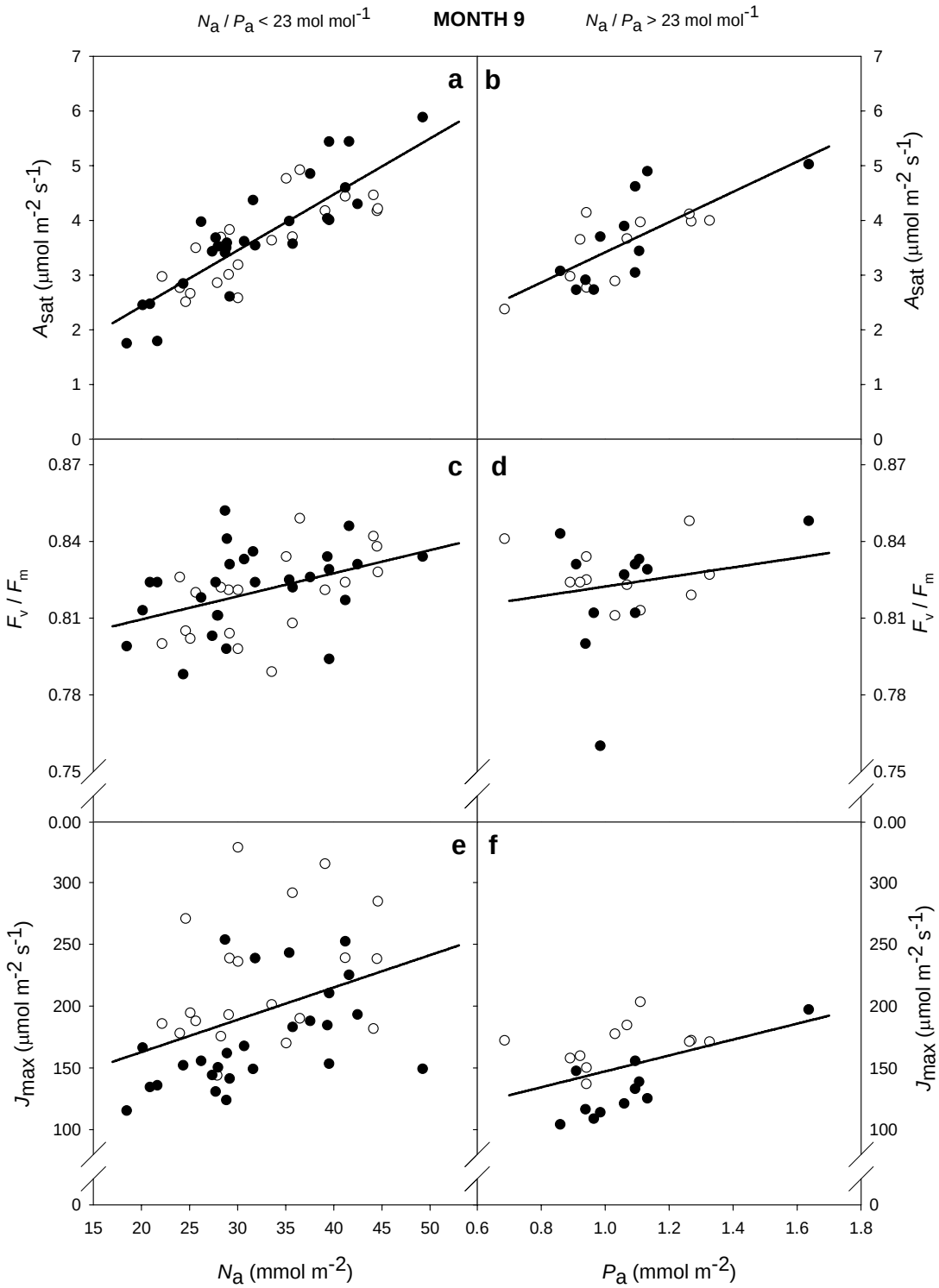


Figure 5. Relationship between **a, b** the rate of photosynthesis at saturating irradiance and ambient CO_2 concentration, A_{sat}, **c, d** maximal photochemical efficiency of PSII of dark-adapted leaves, F_v / F_m, and **e, f** apparent rate of electron transport, J_{max}, and foliage nitrogen and phosphorus concentration on an area basis (N_a, P_a) for clones B (○) and E (●) at **month 9**. On the left side measurements are nitrogen limited ($N_a/P_a \leq 23$) while on the right phosphorus limited ($N_a/P_a > 23$). Under nitrogen limitation: $A_{sat} = 0.1023\ N_a + 0.3819$, $r^2 = 0.70$, $P < 0.001$; $F_v / F_m = 0.0009027\ N_a + 0.7914615$, $r^2 = 0.18$, $P = 0.003$; $J_{max} = 2.6155\ N_a + 110.4772$, $r^2 = 0.14$, $P = 0.009$. Under phosphorus limitation: $A_{sat} = 2.7627\ P_a + 0.6532$, $r^2 = 0.53$, $P < 0.001$; $F_v / F_m = 18.7273\ P_a + 803.6312$, $r^2 = 0.04$, $P = 0.38$; $J_{max} = 64.3728\ P_a + 82.7943$, $r^2 = 0.19$, $P = 0.04$.

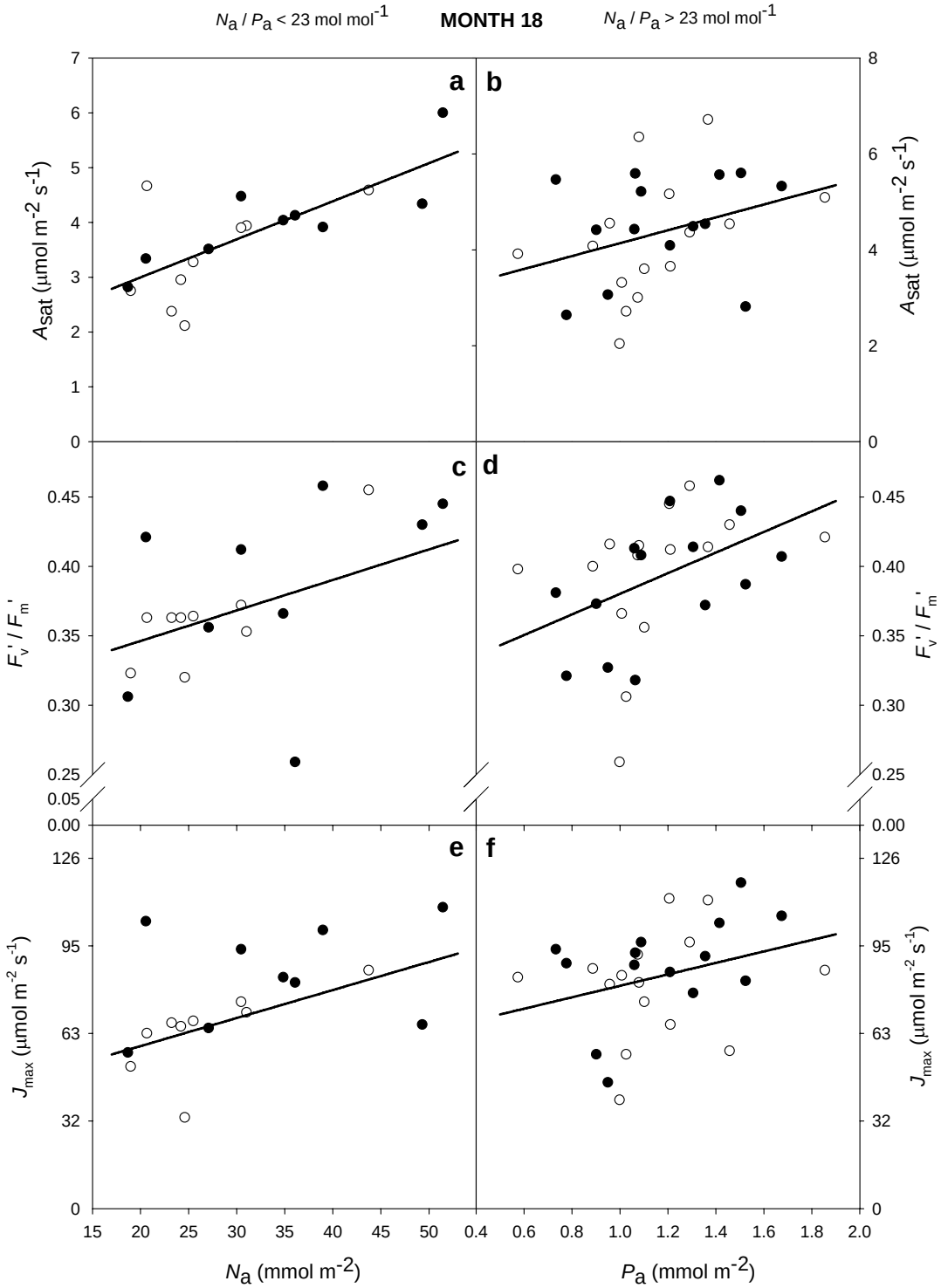


Figure 6. Relationship between **a, b** the rate of photosynthesis at saturating irradiance and ambient CO_2 concentration, A_{sat}, **c, d** maximal photochemical efficiency of PSII in the light, F_v' / F_m', and **e, f** apparent rate of electron transport, J_{max}, and foliage nitrogen and phosphorus concentration on an area basis (N_a, P_a) for clones B (○) and E (●) at **month 18**. On the left side measurements are nitrogen limited ($N_a/P_a \leq 23$) while on the right phosphorus limited ($N_a/P_a > 23$). Under nitrogen limitation: $A_{sat} = 0.0695\ N_a + 1.6061$, $r^2 = 0.54$, $P < 0.001$; $\Phi_{PSII} = 0.0016\ N_a + 0.0688$, $r^2 = 0.27$, $P = 0.02$; $F_v' / F_m' = 0.0022\ N_a + 0.3023$, $r^2 = 0.24$, $P = 0.04$. Under phosphorus limitation: $A_{sat} = 1.3447\ P_a + 2.7937$, $r^2 = 0.11$, $P = 0.08$; $\Phi_{PSII} = 0.0327\ P_a + 0.0945$, $r^2 = 0.09$, $P = 0.10$; $F_v' / F_m' = 0.0743\ P_a + 0.3060$, $r^2 = 0.19$, $P = 0.02$. F_v' / F_m', $= (F_m' - F_o') / F_m'$, where F_o' and F_m' are the minimal and maximal fluorescence in the light-adapted sample respectively (Schreiber et al. 1994).

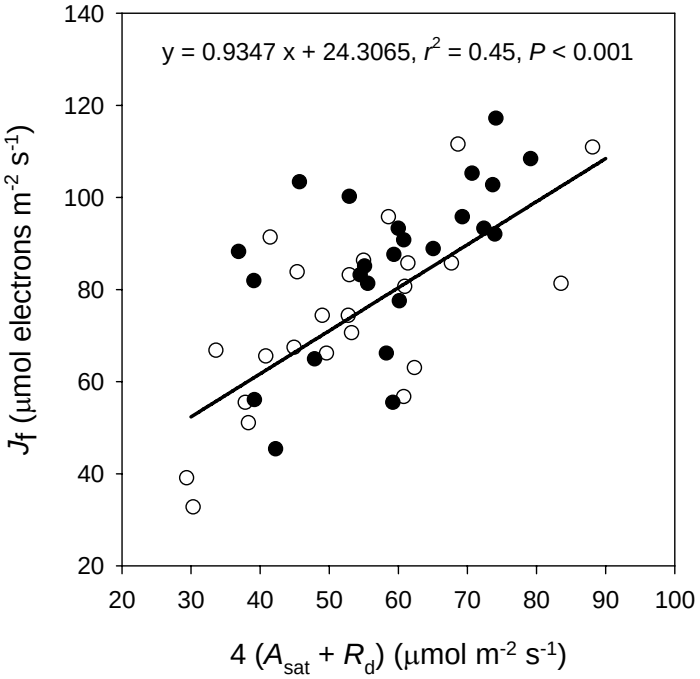


Figure 7. Relationship between the rate of electron transport calculated from measurements of chlorophyll fluorescence (J_f) versus electron transport rates calculated from gas exchange as: 4(A_{sat} + R_d), where A_{sat} is the rate of photosynthesis at ambient CO_2 and saturating irradiance and R_d is the rate of day respiration (both projected leaf area basis), following von Caemmerer (2000). Gas exchange and chlorophyll fluorescence measurements were carried out concurrently on 48 plants of *Pinus radiata* at 20ºC, ambient CO_2 (360 µmol mol^{-1}) and O_2 (0.21 mol mol^{-1}) and irradiance of 1500 µmol m^{-2} s^{-1} at month 18 of the experiment

Is chlorophyll fluorescence a good surrogate for gas exchange?

Gas exchange and chlorophyll fluorescence measurements were carried out concurrently on 48 plants of *Pinus radiata* at 20ºC, ambient CO_2 (360 µmol mol^{-1}) and O_2 (0.21 mol mol^{-1}) and irradiance of 1500 µmol m^{-2} s^{-1} at month 18 of the experiment. We calculated the rate of electron transport based on measurements of chlorophyll fluorescence (J_f) comparing them to electron transport rates estimated from gas exchange (J_g). Values of J_g were calculated as: 4(A_{sat} + R_d), where A_{sat} is the rate of photosynthesis at ambient CO_2 and saturating irradiance and R_d is the rate of day respiration calculated by the Laisk method (von Caemmerer 2000). Chlorophyll fluorescence and gas exchange estimates of electron transport were well correlated ($F_{1,47}$ = 37.8, P <0.001), while slopes ($F_{3,40}$ < 1.24, P > 0.31) and intercepts ($F_{3,40}$ < 1.84, P > 0.15) of this linear relationship were not influenced by nutrient treatment or clone (Figure 7). This relationship has been described previously by Genty et al. (1989) and von Caemmerer (2000) under non-photorespiratory conditions (elevated CO_2 and low 0.01-0.02 mol mol^{-1} O_2 concentration) showing very high correlations (r^2 > 0.98). Our measurements were carried out under normal photorespiratory conditions (ambient CO_2 and O_2

concentration), and the relationship found was still highly significant (Figure 7). This suggests that under our experimental conditions, chlorophyll fluorescence variables were a good surrogate for gas exchange measurements.

DISCUSSION

In this study nitrogen and phosphorus deficiencies reduced the proportion of open PSII reaction centres in the light, reduced the efficiency of energy trapping by open PSII centres and increased the proportion of energy dissipated as heat in *Pinus radiata*. In a landmark paper, Genty et al. (1989) showed that the quantum yield of non-cyclic electron transport was influenced by both the concentration of open PSII reaction centres and the efficiency of energy capture by these centres, and that deactivation of PSII by non-photochemical processes must reduce the quantum yield of non-cyclic electron transport, providing a framework for this study. Several authors have observed increases in photochemical efficiency with higher nitrogen (e.g. Gough et al. 2004, Niinemets et al. 2001) and phosphorus (e.g. Conroy et al. 1986, Conroy et al. 1990, Loustau et al. 1999) supply in conifers, with likely decreases in captured energy dissipated as heat. In this study, Stern-Volmer non-photochemical quenching tended to increase (plants dissipated more heat) with N and P deficiencies, being *c.* 3.3 at low N low P compared to *c.* 2.6 at high N high P supply (typical values 0.5-3.5, Maxwell and Johnson 2000). The activity of PSII is highly regulated by irradiance and the use of ATP and NADPH in the Calvin cycle and other metabolic processes in the chloroplast (Rosenqvist and van Kooten 2003), and therefore the photochemical efficiency of PSII and the rate of heat dissipation must adjust so that the electron transport rates match the capacity of the photosynthetic carbon reduction (Calvin) cycle (Ruban and Horton 1995).

Only a few studies have reported genetic variation in chlorophyll fluorescence traits in forest species. For instance, Koehn et al. (2003) observed genetic variation in photochemical quenching (q_P) and Φ_{PSII} but not in non-photochemical quenching (q_N) among slash pine families (*Pinus elliottii* Engelm. var. *elliottii*) from Mississippi, United States, while Marshall et al. (2001) did not find significant differences in maximum photochemical efficiency (F_v/F_m) between tall and short (i.e. height growth traits) open-pollinated families of *Pseudotsuga menziesii*, *Pinus ponderosa* and *Pinus monticola* from Idaho, United Staes. We found that no measured chlorophyll fluorescence variables were influenced by genotype. Pearcy et al. (1987) points out that environmental variation in resource availability influences photosynthetic responses more strongly than do genetic differences between individuals. Schreiber et al. (1994) and Bolhar-Nordenkampft and Oquist (1993) have argued that chlorophyll fluorescence methods may be useful for selecting for optimal performance to

different environmental stresses. However, in the particular case of *Pinus radiata* plantations in New Zealand, the results of our study suggest that screening genotypes for nutritional stresses using chlorophyll fluorescence techniques does not seem promising.

The range in N_a and P_a in the foliage sampled was wide, and more than covered the range of low, marginal and satisfactory levels of *Pinus radiata* foliage nitrogen and phosphorus concentrations as proposed by Will (1985) as indicative of the nutrient status of plants. In this study photochemical efficiency of PSII correlated well with foliage N and P when we used a ratio of N_a / P_a equal to 23 mol mol^{-1} as separating N from P deficiencies. Stoichiometry ratios have been extensively used to address optimum nutrition and explain particular nutrient limitations (e.g. Ingestad 1971, 1979, Ingestad and Lund 1986). Knecht and Göransonn (2004) argue that the optimum ratio of nitrogen to phosphorus in terrestrial plants is similar for a wide range of species and about 100:10 g g^{-1} (23 mol mol^{-1}), and several authors (Reich and Schoettle 1988, Marschner 1995, Aerts and Chapin, 2000) have suggested that deviations should therefore lead to nitrogen ($N_a/P_a \leq 23$) or phosphorus ($N_a/P_a > 23$) deficiencies. We found this partitioning approach to give consistent results at different times of analysis. The ratio of N_a / P_a for most observations was below 23 mol mol^{-1} at month 6, and consistently we observed that chlorophyll fluorescence variables were correlated to N_a but not to P_a. However, chlorophyll fluorescence variables below an N_a / P_a ratio of 23 mol mol^{-1} correlated well with N_a but not with P_a while observations over 23 mol mol^{-1} scaled positively and linearly with P_a but not with N_a, and this was consistent at months 9 and 18.

Fascicle size and plant leaf area in this study scaled with nitrogen and phosphorus supply with slow growing clones generally exhibiting smaller fascicles and less leaf area than faster growing clones. Cookson et al. (2005) argue that leaf development is influenced by genetic, hormonal, nutritional and environmental conditions. Fife and Nambiar (1997) argue that N supply increase leaf mass by developing larger and greater density of needles, and observed that needle mass increased with N supply in *Pinus radiata*. Similarly, Niinemets et al. (2001) observed that needles were shorter in a N and P poor site compared to a N and P rich site in *Pinus sylvestris*. Gough et al. (2004) argued that fertilization may increase productivity by initially increasing photosynthesis, which is re-invested in leaf area consequently increasing overall C assimilation. Differences in productivity among nutrient treatments was, at least partially explained, by greater C allocation to leaf area and greater photosynthetic capacity at high N high P nutrient supply compared to lower N and P addition rates. Differences in growth performance among clones in this study were attributed, at least partially, to leaf area and fascicle size but not to photosynthetic performance.

Electron transport rates calculated using chlorophyll fluorescence techniques is generally indicative of the overall rate of photosynthesis with the advantage that can be

determined almost instantaneously (Maxwell and Johnson 2000). Genty et al. (1989) showed that under experimental non-photorespiratory conditions Φ_{PSII} is well correlated to Φ_{CO2} (r^2 = 0.99) and we found that this relationship was still highly significant for electron transport (r^2 = 0.45) at ambient O_2 concentration. Maxwell and Johnson (2000) argue that this relationship breaks down under field conditions, but we suggest that concurrent measurements of chlorophyll fluorescence and gas exchange over a small sample may provide the means for validating chlorophyll fluorescence observations in the field.

Because most photosynthesis occurs below light saturation levels within forest canopies, carbon assimilation is strongly influenced by quantum yield (mol of CO_2 fixed per mol of quanta absorbed), which accounts for the strong correlation usually observed between dry matter production and intercepted photosynthetically active radiation (Ball et al. 1994). The quantum yield is a parameter usually required in productivity-process based models (e.g. 3PG, Landsberg and Waring 1997) and two scenarios to estimate this parameter can be seen: light-limited (as in closed canopies) and light unlimited (as in open canopies) conditions. The quantum yield of PSII (initial slope of the J/Q response curve) was found to be 0.258 ± 0.001 mol electrons mol quanta^{-1} (n = 182) independent of nutrient treatment and clone. If we consider that 4 mol of electrons are required to evolve 1 mol of O_2, and that approximately 1 mol of O_2 is evolved for each mol of CO_2 fixed (von Caemmerer 2000), then the quantum yield of CO_2 fixation would be 0.0645 mol CO_2 mol quanta^{-1} independent of nutrient treatment or clone, which is very similar to the 0.060 mol CO_2 mol quanta^{-1} (in normal air, at 25ºC) suggested by Bjorkman and Demmig (1987) based on data from 37 C_3 species. This is a figure that could be used for modeling closed canopies, while quantum yields equal to (A + R_d) / Q may be estimated from electron transport response curves for open canopies, but likely to be lower than 0.0645 mol CO_2 mol quanta^{-1} and to scale with foliage N and P in a similar way to that described in figures 5 (e,f) and 6 (e,f).

We are aware that steady-state light response curves will be different to the ones obtained with the Mini-Pam as shown by Rascher et al. (2000), as steady-state photosynthesis was most likely not reached at each irradiance step, being the response dependent on the light activation state of the photosynthetic apparatus (Rosenqvist and van Kooten 2003). Nevertheless we believe the trends we found using rapid light curves are valid means of comparison among treatments and genotypes. Additionally, when chlorophyll fluorescence responses are measured at steady state photosynthesis, the time necessary to obtain stable values at each light level may cause photoinhibition (White and Critchley 1999).

In conclusion, this paper examined chlorophyll fluorescence responses to nitrogen and phosphorus supply in *Pinus radiata* clones. The study showed that nutrient deficiencies reduce the proportion and efficiency of open PS II centre in the light concomitantly with

increases in heat dissipation, and that the effects of nitrogen and phosphorus supply on chlorophyll fluorescence variables are statistically independent and not influenced by genotype. Representing fertility in process based models can be achieved by increasing leaf area and photosynthetic capacity. Representing genotypes in process-based models can be achieved by increasing carbon allocation to foliage but not photosynthetic performance in faster growing genotypes.

REFERENCES

Aerts, R. and F.S.I. Chapin 2000. The mineral nutrition of wild plants revisited: A re-evaluation of processes and patterns. Advances in Ecological Research. 30:1-5767.

Ball, M.C., J.A. Butterworth, J.S. Roden, R. Christian and J.J.G. Egerton 1994. Applications of chlorophyll fluorescence to forest ecology. Australian Journal Plant Physiology. 1994:311-319.

Beets, P.N., G.R. Oliver, M.O. Kimberley, S.H. Pearce and B. Rodgers 2004. Genetic and soil factors associated with variation in visual magnesium deficiency symptoms in *Pinus radiata*. Forest Ecology and Management. 189:263-279.

Bilger, W., U. Schreiber and M. Bock 1995. Determination of the quantum efficiency of photosystem II and of non-photochemical quenching of chlorophyll fluorescence in the field. Oecologia. 102:425-432.

Bjorkman, O. and B. Demmig 1987. Photon yield of O2 evolution and chlorophyll fluorescence characteristics at 77 K among vascular plants of diverse origins. Planta. 170:489-504.

Blankenship, R.E. 2002. Photosynthesis: the light reactions. *In* Plant Physiology Eds. L. Taiz and E. Zeiger. Sinauer Associates, Inc., Sunderland, Massachussets, pp. 111-143.

Bolhar-Nordenkampf, H.R. and G. Oquist 1993. Chlorophyll fluorescence as a tool in photosynthesis research. *In* Photosynthesis and production in a changing environment: a field and laboratory manual Eds. D.O. Hall, J.M.O. Scurlock, H.R. Bolhar-Nordenkampf, R.C. Leegood and S.P. Long. Chapman&Hall, London, pp. 193-206.

Close, D.C. and C.L. Beadle 2003. Chilling-dependent photoinhibition, nutrition and growth analysis of *Eucalyptus nitens* seedlings during establishment. Tree Physiology. 23:217-226.

Conroy, J.P., P.J. Milham, M.L. Reed and E.W. Barlow 1990. Increases in phosphorus requirements for CO_2-enriched Pine species. Plant Physiology. 92:977-982.

Conroy, J.P., R.M. Smillie, M. Kuppers, D.I. Bevege and E.W. Barlow 1986. Chlorophyll a fluorescence and photosynthetic and growth responses of *Pinus radiata* to phosphorus deficiency, drought stress, and high CO_2. Plant Physiology. 81

Cookson, S.J., M. Van Lijsebettens and C. Granier 2005. Correlation between leaf growth variables suggest intrinsic and early controls of leaf size in *Arabidopsis thaliana*. Plant Cell and Environment. 28:1355-1366.

Demmig-Adams, B., W.W. Adams III, B.A. Logan and A.S. Verhoeven 1995. Xantophyll cycle-dependent energy dissipation and flexible photosystem II efficiency in plants acclimated to light stress. Australian Journal Plant Physiology. 22:249-260.

Fife, D.N. and E.K.S. Nambiar 1997. Changes in the canopy and growth of *Pinus radiata* in response to nitrogen supply. Forest Ecology and Management. 93:137-152.

Genty, B., J. Briantais and N.R. Baker 1989. The relationship between the quantum yield of photosynthetic electron transport and quenching of chlorophyll fluorescence. Biochimica et Biophysica Acta. 990:87-92.

Gough, C.M., J.R. Seiler and C.A. Maier 2004. Short-term effects of fertilization on loblolly pine (*Pinus taeda* L.) physiology. Plant Cell and Environment. 27:876-886.

Greer, D.H. 1995. Effect of canopy position on the susceptibility of kiwifruit (*Actinidia deliciosa*) leaves on vines in an orchard environment to photoinhibition throughout the growing season. Australian Journal Plant Physiology. 22:299-309.

Guo, D.P., Y.P. Guo, J.P. Zhao, H. Liu, Y. Peng, Q.M. Wang, J.S. Chen and G.Z. Rao 2005. Photosynthetic rate and chlorophyll fluorescence in leaves of stem mustard (*Brassica juncea* var. *tsaitsai*) after turnip mosaic virus infection. Plant Science. 168:57-63.

Hall, S.R., V.H. Smith, D.A. Lytle and M.A. Leibold 2005. Constraints on primary producer N:P stoichiometry along N:P supply ratio gradients. Ecology. 86:1894-1904.

Ingestad, T. 1971. A definition of optimum nutrient requirements in Birch Seedlings. II. Physiologia Plantarum. 24:118-125.

Ingestad, T. 1979. Mineral nutrient requirements of *Pinus silvestris* and *Picea abies* seedlings. Physiologia Plantarum. 45:373-380.

Ingestad, T. and A. Lund 1986. New concepts on soil fertility and plant nutrition as illustrated by research on forest trees and stands. Geoderma. 40:237-252.

Knecht, M.F. and A. Goransonn 2004. Terrestrial plants require nutrients in similar proportions. Tree physiology. 24:447-460.

Koehn, A.C., J.H. Roberds and R.L. Doudrick 2003. Variation among slash pine families in chlorophyll fluorescence traits. Canadian Journal of Forest Research. 33:1102-1109.

Krause, G.H. and E. Weis 1991. Chlorophyll fluorescence and photosynthesis: The basics. Annu. Rev. Plant Physiol. Plant Mol. Biol. 42:313-349.

Landsberg, J.J. 1986. Experimental approaches to the study of the effect of nutrients and water on carbon assimilation by trees. Tree Physiology. 2:427-444.

Landsberg, J.J. and R.H. Waring 1997. A generalized model of forest productivity using simplified concepts of radiation-use efficiency, carbon balance and partitioning. Forest Ecology and Management. 95:209-228.

Lavigne, M.B., C.H.A. Little and J.E. Major 2001. Increasing the sink:source balance enhances photosynthetic rate of 1-year-old balsam fir foliage by increasing allocation of mineral nutrients. Tree Physiology. 21:417-426.

Lewis, N.B. and I.S. Ferguson 1993. Management of radiata pine. Inkata Press, Melbourne. 404 p.

Loustau, D., M. Brahim, J.P. Gaudillere and E. Dreyer 1999. Photosynthetic responses to phosphorus nutrition in two-year-old maritime pine seedlings. Tree Physiology. 19:707-715.

Marschner, H. 1995. Mineral nutrition of higher plants. Academic Press, London. xv, 889 pp.

Marshall, J.D., G.E. Rehfeldt and R.A. Monserud 2001. Family differences in height growth and photosynthetic traits in three conifers. Tree Physiology. 21:727-734.

Maxwell, K. and G.N. Johnson 2000. Chlorophyll fluorescence - a practical guide. Journal of Experimental Botany. 51:659-668.

Niinemets, U., D.S. Ellsworth, A. Lukjanova and M. Tobias 2001. Site fertility and the morphological and photosynthetic acclimation of *Pinus sylvestris* needles to light. Tree Physiology. 21:1231-1244.

Pearcy, R.W., O. Bjorkman, M.M. Caldwell, J.E. Keeley, R.K. Monson and B.R. Strain 1987. Carbon gain by plants in natural environments: carbon assimilation analysis provides an understanding of how plants function in diverse environments. Bioscience. 37:21-29.

Prioul, J.L. and P. Chartier 1977. Partitioning of transfer and carboxilation components of intracellular resistance to photosynthesis CO_2 fixation: a critical analysis of the methods used. Ann. Bot. 41:789-800.

Puertolas, J., L. Gil and J.A. Pardos 2005. Effects of nitrogen fertilization and temperature on frost hardiness of Aleppo pine (Pinus halepensis Mill.) seedlings assessed by chlorophyll fluorescence. Forestry. 78:501-511.

Rascher, U., M. Liebig and U. Luttge 2000. Evaluation of instant light-response curves of chlorophyll fluorescence parameters obtained with a portable chlorophyll fluorometer on site in the field. Plant Cell and Environment. 23:1397-1405.

Reich, P.B. and A.W. Schoettle 1988. Role of phosphorus and nitrogen in photosynthetic and whole plant carbon gain and nutrient use efficiency in eastern white pine. Oecologia. 77:25-33.

Rosenqvist, E. and O. van Kooten 2003. Chlorophyll fluorescence: a general description and nomenclature. *In* Practical applications of chlorophyll fluorescence in plant biology Eds. J.R. DeEll and P.M.A. Toivonen. Kluwer Academic Publishers, Norwell, Massachusetts, pp. 30-77.

Ruban, A.V. and P. Horton 1995. Regulation of non-photochemical quenching of chlorophyll fluorescence in plants. Australian Journal Plant Physiology. 22:221-230.

Schreiber, U., W. Bilger and C. Neubauer 1994. Chrorophyll fluorescence as a nonintrusive indicator for rapid assessment of in vivo photosynthesis. *In* Ecophysiology of photosynthesis Eds. E.D. Schulze and M.M. Caldwell. Springer Verlag, Berlin, pp. 49-70.

Seibert, M. 1995. Reflections on the nature and function of the photosystem II reaction centre. Australian Journal Plant Physiology. 22:161-166.

Turnbull, M.H., D.T. Tissue, K.L. Griffin, G.N.D. Rogers and D. Whitehead 1998. Photosynthetic acclimation to long-term exposure to elevated CO_2 concentration in Pinus radiata D.Don is related to age of needles. Plant, Cell and Environment. 21:1019-1028.

von Caemmerer, S. 2000. Biochemical models of leaf photosynthesis. CSIRO Publishing, Collingwood,Victoria, Australia. 165 p.

Watt, M.S., G. Coker, P.W. Clinton, M.R. Davis, R. Parfitt, R. Simcock, L. Garret, T.W. Payn, B. Richardson and A. Dunningham 2005. Defining sustainability of plantation forests through identification of site quality indicators influencing productivity- a national view for New Zealand. Forest Ecology and Management. 216:51-63.

White, A.J. and C. Critchley 1999. Rapid light curves: A new fluorescence method to assess the state of the photosynthetic apparatus. Photosynthesis research. 59:63-72.

Will, G.M. 1985. Nutrient deficiencies and fertiliser use in New Zealand exotic forests. Forest Research Institute Bulletin No 97, Rotorua, New Zealand, p. 53.

Appendix. Leaf area to mass ratio (M, , foliage nitrogen (N_m, and phosphorus (P_m, concentration on a mass basis and their ratio (N_m / P_m,) across nutrient treatments, clones and times of analysis. Nutrient treatments comprised two nitrogen supply regimes (N_0=1.43 and N_1=7.14 mM) and two phosphorus supply regimes (P_0=0.084 and P_1=0.420 mM). Values are presented as means (± 1 SE) for each treatment and clone. Significance of main effects of clones (C) and nutrient treatments (T) or the interaction between clones and treatments (C × T) are shown as: ns, non significant; *, significant at $P < 0.05$; ***, significant at $P < 0.001$. Separation of means was determined by a Tukey test. Different letters indicate significant differences at $P < 0.05$.

C	T	*n*	M (m^2 kg^{-1})	N_m (mg g^{-1})	P_m (mg g^{-1})	N_m / P_m (g g^{-1})
Month 6						
A	N_0P_0	10	20.9 ± 0.9 a	8.1 ± 0.3 a	1.36 ± 0.05 a	6 ± 0.2 b
A	N_0P_1	9	20.2 ± 0.7 a	8 ± 0.4 a	2.27 ± 0.11 c	3.5 ± 0.2 a
A	N_1P_0	10	21.3 ± 0.8 a	13.5 ± 0.6 b	1.49 ± 0.08 a	9.2 ± 0.5 c
A	N_1P_1	10	21.3 ± 0.6 a	12.3 ± 0.5 b	1.72 ± 0.09 b	7.3 ± 0.4 b
	Mean	**39**	**20.9 ± 0.4 a**	**10.5 ± 0.5**	**1.7 ± 0.07 b**	**6.6 ± 0.4**
B	N_0P_0	8	17.8 ± 1.2 a	6.9 ± 0.3 a	1.36 ± 0.09 a	5.2 ± 0.5 a
B	N_0P_1	8	16.5 ± 0.2 a	6.9 ± 0.3 a	1.79 ± 0.11 b	3.9 ± 0.2 a
B	N_1P_0	7	18.9 ± 0.7 a	13.1 ± 0.6 b	1.22 ± 0.06 a	10.9 ± 0.6 c
B	N_1P_1	8	20.9 ± 0.8 a	11.8 ± 0.5 b	1.45 ± 0.07 a	8.2 ± 0.2 b
	Mean	**31**	**18.5 ± 0.5 b**	**9.6 ± 0.6**	**1.46 ± 0.06 a**	**6.9 ± 0.5**
C	N_0P_0	9	19.5 ± 0.8 a	7.8 ± 0.3 a	1.82 ± 0.09 b	4.4 ± 0.3 a
C	N_0P_1	9	20.8 ± 0.7 ab	8.5 ± 0.3 a	2.3 ± 0.09 c	3.7 ± 0.2 a
C	N_1P_0	9	21.1 ± 0.6 ab	15.4 ± 0.5 b	1.45 ± 0.05 a	10.6 ± 0.5 c
C	N_1P_1	10	24.0 ± 0.7 b	15.9 ± 0.6 b	2.2 ± 0.17 c	7.5 ± 0.6 b
	Mean	**37**	**21.4 ± 0.4 a**	**12 ± 0.7**	**1.95 ± 0.08 c**	**6.6 ± 0.5**
D	N_0P_0	10	18.1 ± 0.5 a	8.1 ± 0.2 a	1.64 ± 0.08 a	5.1 ± 0.3 a
D	N_0P_1	10	19.3 ± 0.5 ab	7.9 ± 0.2 a	2.28 ± 0.11 b	3.5 ± 0.1 a
D	N_1P_0	8	21.2 ± 0.6 ab	14.6 ± 0.3 c	1.5 ± 0.05 a	9.8 ± 0.2 c
D	N_1P_1	10	21.5 ± 0.6 b	12.2 ± 0.7 b	1.64 ± 0.08 a	7.4 ± 0.2 b
	Mean	**38**	**20 ± 0.4 a**	**10.6 ± 0.5**	**1.75 ± 0.06 b**	**6.4 ± 0.4**
E	N_0P_0	9	26.5 ± 1.7 a	8.6 ± 0.4 a	2.13 ± 0.11 a	4.1 ± 0.3 a
E	N_0P_1	10	25.1 ± 1.3 a	8.6 ± 0.4 a	2.44 ± 0.1 b	3.5 ± 0.2 a
E	N_1P_0	10	24.7 ± 1.2 a	13.7 ± 0.6 b	1.85 ± 0.18 a	7.8 ± 0.5 b
E	N_1P_1	8	27.7 ± 1.0 a	13.7 ± 0.4 b	2.31 ± 0.12 c	6.1 ± 0.4 b
	Mean	**37**	**25.9 ± 0.7 c**	**11.1 ± 0.5**	**2.18 ± 0.07 d**	**5.4 ± 0.3**
All	N_0P_0	46	20.6 ± 0.7 a	7.9 ± 0.2	1.66 ± 0.06 a	5 ± 0.2
	N_0P_1	46	20.6 ± 0.5 a	8 ± 0.2	2.23 ± 0.06 c	3.6 ± 0.1
	N_1P_0	44	21.6 ± 0.5 ab	14.1 ± 0.3	1.52 ± 0.05 a	9.5 ± 0.3
	N_1P_1	46	23.0 ± 0.5 b	13.2 ± 0.3	1.86 ± 0.07 b	7.3 ± 0.2
Overall Mean		**182**	**21.4 ± 0.3**	**10.8 ± 0.2**	**1.82 ± 0.04**	**6.4 ± 0.2**
Anova						
T			10.64,<0.001 ***	237.7,<0.001 ***	45.02,<0.001 ***	208.8,<0.001 ***
C			36.97,<0.001 ***	10.72,<0.001 ***	26.3,<0.001 ***	9.43,<0.001 ***
C × T			1.36,0.23 ns	2.82,0.007 **	1.98,0.055 ns	2.71,0.0096 **

Table 4 continuation.

C	T	*n*	*M* (m^2 kg^{-1})	N_m (mg g^{-1})	P_m (mg g^{-1})	N_m / P_m (g g^{-1})
Month 9						
B	N_0P_0	8	15.6 ± 0.2 a	6.4 ± 0.4 a	0.65 ± 0.04 ab	10.1 ± 0.8 b
B	N_0P_1	8	16.0 ± 0.5 a	6.1 ± 0.3 a	1.91 ± 0.12 c	3.3 ± 0.3 a
B	N_1P_0	7	16.5 ± 0.6 a	11.5 ± 0.5 b	0.49 ± 0.02 a	23.7 ± 0.7 c
B	N_1P_1	8	15.8 ± 0.5 a	8.9 ± 0.3 a	0.93 ± 0.04 b	9.6 ± 0.4 b
	Mean	**31**	**16 ± 0.2**	**8.1 ± 0.4 a**	**1.01 ± 0.11**	**11.3 ± 1.4 a**
E	N_0P_0	9	20.6 ± 0.6 a	7.8 ± 0.6 a	0.83 ± 0.06 a	9.4 ± 0.5 b
E	N_0P_1	10	20.2 ± 0.9 a	8.2 ± 0.5 a	1.71 ± 0.14 c	5 ± 0.3 a
E	N_1P_0	10	17.3 ± 0.4 b	12.7 ± 1.1 b	0.6 ± 0.05 a	22.1 ± 2.2 c
E	N_1P_1	8	19.7 ± 0.6 ab	10.5 ± 0.4 a	1.21 ± 0.06 b	8.9 ± 0.6 b
	Mean	**37**	**19.4 ± 0.4**	**9.8 ± 0.5 b**	**1.09 ± 0.08**	**11.5 ± 1.3 a**
All	N_0P_0	17	18.2 ± 0.7	7.1 ± 0.4 a	0.74 ± 0.04	9.8 ± 0.4 b
	N_0P_1	18	18.3 ± 0.7	7.3 ± 0.4 a	1.8 ± 0.09	4.2 ± 0.3 a
	N_1P_0	17	17.0 ± 0.4	12.2 ± 0.7 c	0.55 ± 0.03	22.8 ± 1.3 c
	N_1P_1	16	17.8 ± 0.6	9.7 ± 0.3 b	1.07 ± 0.05	9.2 ± 0.4 b
Overall Mean		**68**	**17.8 ± 0.3**	**9 ± 0.3**	**1.05 ± 0.07**	**11.4 ± 0.9**
Anova						
T			1.82, 0.15 ns	29.2,<0.001 ***	95.8,<0.001 ***	114.6,<0.001 ***
C			70.0, <0.001 ***	12.85,<0.001 ***	2.71,0.10 ns	0.20,0.65 ns
C × T			4.81, 0.005 **	0.22,0.88 ns	3.19,0.03 *	0.89,0.45 ns
Month 18						
B	N_0P_0	6	16.9 ± 0.8 a	6.5 ± 0.4 a	0.63 ± 0.06 a	10.6 ± 0.5 b
B	N_0P_1	6	16.1 ± 1.1 a	5.8 ± 0.3 a	1.86 ± 0.05 b	3.2 ± 0.2 a
B	N_1P_0	6	17.2 ± 1.3 a	17.6 ± 0.2 c	0.54 ± 0.02 a	32.4 ± 1 c
B	N_1P_1	6	14.9 ± 1.1 a	9.3 ± 0.8 b	0.72 ± 0.06 a	13 ± 0.8 b
	Mean	**24**	**16.3 ± 0.6 a**	**9.8 ± 1 a**	**0.94 ± 0.11**	**14.8 ± 2.3 a**
E	N_0P_0	6	20.3 ± 2.2 a	10.3 ± 0.4 ab	1.00 ± 0.13 b	11.2 ± 1.6 b
E	N_0P_1	6	18.9 ± 1.1 a	7.9 ± 0.6 a	1.68 ± 0.13 c	4.9 ± 0.6 a
E	N_1P_0	6	20.8 ± 2.2 a	18.6 ± 0.8 c	0.59 ± 0.04 a	32.3 ± 2.3 c
E	N_1P_1	6	20 ± 2.3 a	12.5 ± 1 b	0.97 ± 0.07 b	12.9 ± 0.6 b
	Mean	**24**	**20 ± 0.9 b**	**12.5 ± 0.9 b**	**1.03 ± 0.09**	**15.8 ± 2.3 a**
All	N_0P_0	12	18.6 ± 1.2 a	8.4 ± 0.6 a	0.82 ± 0.09	10.9 ± 0.8 b
	N_0P_1	12	17.5 ± 0.9 a	6.8 ± 0.5 a	1.78 ± 0.07	3.9 ± 0.4 a
	N_1P_0	12	19 ± 1.4 a	18.1 ± 0.4 c	0.57 ± 0.02	32.3 ± 1.2 c
	N_1P_1	12	17.5 ± 1.4 a	10.9 ± 0.8 b	0.85 ± 0.06	12.9 ± 0.5 b
Overall Mean		**48**	**18.1 ± 0.6**	**11.1 ± 0.7**	**0.98 ± 0.07**	**15.3 ± 1.6**
Anova						
T			0.33,0.80 ns	125.6,<0.001 ***	97.2,<0.001 ***	214.7,<0.001 ***
C			17.26,<0.001 ***	32.6,<0.001 ***	5.56,0.024 *	0.37,0.55 ns
C × T			0.99,0.41 ns	1.94,0.14 ns	5.27,0.004 **	0.26,0.85 ns

APPENDIX B

INFLUENCE OF AMMONIUM AND NITRATE SUPPLY ON GROWTH AND PHYSIOLOGICAL RESPONSES OF *PINUS RADIATA* SEEDLINGS

Summary Growth and physiological responses of *Pinus radiata* D. Don seedlings grown hydroponically in a factorial combination of N supply regimes (LN = 1.78 mol m^{-3}, HN = 7.14 mol m^{-3}) and ammonium:nitrate ratios (80:20, 50:50 and 20:80) were measured. Plant growth in diameter, height, leaf area and dry mass increased with N supply and also as the proportion of nitrate increased compared to ammonium. Ammonium and nitrate uptake was significantly influenced by N supply and N form and conformed to ammonium and nitrate concentrations in nutrient solution. Uptake rates of ammonium were twice those of nitrate at comparable concentrations, suggesting that *Pinus radiata* is in the lower end of the ratio of uptake of ammonium to nitrate reported for conifers (ranging from 2 to 20). Despite this, plants grown with higher proportions of ammonium than nitrate were smaller and exhibited luxurious N consumption. Differences in productivity among treatments were partially explained by greater rates of light-saturated photosynthesis associated with nitrate nutrition. Root-shoot biomass partitioning was influenced by N supply according with accepted partitioning theory, but not by N form. Luxurious N consumption, nitrogen use efficiency and ^{15}N discrimination values were strongly influenced by N supply and N form.

Key words: photosynthesis, stable isotopes, uptake

INTRODUCTION

Nitrogen (N) availability is the primary factor limiting primary productivity in most natural and managed ecosystems (Berendse and Aerts 1987; Aerts and Chapin 2000). Most N becomes available to plants through ammonification and nitrification (Haynes and Goh 1978; Bloom 1985; Chapin *et al.* 1987; Marschner 1995) despite some communities relying considerably on organic N forms (Ohlund and Nasholm 2004). Nitrification plays a minor role in climax communities whereas in most disturbed and cultivated soils it may assume a major role (Haynes and Goh 1978). Consequently plants exhibit great differences in their ability to take up and use ammonium and nitrate as sources of N (Haynes and Goh 1978), which reflects the environment to which the species are adapted (Kronzucker *et al.* 1997, 2003; Min *et al.* 1999).

Conifers are usually reported to grow better under ammonium than nitrate (McFee and Stone 1968; van den Driessche 1971; Kronzucker *et al.* 1997). This finding seems to be generalized to include all conifers, probably because most species reported are from temperate and boreal ecosystems in the Northern hemisphere (e.g. Lavoie *et al.* 1992; Downs *et al.* 1993) or mature plantation forests in the Southern hemisphere where nitrification is minimal (e.g. Adams and Attiwill 1982 *a*,*b*). However, *Pinus radiata* D. Don, being the most widely planted conifer species in the southern hemisphere (Lewis and Ferguson 1993), shows enhanced growth on disturbed sites such as old-fields and pastures (Skinner and Attiwill 1981) where nitrification plays a major role (Haynes and Goh 1978; Vitousek *et al.* 1989; Parfitt *et al.* 2003). This contradicts previous work with *Pinus radiata* where the species grew poorly when nitrate was the sole N source (i.e. McFee and Stone 1968). In order to test this apparent paradox we installed a hydroponic experiment under controlled conditions in which seedlings of *Pinus radiata* were grown under different N supply regimes and ratios of ammonium to nitrate. Testing different proportions of ammonium to nitrate was relevant because there is evidence that plant growth performance is enhanced by a mixture of ammonium and nitrate rather than either source alone (Haynes and Goh 1978; Chapin *et al.* 1987; Marschner 1995; Warren and Adams 2002*a*; Rothstein and Cregg 2005).

The aim of the study was to examine the influence of ammonium and nitrate supply on growth, uptake and photosynthetic capacity of *Pinus radiata* seedlings, and to determine if differences in productivity can be attributed to shoot-root biomass partitioning and photosynthetic characteristics. A third objective of the study was to test the extent to which seedlings exposed to combined effects of N supply and N form may exhibit differences in tissue ^{13}C and ^{15}N discrimination.

MATERIALS AND METHODS

Plant material

Pinus radiata D. Don seedlings with a genetic growth and form factor of 19 (Vincent and Dunstan 1989) were hydroponically grown under a factorial combination of N supply regimes (LN = 1.78 mol m^{-3} and HN = 7.14 mol m^{-3}) and ammonium to nitrate ratios (80:20, 50:50 and 20:80). Seeds were germinated in free-draining containers with vermiculite watered daily with $CaSO_4$ 10 mol m^{-3} and then cultured hydroponically for two weeks at ¼ and ½ strength Ingestad (1971, 1979) complete nutrient solution before starting the treatments. Uniform 14-day old seedlings were randomly assigned to groups of four and each group was transplanted to one of forty-eight 4.25 dm^3 light-tight root boxes (192 plants). The boxes were then assigned randomly to nutrient treatments. Foam-plugs were inserted for each plant at the root-shoot junction, and placed hanging from the lids of the root boxes to support the seedlings. Roots were immersed in 4 dm^3 treatment solutions which were continuously aerated and changed weekly during the experiment. Nutrients other than N were provided at 0.420 mol m^{-3} P, 0.512 mol m^{-3} K, 0.250 mol m^{-3} Ca, 0.411 mol m^{-3} Mg, 0.281 mol m^{-3} S, 12.535 mmol m^{-3} Fe, 0.459 mmol m^{-3} Zn, 0.472 mmol m^{-3} Cu, 7.281 mmol m^{-3} Mn, 0.072 mmol m^{-3} Mo, 18.501 mmol m^{-3} B, 0.846 mmol m^{-3} Cl and 0.130 mmol m^{-3} Na following Ingestad (1979). In some treatments, values of Ca, Mg and Cl exceeded those suggested by Ingestad (1979) to account for chemical sources and pH regulation.

Plants were equally divided, with respect to treatment, into two controlled growth cabinets (Contherm Phytotron Climate Simulator, Petone, New Zealand) at 22/18 ºC day/night temperature, 16/8 h day/night regime, irradiance 333 ± 49 µmol m^{-2} s^{-1} photosynthetically active radiation at plant height and 75% relative humidity day and night. Roots of all plants were confirmed by visual inspection to be non-mycorrhizal at each sequential harvest.

Plants within each root box were sequentially harvested at days 32, 57, 77 and 105 (48. plants each time). Dry mass of roots, stems and foliage were measured after oven-drying at 70 °C for 96h. Tissue nitrogen concentration at day 105 was determined based on Kjeldahl digestion and colorimetric methods using a Segmented Flow Analyser (SKALAR Analytical BV, Breda, The Netherlands) at Veritec Laboratories, Rotorua. Foliage N concentrations were expressed as millimoles of N per gram of dry tissue (N_m, mmol g^{-1}) or square meter of total leaf area (N_a, mmol m^{-2}). Plant net N uptake was calculated as the difference between N content at day 105 and day 0, and expressed as millimoles of N per plant (mmol).

Tissue C and N isotope composition

Oven-dried samples from harvest at day 105 were ball-milled, and the carbon and nitrogen ($\delta^{13}C$ and $\delta^{15}N$) isotope composition determined using a mass spectrometer at the Stable Isotope Laboratory at the University of Waikato, New Zealand. The δ values were calculated as: δ (‰) = [(R_{sp} / R_{st}) - 1] x 1000, where R_{sp} and R_{st} are the $^{13}C/^{12}C$ or $^{15}N/^{14}N$ ratios of the sample and standard (PDB for $\delta^{13}C$ and N_2 in air for $\delta^{15}N$), respectively. Using δ values, ^{13}C and ^{15}N discrimination values ($\Delta^{13}C$ and $\Delta^{15}N$) were calculated following Farquhar *et al.* (1989) as: $\Delta = (\delta_{source} - \delta_{plant})/(1+\delta_{plant})$, where δ_{source} are C isotope composition of ambient air (-8‰) and N isotope composition of nutrient solution (0.4-1.6 ‰), respectively. Farquhar *et al.* (1982) found that values of $\Delta^{13}C$ are related to the ratio of internal to external CO_2 concentration (C_i/C_a) during photosynthesis averaged over the life span of the leaf analyzed, and related by the equation: $C_i/C_a = (\Delta-a)/(b-a)$, where a is the discrimination during diffusion in air (4.4‰) and b is the net discrimination during carboxylation (27‰).

Ammonium and nitrate uptake and water use measurements

Nutrient solution was sampled (50 cm^3) at the beginning and at the end of each of seven weeks from day 54 to the end of the experiment on day 105, on 24 roots boxes (4 boxes per nutrient treatment per week) comprising 168 observations. Uptake rates (24 h) were determined based on ammonium and nitrate depletion in nutrient solution corrected by evapotranspiration, and expressed in micromoles of N per gram of dry root tissue (μmol g^{-1}) to account for differences in root mass between root boxes. Ammonium and nitrate concentration in sample solutions was determined by steam-distillation methods (Bremner 1965). Values of pH were determined in all samples with a pH and conductivity meter. By comparison of initial and final hydroponic solution in three root-boxes without plants over a week, ammonium volatilization was found to be negligible. Water depletion by evapotranspiration was measured weekly in all root boxes using a graduated cylinder, and values used in calculating long-term water use efficiency measured as milligrams of plant dry matter produced per gram of water consumed (mg g^{-1}).

Gas exchange measurements and calculations

The rate of light saturated photosynthesis at ambient CO_2 concentration (A_{sat}) was measured in 6-8 plants per treatment before the final harvest using a portable photosynthesis system (Model 6400, Li-Cor, Lincoln, NE, USA). For each plant, three fascicles were placed inside a 6 cm^2 cuvette avoiding shading between needles. Temperature in the growth chamber

and the cuvette was maintained at 20ºC while leaf-to-air vapour pressure deficit (*VPD*) ranged from 1 to 1.5 KPa. Foliage samples were left to equilibrate for 10 minutes at ambient C_a (360 μmol mol^{-1}) with saturating irradiance Q, (400-700 nm) maintained at 2000 μmol m^{-2} s^{-1}. Once values of A, C_i and g_s became stable (coefficient of variation ≤ 2%) values of A_{sat} were recorded. Following the measurement of A_{sat}, foliage samples were carefully removed from the cuvette and cut to match the leaf area exposed to gas exchange. Total surface area of needles was determined based on water volume displacement as described by Johnson (1984). All measurements and analysis are reported on a total leaf area basis. Measurements were carried out from April 10 to 24, 2004.

Photosynthetic nitrogen use efficiency was determined as the ratio of A_{sat} to N_a (E_N) and expressed as micromoles of CO_2 per mol of N second (μmol mol^{-1} s^{-1}), while water use efficiency (E_W) was calculated as the ratio of A_{sat} to transpiration (E) and expressed as millimoles of CO_2 assimilated per mol of H_2O transpired (mmol mol^{-1}). Long-term nitrogen use efficiency (*NUE*) was determined as plant mass divided by N content at day 105, and expressed as grams of plant dry mass per gram of N (g g^{-1}).

Data analysis

All analyses were made at the plant level with SAS software (1996; SAS Institute, Cary, NC). Variables were tested for normality and homogeneity of variance and transformations were made as necessary to meet the underlying statistical assumptions of the models used. The main and interactive effects of N supply and N form on growth and physiological responses at the end of the experiment at day 105 were examined by analysis of variance and covariance. Adjusted means were presented in the results when covariate analysis showed them to be significant. Tukey's least significant difference test was used to distinguish among individual means where applicable with a confidence level of $P \leq 0.05$.

Allometric analysis was used to remove the influence of growth differences on allometry, so that the direct influence of N supply and N form on allocation could be determined. Using data from all harvests at days 32, 57, 77 and 105 (192 plants), the relationship between a particular component, y, (root, stem and foliage dry mass), and total plant mass, x, was modelled using the following linear model; $\text{Ln}\, y = b_0 + b_1 \text{Ln}\, x$. Analysis of covariance was used to determine if slopes or intercepts of fitted equations significantly differed between treatments.

The main and interactive effects of N supply and N form on weekly measurements of ammonium and nitrate uptake and pH over seven weeks were modelled using both a mixed model of repeated measures over time and analysis of variance over averages of repeated

measures. Covariance structure for the mixed model was selected using the Akaike's Information Criterion (Littell *et al.* 1996). As both analyses were equivalent results from the simpler fixed-effects model are presented.

RESULTS

Treatment influences on growth and tissue N concentration

Plant growth in diameter, height, leaf area and dry mass were strongly influenced by N form ($F_{2,41} > 9.51$, $P < 0.001$), while all characteristics apart from diameter ($F_{1,41} = 1.52$, $P = 0.22$), were significantly influenced by N supply ($F_{1,41} > 4.57$, $P < 0.04$). None of these characteristics were influenced by the interaction of N form and N supply ($F_{2,41} > 1.95$, $P > 0.16$) during the 105-days experiment. All four growth variables scaled positively with N supply and negatively with ammonium:nitrate ratio (Table 1). However, the effect of the ammonium:nitrate ratio on growth variables was two to four-fold greater than the effect of N supply. Plant net N uptake was significantly influenced by the main effects of N supply ($F_{1,41} = 35.42$, $P < 0.001$) and N form ($F_{2,41} = 4.60$, $P = 0.02$) but not by their interaction ($F_{2,41} = 2.76$, $P = 0.08$). Plant net N uptake increased with N supply and as the ammonium:nitrate ratio decreased (Table 1).

Stem, foliage and root N concentrations were significantly influenced by N supply ($F_{1,41}$ = 39-75, $P < 0.001$) and N form ($F_{2,41}$ = 3.43-20.54, $P < 0.04$) but not by their interaction ($F_{2,41}$ = 0.07-2.71, $P > 0.08$). Tissue N concentration was higher in plants grown in ammonium- rather than nitrate-dominated solution, but plant N uptake was greater in nitrate dominated solutions (Table 1, only foliage N shown). Values of root N concentration were on average 1.2 times greater than those in foliage and 2.8 times greater than those in stems (data not shown). The leaf area to mass ratio (M) was not significantly influenced by main or interactive effects of N supply and N form ($F_{5,37} = 0.21$, $P = 0.95$) being on average (± SE) 18.5 ± 0.4 m^2 kg^{-1}, hence values of foliage N concentration on a mass (N_m) and area basis (N_a) followed the same pattern (Table1).

Table 1. Plant, foliage and photosynthetic characteristics of *Pinus radiata* growing in a factorial combination of N supply regimes (LN = 1.78 mol m^{-3}, HN = 7.14 mol m^{-3}) and NH_4^+ : NO_3^- ratios (80:20, 50:50 and 20:80). Values are presented as means (± 1 SE) of plant growth in basal diameter, height, mass and leaf area after 105 days, plant net N uptake, leaf area to mass ratio (*M*), foliage N concentration on a mass (N_m) and area (N_a) basis, stomatal conductance to CO_2 diffusion (g_s), light-saturated rate of photosynthesis at ambient CO_2 (A_{sat}), instantaneous photosynthetic nitrogen use efficiency (E_N), long-term nitrogen use efficiency (*NUE*), ^{15}N foliage isotope discrimination ($\Delta^{15}N$), instantaneous photosynthetic water-use efficiency (E_W), long-term water use efficiency (*WUE*), ^{13}C foliage isotope discrimination ($\Delta^{13}C$) and long-term intercellular to ambient CO_2 concentration (C_i/C_a); *n*=7-8 for all plant and foliage variables and *n*=6-8 for all photosynthetic variables. Significance of main effects of N supply (S) and N form (F) or the interaction between N supply and N form (S x F) are shown as: ns, non significant; *, significant at $P < 0.05$; **, significant at $P < 0.01$; ***, significant at $P < 0.001$. Separation of means was determined by a Tukey test where applicable. Different letters within N supply regimes or N forms indicate that means were significantly different at $P < 0.05$. Covariates were significant for plant mass growth (initial plant mass) and $\Delta^{15}N$ (N_m) and therefore adjusted means are presented.

Variable	N form			N supply		ANOVA statistics		
	80:20	50:50	20:80	LN	HN	S	F	S x F
Diameter growth (mm)	9.4 ± 0.3 a	10.0 ± 0.3 a	11.6 ± 0.3 b	10.2 ± 0.2 a	10.6 ± 0.4 a	ns	***	ns
Height growth (mm)	269 ± 11 a	303 ± 13 a	346 ± 13 b	291 ± 10 a	323 ± 13 b	*	***	ns
Plant mass growth (g)	22.8 ± 1.4 a	27.3 ± 1.8 b	36.2 ± 2.0 c	26.8 ± 1.4 a	31.1 ± 2.1 b	*	***	ns
Leaf area growth (m^2)	0.26 ± 0.02 a	0.27 ± 0.02 a	0.37 ± 0.03 b	0.27 ± 0.02 a	0.33 ± 0.02 b	*	***	ns
Plant net N uptake (mmol)	38.6 ± 3.0 a	42.7 ± 3.3 ab	49.5 ± 3.7 b	35.3 ± 1.4 a	52.5 ± 2.9 b	***	*	ns
M (m^2 kg^{-1})	18.9 ± 0.4 a	18.2 ± 0.7 a	18.5 ± 0.9 a	18.4 ± 0.5 a	18.6 ± 0.6 a	ns	ns	ns
N_m (mmol g^{-1})	1.78 ± 0.10 a	1.67 ± 0.07 a	1.35 ± 0.06 b	1.40 ± 0.06 a	1.80 ± 0.06 b	***	***	ns
N_a (mmol m^{-2})	94.3 ± 4.8 a	93.1 ± 4.9 a	74.8 ± 4.7 b	76.3 ± 3.2 a	98.7 ± 4.9 b	***	***	ns
g_s (mmol m^{-2} s^{-1})	50.0 ± 8.2 a	65.6 ± 7.9 a	78.0 ± 9.2 a	60.9 ± 6.9 a	67.2 ± 7.3 a	ns	ns	ns
A_{sat} (μmol m^{-2} s^{-1})	6.2 ± 0.5 a	8.0 ± 0.5 b	9.5 ± 0.7 b	7.3 ± 0.5 a	8.3 ± 0.6 a	ns	**	ns
E_N (μmol mol^{-1} s^{-1})	63.7 ± 4.7 a	89.7 ± 8.0 b	120.2 ± 7.0 c	94.6 ± 7.8 a	86.7 ± 7.0 a	ns	***	ns
NUE (g g^{-1})	43.7 ± 2.1 a	46.8 ± 2.0 a	54.0 ± 2.1 b	54.2 ± 1.6 a	42.0 ± 1.2 b	***	***	ns
$\Delta^{15}N$ (‰)	0.62 ± 0.28 a	-0.09 ± 0.25 a	-0.75 ± 0.29 b	-1.22 ± 0.23 a	1.07 ± 0.25 b	***	*	ns
E_W (mmol mol^{-1})	5.9 ± 0.5 a	6.3 ± 0.4 a	5.3 ± 0.4 a	5.8 ± 0.4 a	5.9 ± 0.3 a	ns	ns	ns
WUE (mg g^{-1})	2.86 ± 0.10 a	2.78 ± 0.12	2.87 ± 0.10 a	2.87 ± 0.09 a	2.80 ± 0.08 a	ns	ns	ns
$\Delta^{13}C$ (‰)	15.8 ± 0.4 a	16.4 ± 0.2 a	16.6 ± 0.3 a	16.3 ± 0.3 a	16.2 ± 0.2 a	ns	ns	ns
Long-term C_i / C_a (%)	50.6 ± 1.6 a	53.1 ± 0.8 a	54.0 ± 1.3 a	52.8 ± 1.1 a	52.4 ± 1.0 a	ns	ns	ns

Treatment influences on photosynthetic characteristics

The rate of photosynthesis at saturating irradiance and ambient CO_2 (A_{sat}) was significantly influenced by N form ($F_{2,36} = 7.64$, $P = 0.002$) but not by N supply ($F_{1,36} = 0.96$, $P = 0.33$) or their interaction ($F_{2,36} = 0.43$, $P = 0.65$). Values of A_{sat} increased as the ammonium:nitrate ratio decreased, being 53% greater at 20:80 (9.5 ± 0.7 μmol m^{-2} s^{-1}) than at 80:20 (6.2 ± 0.5 μmol m^{-2} s^{-1}). Also, values of A_{sat} tended (not significantly though) to increase with nutrient supply from 7.3 ± 0.5 μmol m^{-2} s^{-1} at LN to 8.3 ± 0.6 μmol m^{-2} s^{-1} at HN (Table 1, Figure 1). Stomatal conductance to CO_2 diffusion (g_s) was not significantly influenced by main or interactive effects of N supply and N form ($F_{5,36} = 1.17$, $P = 0.34$). However, values of g_s tended to increase with N supply (from about 61 mmol m^{-2} s^{-1} at LN to 67 mmol m^{-2} s^{-1} at HN) and also as the ammonium:nitrate ratio decreased (from about 50 mmol m^{-2} s^{-1} at 80:20 to 78 mmol m^{-2} s^{-1} at 20:80) (Table 1).

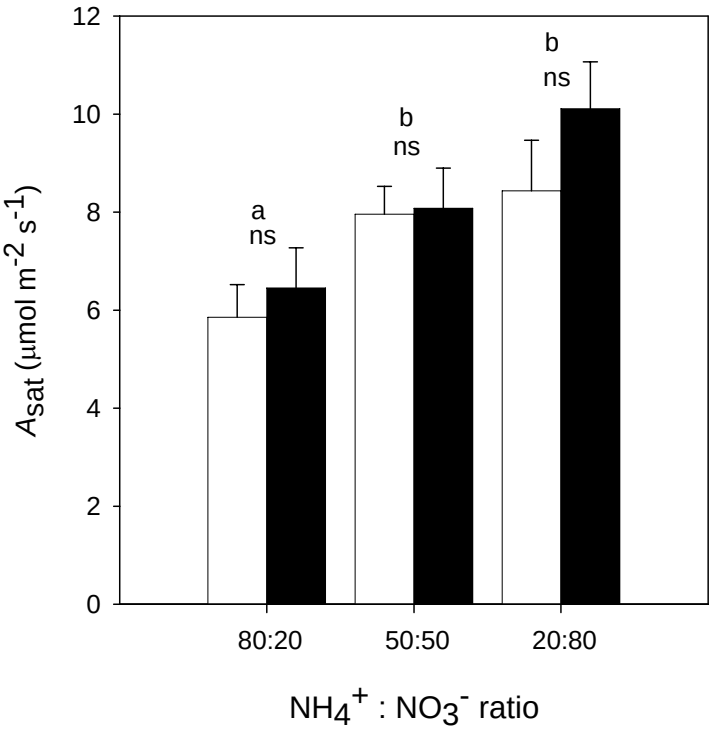


Figure 1. Influence of N supply regimes (LN = 1.78 mol m^{-3}, HN = 7.14 mol m^{-3}) and NH_4^+ : NO_3^- ratios (80:20, 50:50 and 20:80) on the rate of light saturated photosynthesis (A_{sat}) at ambient CO_2 concentration (360 μmol mol^{-1}) of *Pinus radiata* grown for 105 days. Values are presented as means (± 1 SE; n = 6-8) for each treatment. Different letters indicate significant differences among NH_4^+ : NO_3^- ratios at $P < 0.05$. Differences between N supply regimes were not significant (ns). Open bars represent LN and closed bars HN. Interactive effects between N supply regime and NH_4^+ : NO_3^- ratio were not significant ($P > 0.05$). Photosynthesis was measured as moles of CO_2 per square meter of total leaf area second (μmol m^{-2} s^{-1}).

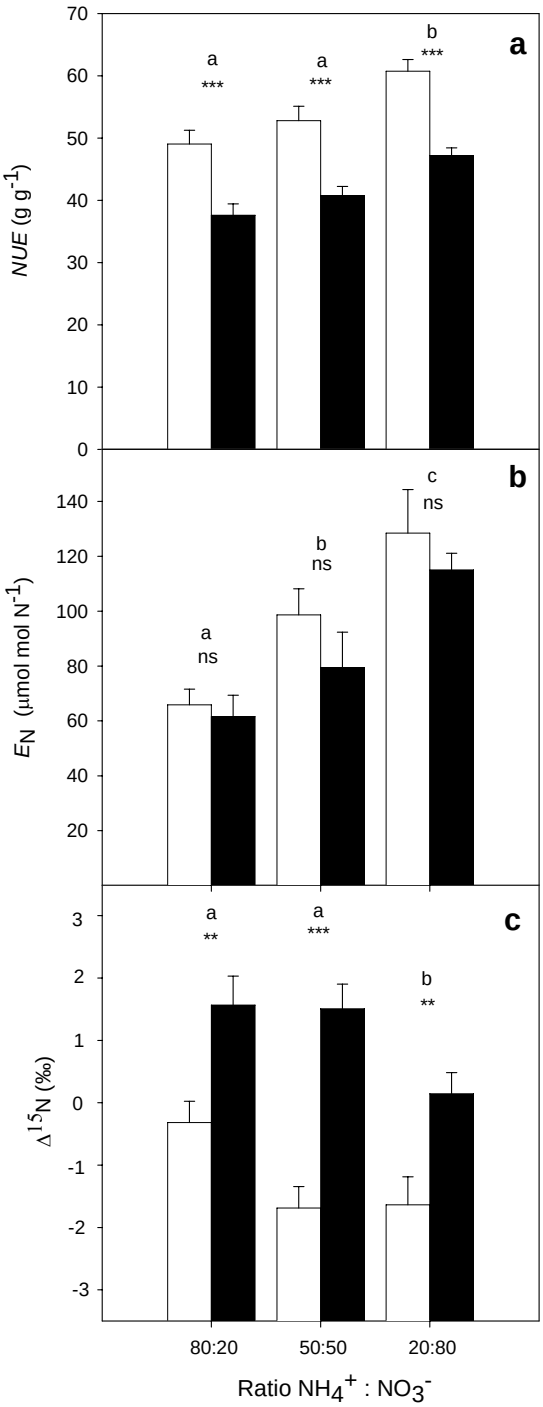


Figure 2. Influence of N supply regimes (LN = 1.78 mol m^{-3}, HN = 7.14 mol m^{-3}) and NH_4^+ : NO_3^- ratios (80:20, 50:50 and 20:80) on **a** long-term nitrogen use efficiency (*NUE*), **b** photosynthetic nitrogen use efficiency (E_N) and **c** ^{15}N discrimination ($\Delta^{15}N$) of *Pinus radiata* grown for 105 days. Values are presented as means (± 1 SE; n = 6-8) for each treatment. Different letters indicate significant differences among NH_4^+ : NO_3^- ratios at $P < 0.05$. Differences between N supply regimes within the same NH_4^+ : NO_3^- ratio are shown as: ns, non significant; *, significant at $P < 0.05$; **, significant at $P < 0.01$; ***, significant at $P < 0.001$. Open bars represent LN and closed bars HN. Interactive effects between N supply regime and NH_4^+ : NO_3^- ratio were not significant ($P > 0.05$). Values of *NUE* are presented as grams of plant dry mass per gram of N (g g^{-1}), E_N as micro moles of CO_2 per mole of N second (µmol mol^{-1} s^{-1}) and $\Delta^{15}N$ as parts per thousand (‰).

Values of photosynthetic nitrogen use efficiency (E_N), long-term Nitrogen use efficiency (*NUE*) and leaf ^{15}N discrimination ($\Delta^{15}N$) were strongly influenced by N form ($F_{2,36\text{-}41} > 4.7$, $P < 0.02$), while all these characteristics apart from E_N ($F_{1,36} = 2.41$, $P = 0.13$), were significantly influenced by N supply ($F_{1,39\text{-}41} > 34$, $P < 0.001$). The interaction between N form and N supply on all these characteristics was not significant ($F_{2,36\text{-}41} = 2.54$, $P > 0.09$). Values of E_N and *NUE* decreased with N supply and as the proportion of ammonium in nutrient solution increased (Figure 2 a,b). Values of $\Delta^{15}N$ followed the opposite pattern to E_N and *NUE*, increasing with N supply and as the proportion of ammonium in nutrient solution increased (Figure 2 c). The effect of N supply was almost twice than that of N form on $\Delta^{15}N$ values (Table 1).

Values of photosynthetic water use efficiency (E_W), long-term water use efficiency (*WUE*), foliage C isotope discrimination ($\Delta^{13}C$) and long-term C_i/C_a values were not significantly influenced by main or interactive effects of N supply and N form ($F_{5,35\text{-}41} = 0.18\text{-}0.84$, $P > 0.52$) (Table 1).

Treatment influences on dry matter partitioning

Foliage and root mass fractions decreased while stem mass fraction increased with plant size (Figure 3). Root and foliage mass fractions were significantly influenced by N supply ($F_{1,181} > 15$, $P < 0.001$) but not by N form ($F_{2,181} < 1.1$, $P > 0.34$) or their interaction ($F_{2,181} < 1.1$, $P > 0.33$). Root mass fraction decreased with N supply (Figure 3a), being on average (± 1 SE) 0.294 ± 0.005 (R/S = 0.425 ± 0.011) at LN and 0.266 ± 0.005 (R/S = 0.367 ± 0.009) at HN supply (data not shown). In contrast, foliage mass fraction increased with N supply (Figure 3c), being 0.567 ± 0.005 at LN and 0.595 ± 0.004 at HN supply (data not shown). Stem mass fraction was not influenced by main or interactive effects of N supply and N form ($F_{6,181} = 1.63$, $P = 0.14$) being on average 0.138 ± 0.002 (Figure 3b), and therefore a single equation was fitted to represent all data. Slopes ($F_{1,184} > 4.3$, $P < 0.04$), but not intercepts ($F_{1,184} < 0.85$, $P > 0.35$), of the linear relationships between log-transformed foliage and root mass to log-transformed total plant mass were significantly different between N supply regimes (Figure 3 a,c).

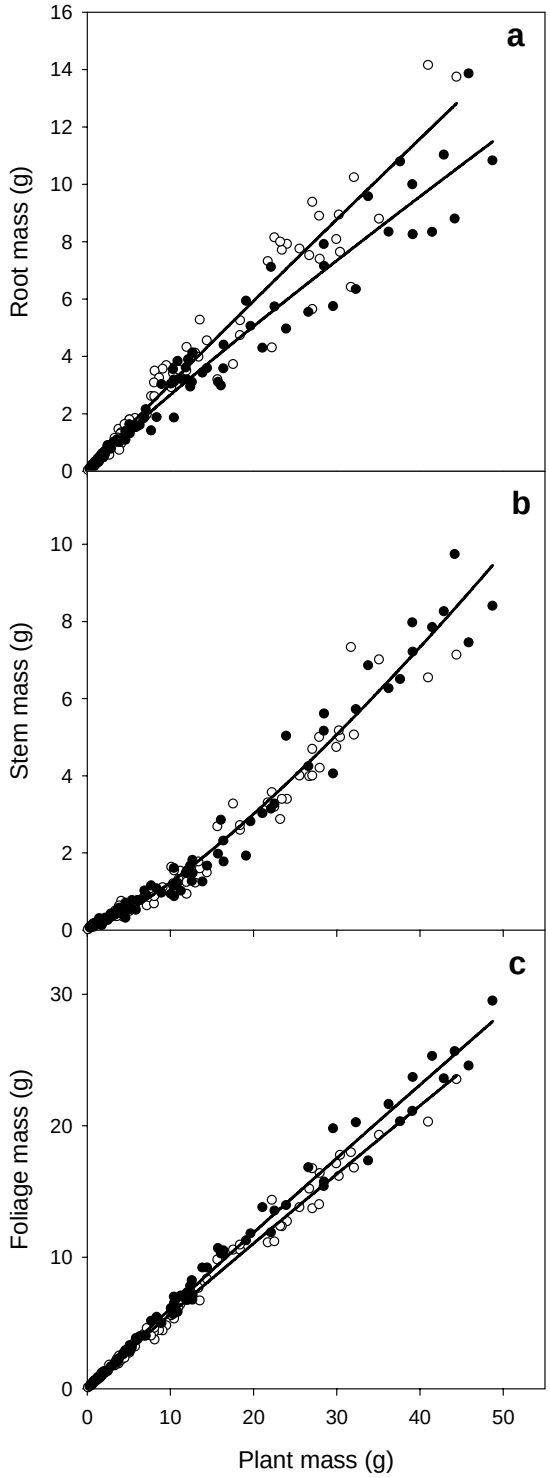


Figure 3. Relationships between **a** root (W_R), **b** stem (W_S) and **c** foliage (W_F) mass to total plant mass (W_T) as influenced by N supply regimes (n = 182). Open symbols represent LN (1.78 mol m^{-3}) and closed symbols HN (7.14 mol m^{-3}). Data was fitted by non-linear regression. For Figure 3a : $W_R = 0.3277\ W_T^{\ 0.9667}$, $r^2 = 0.97$, $P < 0.001$ (LN); $W_R = 0.3176\ W_T^{\ 0.9234}$, $r^2 = 0.98$, $P < 0.001$ (HN). For Figure 3b: $W_S = 0.0637\ W_T^{\ 1.2866}$, $r^2 = 0.98$, $P < 0.001$. For Figure 3c: $W_F = 0.6187\ W_T^{\ 0.9622}$, $r^2 = 0.99$, $P < 0.001$ (LN); $W_F = 0.6668\ W_T^{\ 0.9612}$, $r^2 = 0.99$, $P < 0.001$ (HN). All these relationships were not influenced by the NH_4^+ : NO_3^- ratio ($P > 0.05$) and additionally, the relationship W_S/W_T was not influenced by N supply regime and therefore a single equation was fitted. Data are presented on a dry-mass basis (g).

Treatment influences on ammonium and nitrate uptake

Ammonium, nitrate and ammonium plus nitrate uptake (24 hours) were significantly influenced by N supply ($F_{1,18} > 11$, $P < 0.004$) and N form ($F_{2,18} > 11$, $P < 0.001$). None of these characteristics were influenced by the interaction between N supply and N form ($F_{2,18} < 2.4$, $P > 0.12$) except for nitrate uptake ($F_{2,18} = 7.8$, $P = 0.004$). The rates of ammonium and nitrate uptake conformed to ammonium and nitrate concentrations in nutrient solutions (Figure 4 a,b). However, the rate of ammonium uptake was on average 2.0 ± 0.2 times greater than that of nitrate at comparable concentrations, and the difference tended to increase with N supply from 1.65 ± 0.15 at LN (50:50) to 2.31 ± 0.30 at HN (50:50). Additionally, the ratio of ammonium to nitrate uptake was consistently greater than the ratio of ammonium to nitrate concentration in treatment solutions (e.g. 0.74 ± 0.18 at 20:80 and 6.04 ± 0.82 at 80:20). Analysis of covariance also showed that nitrate uptake did not influence ammonium uptake and vice versa ($F_{1,17} = 2.11$, $P = 0.16$).

Ammonium plus nitrate uptake (24 hours) increased by 72% with N supply from about 187 ± 12 μmol g^{-1} at LN to about 322 ± 19 μmol g^{-1} at HN. Plants grown at a greater proportion of ammonium exhibited luxurious consumption of nitrogen (Figure 4 c), which translated in greater tissue nutrient concentration (Table 1). Total N uptake increased by 56% from about 202 ± 18 μmol g^{-1} for plants grown at (20:80) to about 315 ± 41 μmol g^{-1} at (80:20).

The pH of nutrient solution measured at the end of each week was significantly influenced by N supply ($F_{1,18} = 257$, $P < 0.001$), N form ($F_{2,18} = 126$, $P < 0.001$) and their interaction ($F_{2,18} = 40$, $P < 0.001$) (Figure 4d). Values of pH were adjusted to lie between 5.0-5.5 at the beginning of each week. We observed a decrease in ending pH with N supply being on average 5.3 ± 0.4 at LN compared to 3.5 ± 0.1 at HN. Ending pH also increased with [NO_3^-] and decreased with [NH_4^+] in nutrient treatments being on average 3.5 ± 0.1 at (80:20) compared to 5.5 ± 0.5 at (20:80).

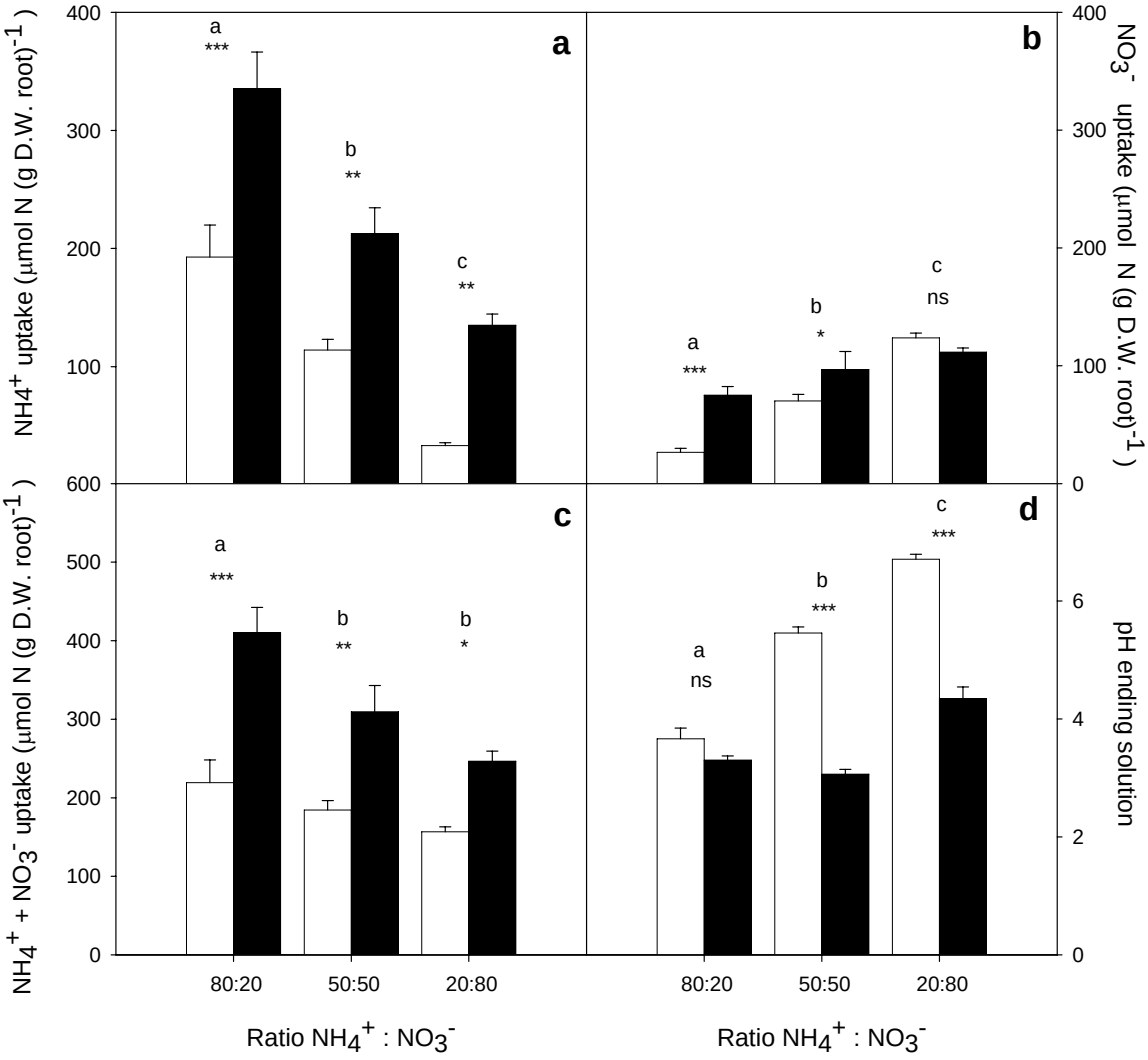


Figure 4. Influence of N supply regimes (LN = 1.78 mol m^{-3}, HN = 7.14 mol m^{-3}) and NH_4^+ : NO_3^- ratios (80:20, 50:50 and 20:80) on **a** ammonium, **b** nitrate, **c** ammonium plus nitrate uptake (24 hours) and **d** pH of nutrient solution (at the end of a week) of *Pinus radiata* grown for 105 days. Values are presented as means (± 1 SE; $n = 4$) for each treatment. Different letters indicate significant differences among NH_4^+ : NO_3^- ratios at $P < 0.05$. Differences between N supply regimes within the same NH_4^+ : NO_3^- ratio are shown as: ns, non significant; *, significant at $P < 0.05$; **, significant at $P < 0.01$; ***, significant at $P < 0.001$. Open bars represent LN and closed bars HN. Interactive effects between N supply regime and NH_4^+ : NO_3^- ratio were not significant ($P > 0.05$) for ammonium and ammonium plus nitrate uptake, and were significant ($P < 0.05$) for nitrate uptake and pH. Values of uptake and pH used in the analysis are averages over seven weeks. An equivalent answer was found using a mixed model with repeated measures over time. Uptake rates (24 h) are presented as micro moles of N per gram of dry root tissue (µmol g^{-1}).

DISCUSSION

This study suggests that *Pinus radiata* seedlings are well adapted to use both NH_4^+ and NO_3^- as sources of N, which may partly explain the success of this species over a wide range of ecological conditions. In a previous study McFee and Stone (1968) showed that *Pinus radiata* performed poorly when supplied with nitrate as the sole source of N. However, it is now widely accepted that most species grow better with a mixture of ammonium and nitrate rather than either source alone (e.g. van den Driesche 1971; Cox and Reisenauer 1973; Bigg and Daniel 1978; Haynes and Goh 1978; Chapin *et al.* 1987; Marschner 1995; Warren and Adams 2002*a*; Rothstein and Cregg 2005). For instance, nitrate as the sole N source was reported to retard growth to a greater extent to that explained by slower uptake or lower photosynthetic rates in *Pinus pinaster* (Warren and Adams 2002*a*). Among the likely reasons are nutrient imbalances (Haynes and Goh 1978; Marschner 1995), greater synthesis of organic acids to restore imbalance of hydroxyl ions (Raven and Smith 1976; Warren and Adams 2002*a*) and excessive carbon loss to the growing media (Vuorinen *et al.* 1995).

In this study, seedlings of *Pinus radiata* developed faster under nitrate- compared to ammonium-dominated N supply. Differences in productivity were at least partially explained by greater photosynthetic rates in plants grown in nitrate dominated solutions. Similarly, Rothstein and Cregg (2005) found that photosynthesis rates of *Abies fraseri* declined markedly under ammonium compared to nitrate dominated N supply. Bloom *et al.* (1989) suggested that nitrate assimilation was not competitive with carbon fixation in barley plants grown at high irradiance, suggesting that chloroplast electron transport has a capacity beyond that immediately required for carbon fixation. Similarly, Zerihun *et al.* (1998) in a study of energy costs of N form acquisition showed that nitrate assimilation in well-illuminated leaves might not be much more expensive than ammonium acquisition. Thus, additional energetic costs associated with nitrate nutrition might be offset by excess reductants supplied from a surplus in electron transport (Bloom *et al.* 1989) or by up-regulation of photosynthesis as suggested by Rothstein and Cregg (2005) and also by the results of this study.

The effects of plant size and age on ontogeny are well documented (e.g. Walters *et al.* 1993; Bartelink 1998; Portsmuth et al 2005) as well as the effect of resource limitations on biomass partitioning (e.g. Bloom *et al.* 1985; Chapin *et al.* 1987; Gedroc *et al.* 1996). However, few studies have characterized the influence of N form on biomass partitioning in forest species (e.g. Heiskanen 2005; Bauer and Berntson 2001), and we are unaware of any investigation

considering simultaneously plant size and N form. We found that biomass partitioning was influenced by plant size and N supply regime but not by N form. This result may be relevant as there is an on-going debate as whether N form may (e.g. Kruse *et al.*, 2003) or may not (e.g. Zerihun *et al.* 1998) influence shoot-root biomass partitioning.

Plants growing under ammonium dominated solutions were smaller and exhibited luxurious consumption of N. Several authors have reported luxurious consumption of N associated with ammonium nutrition (McFee and Stone 1968; Van Den Driessche 1971; Van Den Driesche and Dangerfield 1975; Haynes and Goh 1978; Flaig and Mohr 1991; Lavoie *et al.* 1992; Malhi *et al.* 1988; Warren and Adams 2002*a*). Independent of N form, photosynthesis rates are known to be closely related to foliar nitrogen (Field and Mooney 1986; Walcroft *et al.* 1997; Grassi *et al.* 2002; Ripullone *et al.* 2003), which is explained by the high proportion of total nitrogen partitioned to the carboxylating enzyme Rubisco (Sage and Pearcy 1987; Evans 1989; Warren and Adams 2002*b*; Takashima *et al.* 2004). Considering N form, the results of the study show that photosynthetic rates were smaller and foliar nitrogen greater in plants growing under ammonium rather than nitrate dominated solutions, suggesting that N partitioning to active Rubisco decreased while N storage increased with ammonium nutrition. Warren and Adams (2002*a*) showed that Rubisco concentration remain constant in seedlings of *Pinus pinaster* supplied with ammonium, nitrate or a mixture, while foliage N concentration increased with ammonium nutrition, providing further evidence of N-storage associated with ammonium nutrition in conifers (e.g. Flaig and Mohr 1991; Lavoie *et al.* 1992; Kronzucker *et al.* 1997).

In this experiment, long-term and photosynthetic N use efficiency, luxurious N consumption, and ^{15}N discrimination were significantly influenced by the main effects of N supply regime and N form. Nitrogen use efficiency increased, luxurious N consumption decreased and $\Delta^{15}N$ decreased with nitrate nutrition (as the ratio ammonium to nitrate decreased). On the other hand, N use efficiency decreased, luxurious N consumption increased and $\Delta^{15}N$ increased as N supply increased. External N concentration exerted a greater influence on $\Delta^{15}N$ values than N form within the range of N concentrations studied, which coincides with the results found by Kolb and Evans (2003) in *Hordeum vulgare*. Forest soil nitrification has been reported to increase with disturbance (e.g. Vitousek *et al.* 1982; Vitousek *et al.* 1989; Kronzucker *et al.* 1997) and N availability (e.g. Adams and Attiwill 1982*a*; Carlyle *et al.* 1989), and therefore the results of the study suggest that contrasting effects of increased fertility with increased nitrification may confound the interpretation of $\Delta^{15}N$ values in the field. In this study we did not find evidence to suggest that water-use efficiency was influenced by N supply or N form and this

was confirmed by $\Delta^{13}C$ values. Farquhar and Sharkey (1982) pointed out that stomatal conductance is tuned to respond to a reduced leaf water status by reducing the CO_2 assimilation rate and also to respond to increases in the leaf to air vapour pressure deficit (VPD) by reducing the rate of transpiration. However, similarity in water-use efficiency among treatments might be expected as plants were grown in a hydroponic system in which water was not limiting and VPD inside the growth chambers were maintained at low values.

Ammonium uptake was about two-fold greater than that of nitrate at comparable concentrations. Kronzucker *et al.* (1997) proposed that the ability to use ammonium and nitrate depends on the species successional stage, with early successional species growing better on nitrate (more disturbed soils) and late successional species growing better on ammonium (less disturbed soils). The empirical evidence strongly supports this theory (e.g. Krajina *et al.* 1973; Hangs *et al.* 2003; Chen *et al.* 2005). Thus, the ratio of ammonium to nitrate uptake might be used as an index of this successional ability, with values close to 1 occurring in agricultural species and values in the range for 2-20 in conifers (Kronzucker *et al.* 1997; Kronzucker *et al.* 2003). This would suggests that *Pinus radiata* occurs at the lower end of the range given for conifers, partly explaining its enhanced early growth on disturbed sites such as old-fields and pastures (Skinner and Attiwill 1981) where light is not usually as limiting as it is in mature undisturbed forests. Therefore conifer species could be ordered using the ammonium:nitrate uptake ratio (Φ) as a surrogate for their capacity to use nitrate e.g. *Pinus radiata* (Φ = 2) > *Pseudotsuga menziesii* (Φ = 3, Kronzucker *et al.* 2003) = *Pinus sylvestris* (Φ = 3, Flaig and Mohr 1992) > *Picea abies* (Φ = 4, Buchman *et al.* 1995) > *Pinus contorta* (Φ = 6, Min *et al.* 2000) > *Pinus strobus* (Φ = 12, Bauer and Berntson 2001) > *Picea glauca* (Φ = 20, Kronzucker *et al.* 1997), among others.

Nitrate and ammonium uptake were independent of each other within the range of concentrations used in this study. Some studies suggest that high ammonium concentration will reduce nitrate reductase activity in shoots and roots and therefore inhibit nitrate uptake (Haynes and Goh 1978; Downs *et al.* 1993; Sagi and Lips 1998). Others, like Flaig and Mohr (1992), found that ammonium and nitrate were taken up at the same rate in seedlings of *Pinus sylvestris* as if they were applied separately suggesting that ammonium does not inhibit nitrate uptake (the same as this study) at least in longer-term studies than those involved in uptake kinetics.

In conclusion, we examined the effects of N supply and N form on growth, uptake and photosynthetic characteristics of *Pinus radiata* seedlings grown at high irradiance. Plants grown in ammonium dominated solutions were smaller, had lower photosynthetic rates and exhibited

luxurious consumption of N. Uptake rates of ammonium were about two-fold greater than those of nitrate at comparable concentrations, suggesting that *Pinus radiata* occurs at the lower end expected for conifers (2-20), which may help to explain success of this species in fertile disturbed sites such as ex-pastures where nitrate may represent a high proportion of available N.

REFERENCES

Adams MA, Attiwill PM (1982b) Nitrate reductase activity and growth response of forest species to ammonium and nitrate sources of nitrogen. Plant and Soil 66, 373-381.

Adams MA, Attiwill PM (1982a) Nitrogen mineralization and nitrate reduction in forests. Soil Biology and Biochemistry 14, 197-202.

Aerts R, Chapin FSI (2000) The mineral nutrition of wild plants revisited: A re-evaluation of processes and patterns. Advances in Ecological Research 30, 1-5767.

Bartelink HH (1998) A model of dry matter partitioning in trees. Tree Physiology 18, 91-101.

Bauer GA, Berntson GM (2001) Ammonium and nitrate acquisition by plants in response to elevated CO_2 concentration: the roles of root physiology and architecture. Tree Physiology 21, 137-144.

Berendse F, Aerts R (1987) Nitrogen-use-efficiency: a biologically meaningful definition? Functional Ecology 1, 293-296.

Bigg WL, Daniel TW (1978) Effects of nitrate, ammonium and pH on the growth of conifer seedlings and their production of nitrate reductase. Plant and Soil 50, 371-385.

Bloom AJ (1985) Wild and cultivated barley show similar affinities for mineral nitrogen. Oecologia 65, 555-557.

Bloom AJ, Caldwell RM, Finazzo J, Warner RL, Weissbart J (1989) Oxygen and carbon dioxide fluxes from barley shoots depend on nitrate assimilation. Plant Physiology 91, 352-356.

Bloom AJ, Chapin FS, Mooney HA (1985) Resource limitation in plants - an economic analogy. Annual Review of Ecology and Systematics 16, 363-392.

Bremner JM (1965) Inorganic forms of nitrogen. In 'Methods of soil analysis. Part 2: Chemical and Microbiological properties. Section 84.' (Eds CA Black, DD Evans, JL White, LE Ensminger and FE Clark) pp. 1179-1237. (American Society of Agronomy: New York)

Buchman N, Schulze ED, Gebauer G (1995) ^{15}N-ammonium and ^{15}N-nitrate uptake of a 15-year-old Picea abies plantation. Oecologia 102, 361-370.

Carlyle JC, Turvey ND, Hopmans P, Downes GM (1989) Stem deformation in Pinus radiata associated with previous land use. Canadian Journal of Forest Research 19, 96-105.

Chapin FS, Bloom AJ, Field CB, Waring RH (1987) Plant responses to multiple environmental factors. BioScience 37, 49-57.

Chen W, Luo JK, Shen QR (2005) Effect of NH4+-N/NO3+-N ratios on growth and some physiological parameters of Chinese cabbage cultivars. Pedosphere 15, 310-318.

Cox WJ, Reisenauer HM (1973) Growth and ion uptake by wheat supplied nitrogen as nitrate, or ammonium or both. Plant and Soil 38, 363-380.

Downs MR, Nadelhoffer KJ, Melillo JM, Aber JD (1993) Foliar and fine root nitrate reductase activity in seedlings of four forest tree species in relation to nitrogen availability. Trees 7, 233-236.

Evans JR (1989) Photosynthesis and nitrogen relationships in leaves of C_3 plants. Oecologia 78, 9-19.

Farquhar GD, Hubick KT, Condon AG, Richards RA (1989) Carbon isotope fractionation and plant water-use efficiency. In 'Stable Isotopes in Ecological Research'. (Eds PW Rundel, JR Ehleringer and KA Nagy) pp. 21-40. (Springer-Verlag: New York)

Farquhar GD, O'Leary MH, Berry JA (1982) On the relationship between carbon isotope discrimination and the intercellular carbon dioxide concentration in leaves. Australian Journal of Plant Physiology, 121-137.

Farquhar GD, Sharkey TD (1982) Stomatal conductance and photosynthesis. Annual Review of Plant Physiology. 33, 317-345.

Field C, Mooney HA (1986) The photosynthesis - nitrogen relationship in wild plants. In 'The Economy of Plant Form and Function'. (Ed. TJ Givnish) pp. 25-55. (Cambridge University Press, Cambridge)

Flaig H, Mohr H (1991) Effect of high ammonium supply in Scots Pine seedlings (Pinus sylvestris L.). Allgemeine Forst und Jagdzeitung 162, 35-42.

Flaig H, Mohr H (1992) Assimilation of nitrate and ammonium by the Scots pine (Pinus sylvestris) seedlings under conditions of high nitrogen supply. Physiologia Plantarum 84, 568-576.

Gedroc JJ, McConnaughay KDM, Coleman JS (1996) Plasticity in root/shoot partitioning: optimal, ontogenic or both? Functional Ecology 10, 44-50.

Grassi G, Meier P, Cromer R, Tompkins D, Jarvis PG (2002) Photosynthetic parameters in seedlings of Eucalyptus grandis as affected by rate of nitrogen supply. Plant, Cell and Environment 25, 1677-1688.

Hangs RD, Knight JD, Van Rees KCJ (2003) Nitrogen uptake characteristics for roots of conifer seedlings and common boreal forest competitor species. Canadian Journal of Forest Research 33, 156-163.

Haynes RJ, Goh KM (1978) Ammonium and nitrate nutrition of plants. Biological Reviews 53, 465-510.

Heiskanen J (2005) Effect of nitrate and ammonium on growth of transplanted Norway spruce seedlings: a greenhouse study. Annales Botanici Fennici 42, 1-9.

Ingestad T (1971) A definition of optimum nutrient requirements in Birch Seedlings. II. Physiologia Plantarum 24, 118-125.

Ingestad T (1979) Mineral nutrient requirements of Pinus silvestris and Picea abies seedlings. Physiologia Plantarum 45, 373-380.

Johnson JD (1984) A rapid technique for estimating total surface area of pine needles. Forest Science 30, 913-921.

Kolb KJ, Evans RD (2003) Influence of nitrogen source and concentration on nitrogen isotopic discrimination in two barley genotypes (Hordeum vulgare L.). Plant, Cell and Environment 26, 1431-1440.

Krajina VJ, Madoc-Jones S, Mellor G (1973) Ammonium and nitrate in the nitrogen economy of some conifers growing in Douglas-fir communities of the Pacific North-West of America. Soil Biology and Biochemistry 5, 143-147.

Kronzucker HJ, Siddiqi MY, Glass ADM (1997) Conifer root discrimination against soil nitrate and the ecology of forest succession. Nature 385, 59-61.

Kronzucker HJ, Siddiqi MY, Glass ADM, Britto DT (2003) Root ammonium transport efficiency as a determinant in forest colonization patterns: an hypothesis. Physiologia Plantarum 117, 164-170.

Kruse J, Hetzger I, Mai C, Polle A, Rennenberg H (2003) Elevated pCO_2 affects N-metabolism of young poplar plants (Populus tremula x P. alba) differently at deficient and sufficient N-supply. New Phytologist 157, 65-81.

Lavoie N, Vezina LP, Margolis HA (1992) Absorption and assimilation of nitrate and ammonium ions by jack pine seedlings. Tree physiology 11, 171-183.

Lewis NB, Ferguson IS (1993) 'Management of radiata pine.' (Inkata Press, Melbourne)

Littell RC, Milliken GA, Stroup WW, Wolfinger RD (1996) Analysis of repeated measures data. In 'SAS systems for mixed models' pp. 87-134. (SAS Institute Inc.: Cary, N.C.)

Malhi SS, Nyborg M, Jahn HG, Penney DC (1988) Yield and nitrogen uptake of rapeseed (Brassica campestris L.) with ammonium and nitrate. Plant and Soil 105, 231-239.

Marschner H (1995) 'Mineral nutrition of higher plants.' (Academic Press: London, 889 pp.)

McFee WW, Stone EL (1968) Ammonium and nitrate as nitrogen sources for Pinus radiata and Picea glauca. Soil Science Society of America Journal 32, 879-884.

Min X, Siddiqi MY, Guy RD, Glass ADM, Kronzucker HJ (1999) A comparative study of fluxes and compartmentation of nitrate and ammonium in early-succesional tree species. Plant Cell and Environment 22, 821-830.

Min X, Siddiqi MY, Guy RD, Glass ADM, Kronzucker HJ (2000) A comparative kinetic analysis of nitrate and ammonium influx in two early-succesional tree species of temperate and boreal ecosystems. Plant Cell and Environment 23, 321-328.

Ohlund J, Nasholm T (2004) Regulation of organic and inorganic nitrogen uptake in Scots pine (Pinus sylvestris) seedlings. Tree Physiology 24, 1397-1402.

Parfitt RL, Scott NA, Ross DJ, Salt GJ, Tate KR (2003) Land-use change effects on soil C and N transformations in soils of high N status: comparisons under indigenous forest, pasture and pine plantation. Biogeochemistry 66, 203-221.

Portsmuth A, Niinemets U, Truus L, Pensa M (2005) Biomass allocation and growth rates in Pinus sylvestris are interactively modified by nitrogen and phosphorus availabilities and by tree size and age. Canadian Journal of Forest Research 35, 2346-2359.

Raven JA, Smith FA (1976) Nitrogen assimilation and transport in vascular land plants in relation to intracellular pH regulation. New Phytologist 76, 415-431.

Ripullone F, Grassi G, Lauteri M, Borghetti M (2003) Photosynthesis-nitrogen relationships: interpretation of different patterns between Pseudotsuga menziesii and Populus x euroamericana in a mini-stand experiment. Tree Physiology 23, 137-144.

Rothstein DE, Cregg BM (2005) Effects of nitrogen form on nutrient uptake and physiology of Fraser fir (Abies fraseri). Forest Ecology and Management 219, 69-80.

Sage RF, Pearcy RW (1987) The nitrogen use efficiency of C_3 and C_4 plants. Plant Physiology 84, 959-963.

Sagi M, Lips HS (1998) The levels of nitrate reductase and MoCo in annual ryegrass as affected by nitrate and ammonium nutrition. Plant Science 135, 17-24.

Skinner MF, Attiwill PM (1981) The productivity of pine plantations in relation to previous land use. Plant and Soil 60, 161-176.

Takashima T, Hikosaka K, Hirose T (2004) Photosynthesis or persistence: nitrogen allocation in leaves of evergreen and deciduous Quercus species. Plant Cell and Environment 27, 1047-1054.

Van Den Driessche R (1971) Response of conifer seedlings to nitrate and ammonium sources of nitrogen. Plant and Soil 34, 421-439.

Van Den Driessche R, Dangerfield J (1975) Response of Douglas-fir seedlings to nitrate and ammonium nitrogen sources under various environmental conditions. Plant and Soil 42, 685-702.

Vincent TG, Dunstan JS (1989) 'Register of commercial seedlots issued by the New Zealand Forest Service.' (FRI Bulletin No. 144, Ministry of Forestry, Forest Research Institute: Rotorua, New Zealand)

Vitousek PM, Gosz JR, Grier CC, Melillo JM, Reiners WA (1982) A comparative analysis of potential nitrification and nitrate mobility in forest ecosystems. Ecological Monographs 52, 155-177.

Vitousek PM, Matson PA, Van Cleve K (1989) Nitrogen availability and nitrification during succesion: Primary, secondary, and old-field seres. Plant and Soil 115, 229-239.

Vuorinen AH, Rossi P, Vapaavuori EM (1995) Combined effect of inorganic carbon and different nitrogen sources in the growth media on biomass production and nitrogen uptake in young willow and birch plants. Journal of Plant Physiology 147, 236-242.

Walcroft AS, Whitehead D, Silvester WB, Kelliher FM (1997) The response of photosynthetic model parameters to temperature and nitrogen concentration in Pinus radiata D. Don. Plant Cell and Environment 20, 1338-1348.

Walters MB, Kruger EL, Reich PB (1993) Relative growth rate in relation to physiological and morphological traits for northern hardwood tree seedlings: species, light environments and ontogenetic considerations. Oecologia 96, 219-231.

Warren CR, Adams MA (2002b) Phosphorus affects growth and partitioning of nitrogen to Rubisco in Pinus pinaster. Tree Physiology 22, 11-19.

Warren CR, Adams MA (2002a) Possible casues of slow growth of nitrate-supplied Pinus pinaster. Canadian Journal of Forest Research 32, 569-580.

Zerihun A, McKenzie BA, Morton JD (1998) Photosynthate costs associated with the utilization of different nitrogen forms: influence on the carbon balance of plants and shoot-root biomass partitioning. New Phytologist 1998, 1-11.

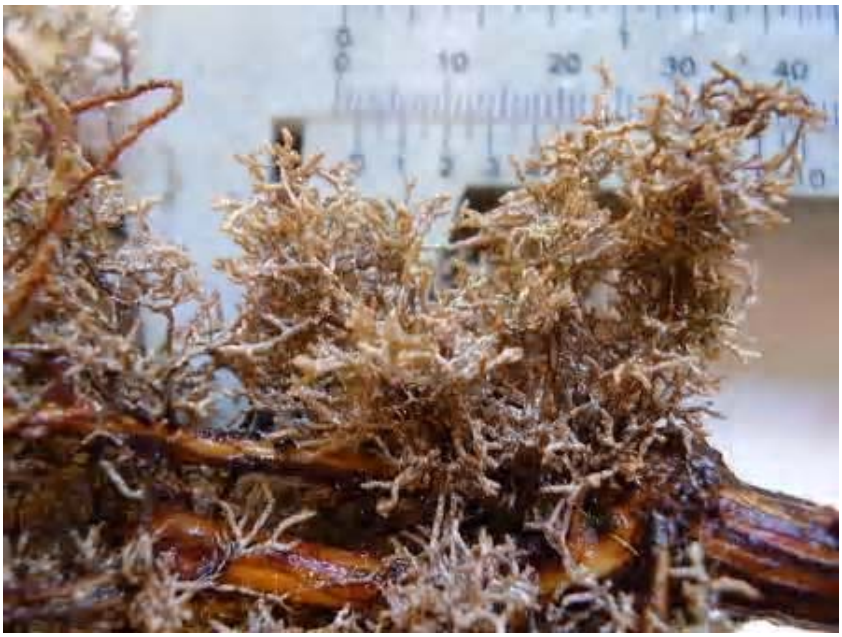
APPENDIX C

INFLUENCE OF NITROGEN AND PHOSPHORUS SUPPLY ON THE CARBON COST OF MYCORRHIZAE OF *PINUS RADIATA* CLONES

Pinus radiata clones were cultivated in silica sand in 42-dm^3 pots in a factorial combination of nitrogen and phosphorus supply to assess patterns of carbon allocation (Chapter *Five*)

Shallow (50 mm) and deep (300 mm) polyvinyl chloride collars 100 mm inner diameter were inserted in all 96 pots at the start of the experiment to assess mycorrhizae respiration.

Fruiting bodies of *Rhyzopogon rubescens* Tul. started to appear three-months after the experiment started and were collected periodically in order to assess fungal biomass



Roots of all plants were artificially inoculated with spores of *Rhizopogon rubescens* Tul. and confirmed as mycorrhizal when plants were harvested at the end of the ten-months experiment

ADDITIONAL MATERIAL AND METHODS

Plant material, climate in the greenhouse, plant and soil respiration measurements, and the carbon balance method were described in Chapter Five, and therefore not explained here. However in order to determine the carbon balance for mycorrhizae, fungal biomass determination and fungal respiration methods are described next.

Fungal biomass determination

Fungal biomass was partitioned as fruiting bodies, fungal biomass in fine roots and hyphae recovered in the sand. Fruiting bodies were collected periodically, oven-dried at 70 °C to constant mass and dry mass recorded. Fungal biomass in fine roots was determined by the intersection method in three subsamples of oven-dried fine roots per tree. Basically, small randomly selected fine-roots samples were placed on a Petri-dish over a 5 × 5 mm grid under a magnifying microscope, and intersections with either mycorrhizae or roots recorded using a tally-counter. Mycorrhizae infection was then recorded as a percentage of total counts, and this fraction was applied to the tree fine-roots biomass to yield an estimate of fungal biomass in fine roots. Coarse-roots (> 2 mm) did not exhibit visual signs of mycorrhizae infection. In Chapter Five it was reported that 2.5 kg subsamples of silica sand were taken from each pot to recover remaining roots by flotation. These same oven-dried samples were used to asses fungal biomass in the silica sand by the intersection method. Carbon content was assumed to be 50% of total mycorrhizae biomass.

Soil respiration measurements

Soil surface CO_2 efflux and mineral soil temperature were measured monthly in all plants using a soil respiration chamber (100 mm inner diameter, Model SRC-1, PP Systems, Herts, UK) connected to an infrared gas analyzer (Model EGM-4, PP Systems, Herts, UK). Soil collars made of polyvinyl chloride (100 mm inner diameter), one shallow (50 mm length) and one deep (300 mm length), were placed in each of the 96 pots at the start of the experiment. Soil respiration measurements were made monthly within the same day between 10 am and 6 pm. The soil respiration chamber was fitted tightly to each soil collar and soil CO_2 efflux and soil temperature

recorded for each plant. The deep collar had a lateral window of 5 × 20 cm cut in the cylinder and covered with 50 μm nylon mesh, to allow the hyphae but not fine roots to enter the cylinder (Figure C.1)

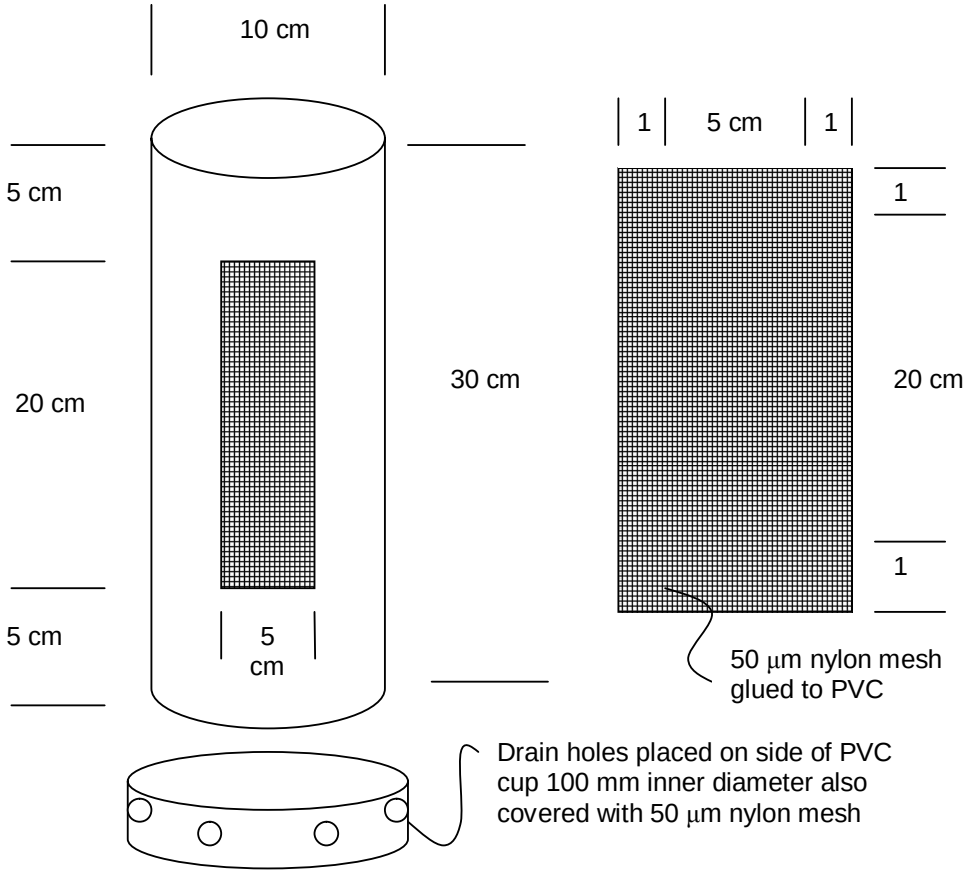


Figure C.1. Description of the soil enclosure used to measure mycorrhizae respiration in the carbon allocation experiment.

Additional to the 96 experimental units, four pots filled with silica sand without plants were set as controls for soil respiration and mineral soil carbon. These controls were subject to the same regimes of nutrition and irrigation as those applied in the experimental pots carrying plants (one pot per nutrient treatment). Monthly soil respiration values in control pots were very small (<0.02) or negative suggesting that CO_2 efflux was zero and that values measured in containers with plants were a true measure of root and mycorrhizae respiration.

Determination of mycorrhizae respiration

Measurements of soil respiration measured in deep collars were generally lower than those recorded using shallow collars. Because CO_2 concentration inside the deep collar was lower than in the surrounding media, CO_2 must be expected to diffuse according to Fick's Law i.e. CO_2 moves across a gradient in CO_2 concentration from high to low. Shallow and deep collars CO_2 efflux measurements were used to estimate mycorrhizal respiration.

Soil respiration rates in shallow and deep collars were zero or very close to zero in control pots without plants. However in pots with plants, CO_2 diffusion from the surrounding soil to the deep collar was observed, and the magnitude of the CO_2 efflux from the deep collar depended on plant size. According to Fick's law, gas flux (F) should be directly proportional to the diffusivity constant (D) and the concentration gradient (ΔC) and inversely proportional to distance for the gas to move (ΔX),

$$F = D\,\Delta C / \Delta X \qquad \text{(C.1)}$$

Values of D are gas specific being 0.1381 $cm^2\ s^{-1}$ for CO_2, and the distance (ΔX) was constant because the positition of the deep collar inside the pot was not changed throughout the experiment. The only condition that changed over the duration of the experiment was the CO_2 concentration gradient (ΔC), as plants developed and hence soil respiration increased over time.

Without the presence of hyphae and fruiting bodies inside the collar, the relationship between shallow and deep collar respiration should be linear according to Fick's law. This was effectively observed before the appearance of fruiting bodies at day 85 (Figure C.2). As expected, Slopes ($F_{3,88} < 2.57$, $P > 0.06$) and intercepts ($F_{3,88} < 1.57$, $P > 0.20$) of the linear relationships between deep and shallow collar CO_2 effluxes were not influenced by nutrient treatment or clone. However we observed a positive departure from the initial slope from day 90 onwards which also coincided with the appearance of fruiting bodies of *Rhyzopogon rubescens* in some pots (Figure C.3).

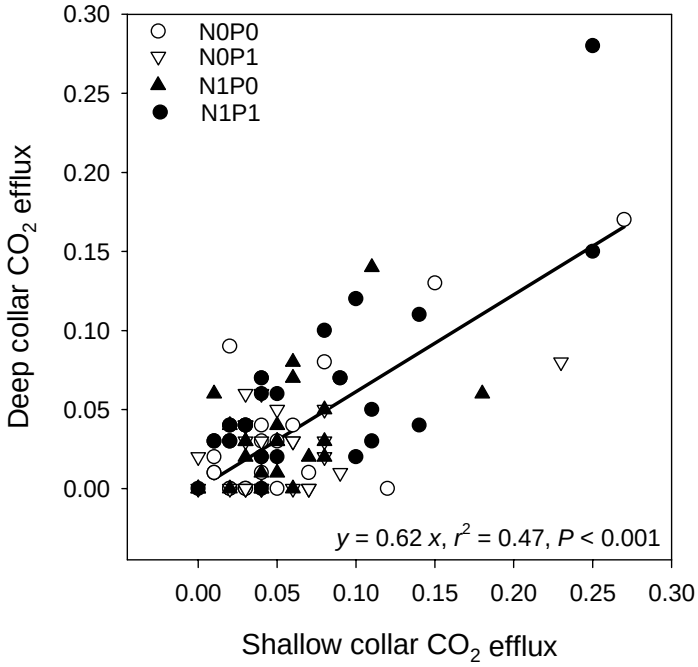


Figure C.2. Relationship between deep and shallow collar CO_2 efflux before hyphae spread inside deep collars. Measurements reported are those collected at day 85 (datum 15 September 2004, day zero). Fruiting bodies appeared after day 90.

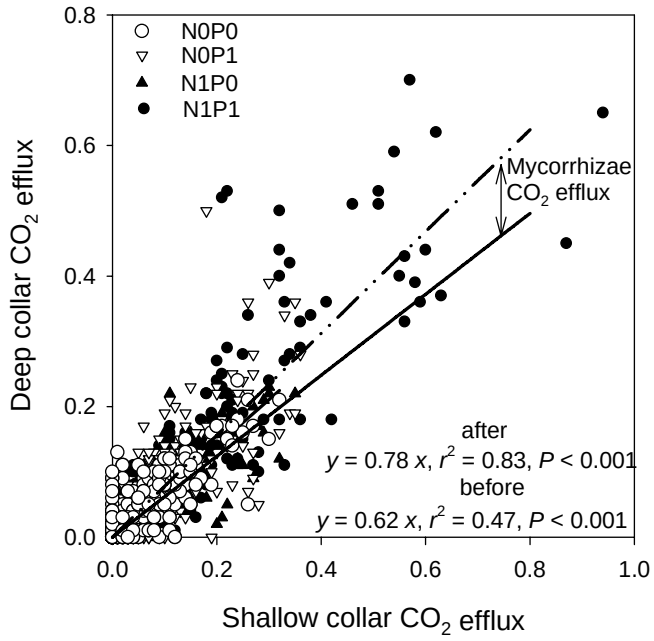


Figure C.3. Relationship between deep and shallow collar CO_2 efflux before and after hyphae spread inside deep collars. The deep collar was a sand enclosure with a window covered by a nylon mesh facing the roots. CO_2 diffusion was observed and explained according to Fick'a law before hyphae spread into the collar. Afterwards CO_2 efflux was explained by CO_2 diffusion from surrounding media and by mycorrhyzal respiration.

Departures from Fick's law were used as an estimation of mycorrhyzal respiration. Before hyphae spread inside the soil enclosure, deep collar respiration (y) was on average 62% of the shallow collar (x) respiration ($y = 0.62\ x$, $r^2 = 0.47$, $P < 0.001$, $n = 96$). After the appearance of fruiting bodies deep collar respiration was on average 78% of the shallow collar respiration ($y = 0.78\ x$, $r^2 = 0.83$, $P < 0.001$, $n = 1152$). As an approximation, mycorrhyzal respiration can be considered a fixed proportion of deep collar respiration (1-0.62/0.78 = 21%).

RESULTS

Fungal carbon content (CM), mycorrhizae respiration (MS), carbon allocation to mycorrhizae (MBCA) and the carbon cost of mycorrhizae (MBCA : GPP) are presented in Table C.1. Values of gross-primary productivity (GPP) were extracted from Chapter *Five*. Micorrhizae infection was significantly influenced by nutrient treatment ($F_{3,48} = 4.20$, $P = 0.014$) and clone ($F_{3,48} = 7.20$, $P < 0.001$) but not by their interaction ($F_{9,48} = 1.15$, $P = 0.36$). Values of infection consistently decreased with nitrogen and phosphorus supply from about 13% in the low-nutrient supply regime to about 8% in the high-nutrient supply regime. Values of infection in imbalanced treatments N_0P_1 and N_1P_0 were generally intermediate between balanced treatments N_0P_0 and N_1P_1, indicating an additive effect of N and P supply on mycorrhizal infection rates. In imbalanced treatments, plants growing with N only exhibited higher rates of infection than plants growing with P only. Rates of mycorrhizae infection were about half in Clone C compared to other clones.

Mycorrhizae production (C_M) and respiration (M_S) and their sum (MBCA) scaled with nitrogen and phosphorus supply and gross-primary productivity (Table C.1), indicating that the development of mycorrhizae was strongly linked to overall carbon assimilation. However, the ratio of MBCA : GPP, the carbon cost of mycorrhizae, declined with nitrogen and phosphorus supply from about 7.0 % in the low-nutrient supply regime to about 4.0 % in the high nutrient supply regime (Table C.1). Values of MBCA : GPP in imbalanced treatments N_0P_1 and N_1P_0 were intermediate between balanced treatments N_0P_0 and N_1P_1, indicating a clear interactive effect of N and P supply on mycorrhizae infection rates. Single deficiencies in nitrogen triggered greater responses in mycorrhizae infection than single P deficiencies. Using carbon use efficiencies determined in Chapter *Five*, the carbon cost of mycorrhizae can be also expressed in terms of net primary productivity (NPP). Then the carbon cost of mycorrhizae in the low-nutrient supply regime can be set at 16.2% of NPP compared to 7.7% of NPP in the high-nutrient supply regime.

Table C.1. Carbon content of mycorrhizae and respiration for all nutrient treatments and clones in the carbon allocation experiment. Nutrient treatments comprised two nitrogen supply regimes (N_0=1.43 and N_1=7.14 mM) and two phosphorus supply regimes (P_0=0.084 and P_1=0.420 mM). Values are presented as means (± 1 SE) for each treatment and clone; n = 6. Significance of main effects of clones (C) and nutrient treatments (T) or the interaction between clones and treatments (C × T) are shown as F values, P values and P range: ns, non significant; *, significant at $P < 0.05$; **, significant at $P < 0.01$; ***, significant at $P < 0.001$. Separation of means was determined by a Tukey test when applicable. Different letters indicate significant differences at $P < 0.05$. Significance of main effects of nutrient treatments and clones are indicated by letters when the C × T interaction showed to be non-significant (omitted otherwise).

C	T		C_M	M_S	MBCA	GPP	MBCA/GPP
		Mycorrhizae Infection in fine-roots (%)	Mycorrhizae C (g C plant^{-1})	Mycorrhizae respiration (g C plant^{-1})	Mycorrhizae C allocation (g C plant^{-1})	Gross-primary productivity (g C)	(%)
A	N_0P_0	0.11 ± 0.03 a	0.45 ± 0.16 a	1.32 ± 0.21 ab	1.78 ± 0.32 a	23 ± 3.4 a	7.6 ± 0.4 a
	N_0P_1	0.12 ± 0.03 a	1.19 ± 0.32 a	2.06 ± 0.31 ab	3.25 ± 0.41 a	47.3 ± 2.6 b	6.9 ± 0.8 ab
	N_1P_0	0.14 ± 0.03 a	0.7 ± 0.1 a	0.91 ± 0.12 a	1.61 ± 0.16 a	32.7 ± 6.4 a	5.5 ± 0.9 ab
	N_1P_1	0.07 ± 0.01 a	1.1 ± 0.27 a	2.65 ± 0.41 b	3.76 ± 0.67 a	86.3 ± 7.0 c	4.3 ± 0.6 b
	Mean	**0.11 ± 0.01 a**	**0.86 ± 0.12 ab**	**1.74 ± 0.19 a**	**2.6 ± 0.28 bc**	**47.3 ± 5.6 a**	**6.1 ± 0.4 a**
B	N_0P_0	0.17 ± 0.05 a	0.71 ± 0.16 a	1.3 ± 0.2 a	2.01 ± 0.18 a	28.4 ± 1.7 a	7.2 ± 0.7 a
	N_0P_1	0.08 ± 0.01 a	0.76 ± 0.06 a	2.01 ± 0.31 a	2.78 ± 0.33 ab	50.5 ± 3.5 b	5.5 ± 0.5 a
	N_1P_0	0.15 ± 0.01 a	1.68 ± 0.4 a	1.82 ± 0.15 a	3.5 ± 0.55 ab	64.6 ± 6.8 b	5.3 ± 0.4 a
	N_1P_1	0.12 ± 0.03 a	2.18 ± 0.61 a	3.76 ± 0.45 b	5.93 ± 1.01 b	119.3 ± 9.6 c	4.9 ± 0.6 a
	Mean	**0.13 ± 0.02 a**	**1.33 ± 0.22 a**	**2.22 ± 0.24 a**	**3.56 ± 0.42 a**	**65.7 ± 7.6 b**	**5.7 ± 0.3 a**
C	N_0P_0	0.09 ± 0.01 a	0.38 ± 0.11 a	1.15 ± 0.26 a	1.54 ± 0.36 a	25.5 ± 5.4 a	5.8 ± 0.7 a
	N_0P_1	0.05 ± 0.01 a	0.59 ± 0.12 a	2.02 ± 0.42 a	2.61 ± 0.52 ab	58.7 ± 6.8 b	4.3 ± 0.7 a
	N_1P_0	0.06 ± 0.01 a	0.58 ± 0.06 a	1.49 ± 0.21 a	2.07 ± 0.26 ab	60.5 ± 8.8 b	3.5 ± 0.4 a
	N_1P_1	0.05 ± 0.02 a	1.16 ± 0.36 a	3.17 ± 0.4 b	4.33 ± 0.7 b	125.4 ± 16.8 c	3.4 ± 0.3 a
	Mean	**0.06 ± 0.01 b**	**0.68 ± 0.11 b**	**1.96 ± 0.22 a**	**2.64 ± 0.32 c**	**67.5 ± 9 b**	**4.3 ± 0.3 b**
D	N0P0	0.15 ± 0.02 a	1.16 ± 0.15 a	1.85 ± 0.22 a	3.02 ± 0.28 a	43.5 ± 5.0 a	7.3 ± 0.9 a
	N0P1	0.13 ± 0.02 a	1.54 ± 0.31 a	2.16 ± 0.19 a	3.69 ± 0.46 a	57 ± 4.4 a	6.4 ± 0.5 ab
	N1P0	0.13 ± 0.01 a	1.04 ± 0.16 a	1.69 ± 0.13 a	2.72 ± 0.27 a	58.2 ± 7.6 a	4.8 ± 0.4 ab
	N1P1	0.08 ± 0.01 a	0.94 ± 0.16 a	2.48 ± 0.22 a	3.42 ± 0.35 a	105.7 ± 15.1 b	3.4 ± 0.2 b
	Mean	**0.12 ± 0.01 a**	**1.17 ± 0.11 ab**	**2.05 ± 0.11 a**	**3.21 ± 0.18 ab**	**66.1 ± 6.5 b**	**5.5 ± 0.4 a**
All Clones	N_0P_0	0.13 ± 0.02 a	0.68 ± 0.09 a	1.41 ± 0.12 a	2.09 ± 0.18 a	30.1 ± 2.5 a	7 ± 0.4 a
	N_0P_1	0.1 ± 0.01 ab	1.02 ± 0.13 ab	2.06 ± 0.15 b	3.08 ± 0.22 b	53.4 ± 2.3 b	5.8 ± 0.4 b
	N_1P_0	0.12 ± 0.01 ab	1.00 ± 0.14 ab	1.48 ± 0.1 a	2.48 ± 0.22 ab	54 ± 4.3 b	4.8 ± 0.3 bc
	N_1P_1	0.08 ± 0.01 b	1.34 ± 0.21 b	3.02 ± 0.2 c	4.36 ± 0.39 c	109.2 ± 6.7 c	4 ± 0.3 c
Overall	**Mean**	**0.11 ± 0.01**	**1.01 ± 0.08**	**1.99 ± 0.1**	**3 ± 0.16**	**61.7 ± 3.7**	**5.4 ± 0.2**
Anova	C	7.2,<0.001 ***	9.7,<0.001 ***	2.28,0.10 Ns	4.6,0.009 **	7.62, <0.001 ***	5.6,0.004 **
	T	4.2, 0.014 *	6.7,0.0014 **	31.0,<0.001 ***	12.8,<0.001 ***	71.48, < 0.001 ***	14.9,<0.001 ***
	C × T	1.15,0.36 ns	2.18,0.053 ns	1.99,0.08 ns	1.77,0.12 ns	2.26, 0.046 *	0.61,0.78 ns

APPENDIX D

COMPLEMENTARY INFORMATION TO CHAPTER *SIX*

Only key findings are presented in Chapter *Six*, while further explanations and additional information are provided in this appendix. This appendix covers the following points:

D1. Plot specific soil chemical and physical properties

D2. Seasonal fluctuations in exchangeable NO_3^- - N, NH_4^+ - N and P for each plot

D3. The response of soil respiration to temperature and water availability, and the partitioning of soil respiration between autotrophic and heterotrophic respiration

D4. Calculation of gross-primary productivity fractions (TBCA, APR, ANPP)

D5. State variables, dry matter partitioning and nutrient content

D6. Tree phenology and leaf area to mass ratio

D7. Chlorophyll fluorescence as an index of photosynthetic performance

D.1 PLOT SPECIFIC SOIL CHEMICAL AND PHYSICAL PROPERTIES

Table D.1.1.. Soil physical and chemical properties of control (C) and fertilized (F) plots of *Pinus radiata* at five sites in the South Island of New Zealand at harvest in August-September 2005. Data was provided by Landcare Research and the ENSIS. Soil analysis are presented for the first mineral soil horizon (A, 0-10 cm deep). Water balance was determined as the average monthly volumetric water content (θ) for the year ending August 2005.

Site	Rai Valley		Golden Downs		Tekapo		Catlins		Longwoods	
	C	F	C	F	C	F	C	F	C	F
Order	Brown	Brown	Brown	Brown	Brown	Brown	Brown	Brown	Alloph.	Alloph.
Coarse sand (%)	16	16	2	2	1	1	1	1	1	1
Medium sand (%)	2	2	8	8	3	3	8	8	1	1
Fine sand (%)	2	2	16	16	19	19	25	25	3	3
Sand (%)	20	20	26	26	23	23	34	34	5	5
Silt (%)	34	34	42	42	62	62	39	39	46	46
Clay (%)	46	46	31	31	15	15	27	27	49	49
Bulk density (g cm^{-3})	0.99	0.99	1.15	1.15	0.92	0.92	0.79	0.79	0.47	0.47
Part. density (g cm^{-3})	2.68	2.68	2.53	2.53	2.53	2.53	2.51	2.51	2.22	2.22
Pen. resistance (MPa)	0.77	0.77	1.23	1.23	0.78	0.78	0.68	0.68	0.71	0.71
Tot. porosity (%, v/v)	63	63	55	55	64	64	69	69	79	79
Macroporosity (%)	24	24	14	14	17	17	22	22	18	18
Carbon (%)	4.27	5.06	6.07	5.62	3.98	4.13	7.01	6.53	26.74	24.47
Total N (%)	0.23	0.23	0.25	0.23	0.29	0.28	0.31	0.34	0.85	0.80
C:N ratio	18.6	21.9	24.6	24.5	13.9	14.6	22.3	19.3	31.3	30.5
pH	4.78	4.41	4.48	4.38	5.12	5.07	4.63	4.50	4.18	4.00
CEC (cmol kg^{-1})	20.15	20.42	16.63	17.18	17.77	17.28	17.40	17.05	42.89	45.77
Exch Ca (cmol kg^{-1})	5.21	3.07	1.67	1.50	5.89	4.06	1.00	1.19	9.27	6.58
Ex Mg (cmol kg^{-1})	2.97	2.23	0.92	1.01	1.19	1.09	0.89	1.30	3.93	4.11
Ex K (cmol kg^{-1})	0.75	0.90	0.49	0.73	0.56	1.05	0.32	0.84	0.44	1.22
Ex Na (cmol kg^{-1})	0.31	0.34	0.18	0.06	0.18	0.17	0.27	0.34	0.00	0.68
Sum bases (cmol kg^{-1})	9.24	6.54	3.25	3.29	7.82	6.38	2.48	3.68	13.59	12.59
Base sat. (%)	45.87	32.01	19.56	19.17	44.00	36.89	14.28	21.56	31.68	27.51
Olsen P (μg g^{-1})	3	10	3	26	13	30	31	65	2.5	16
Bray P (μg g^{-1})	4	28	12	67	37	109	49	106	4	36
Inorg. P (μg g^{-1})	74	148	42	162	143	227	299	409	44	202
Org. P (μg g^{-1})	438	383	280	273	601	587	598	657	477	492
Total P (μg g^{-1})	512	531	322	435	744	814	898	1065	520	694
θ (m^3 m^{-3})	0.236	0.230	0.262	0.252	0.137	0.134	0.238	0.230	0.237	0.217
Rooting depth (mm)	778	386	368	462	539	456	401	427	330	415
Water balance (mm)	183	89	96	116	74	61	96	98	78	90

D.2 SEASONAL FLUCTUATIONS IN EXCHANGEABLE NO_3^- - N, NH_4^+ - N AND P FOR EACH PLOT

There was a strong seasonal effect in exchangeable NO_3^-, NH_4^+ and HPO_4^- across sites and fertilization treatments as measured by ion exchange membranes and soil extracts. In control plots, the main flush of NH_4^+ mineralization occurred during November 2004, while the main flush for NO_3^- occurred one month later being this seasonality consistent across sites (Figure D.2.1).

Fertilization was applied in September 2004 and had an immediate effect on nitrogen and phosphorus availability except at Tekapo where the effect of fertilization was small. The effect of fertilization was more pronounced for NO_3^- than for NH_4^+ and this trend was consistent across all sites (Figure D.2.1). Exchangeable NO_3^- - N availability fluctuated seasonally 29-72 fold compared to 12-31 fold for NH_4^+- N in control plots across sites, while 110-792 fold for NO_3^- - N and 26-116 fold for NH_4^+- N in fertilized plots. Seasonal fluctuations in available phosphorus were small compared to NO_3^- - N and NH_4^+- N oscillating 1.9-4.7 times in control plots and 3.2-17.6 times in fertilized plots (Figure D.2.1).

The area below the curves of exchangeable NO_3^- - N and NH_4^+- N over time might be integrated to yield an overall index of nutrient availability in Figure D.2.1. Ion exchange membranes accumulated ions between burial periods and hence their integration seems to be a valid means to represent nutrient availability. Hence, the magnitude of the fertilization response might be assessed as the area between control and fertilized plots for exchangeable NO_3^- - N and NH_4^+- N. Such fertilization response suggests that sites might be classified in descending order of fertility as: Tekapo > Rai Valley > Golden Downs > Catlins > Longwoods. It seems also relevant to point out that the effect of fertilization on nitrate availability lasted two months at Rai Valley and Golden Downs, five months at Catlins, six months at Longwoods, whereas for Tekapo the effect of fertilization was minimal (Figure D.2.1).

Average monthly values of exchangeable nitrate, ammonium and phosphates as measured by ion exchange membranes are presented in Table D.2.1. Sites were not largely different in exchangeable NO_3^- -N or NH_4^+-N, whereas exchangeable P showed to be substantially higher at Golden Downs compared to other sites. In control plots, total exchangeable nitrogen (NH_4^+-N + NO_3^--N) was highest at Longwoods (5.5), progressively decreasing at Tekapo (4.7), Catlins (4.1), Rai Valley (3.7) and Golden Downs (2.6). Also, the NH_4^+-N : NO_3^--N ratio in control plots was largest at Golden Downs (1.5), progressively decreasing in Longwoods (1.3), Catlins (1.3), Rai Valley (0.8), being lowest at Tekapo (0.6),

suggesting that the proportion of ammonium to nitrate might be a stronger driver of productivity than total N supplied.

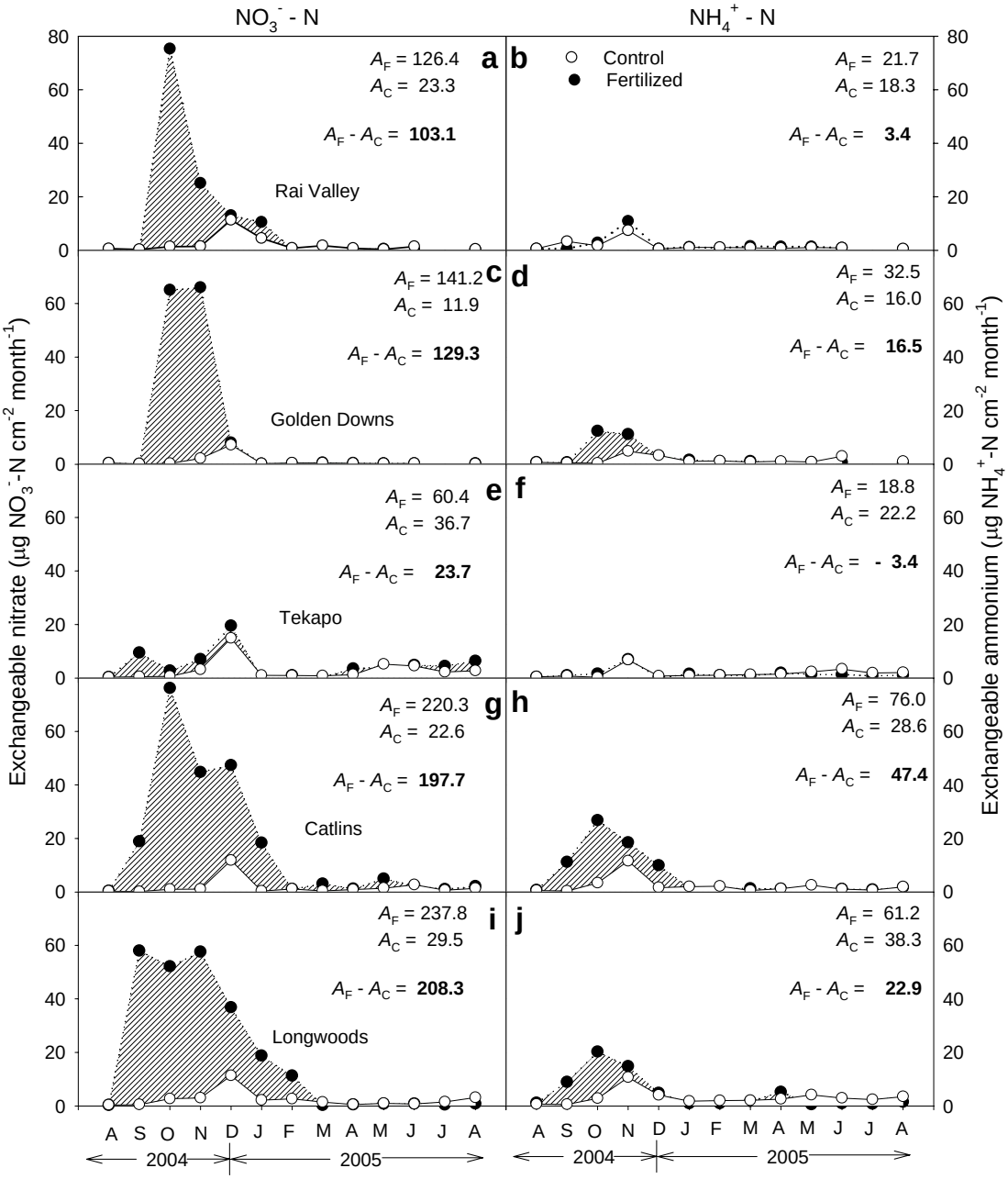


Figure D.2.1 Soil exchangeable NO_3^--N and NH_4^+-N as measured by ion exchange membranes for control (○) and fertilized plots (●) of *Pinus radiata* in five sites in the South Island of New Zealand. In the left column exchangeable NO_3^- is presented and in the right column exchangeable NH_4^+ is presented. Sites are presented from top to bottom as in a latitudinal gradient. Area between the response curves of fertilized (A_F) and control plots (A_C) might be seen as the overall magnitude of the fertilization response.

Table D.2.1. Average monthly values of exchangeable ammonium, nitrate and phosphates as measured by ion exchange membranes in control and fertilized plots of *Pinus radiata* in five sites in the South Island of New Zealand for the year ending August 2005. Main effects of sites (S) and fertilization (F) were assessed by analysis of variance. Significant differences are presented as *F* values, *P* values and *P* range: ns, non significant; *, significant at $P < 0.05$; **, significant at $P < 0.01$; ***, significant at $P < 0.001$. Different letters indicate significant differences at $P < 0.05$. Values are presented as means (± 1SE) for sites and fertilization treatments.

Site	Plot	Exchangeable ions (Membranes)				
		NO_3^--N µg cm^{-2} month^{-1}	NH_4^+-N µg cm^{-2} month^{-1}	NH_4^+-N / NO_3^-N	Total N µg cm^{-2} month^{-1}	P µg cm^{-2} month^{-1}
Rai Valley	Control	2.1	1.6	0.8	3.7	0.25
	Fertilized	10.6	1.9	0.2	12.5	0.37
	Mean	6.4 ± 4.3	1.8 ± 0.2	0.5 ± 0.3	8.1 ± 4.4	0.31 ± 0.06 **b**
Golden Downs	Control	1.0	1.6	1.5	2.6	2.05
	Fertilized	11.8	2.8	0.2	14.6	2.21
	Mean	6.4 ± 5.4	2.2 ± 0.6	0.9 ± 0.7	8.6 ± 6	2.13 ± 0.08 **a**
Tekapo	Control	2.9	1.8	0.6	4.7	0.25
	Fertilized	4.9	1.5	0.3	6.4	0.28
	Mean	3.9 ± 1	1.7 ± 0.2	0.5 ± 0.2	5.6 ± 0.9	0.27 ± 0.02 **b**
Catlins	Control	1.8	2.3	1.3	4.1	0.27
	Fertilized	17.0	5.9	0.3	23.0	0.38
	Mean	9.4 ± 7.6	4.1 ± 1.8	0.8 ± 0.5	13.6 ± 9.5	0.33 ± 0.06 **b**
Longwoods	Control	2.4	3.1	1.3	5.5	0.25
	Fertilized	18.3	4.8	0.3	23.1	0.27
	Mean	10.4 ± 8	4 ± 0.9	0.8 ± 0.5	14.3 ± 8.8	0.26 ± 0.01 **b**
Fertilization	Control	2 ± 0.3 a	2.1 ± 0.3	1.1 ± 0.2 **a**	4.1 ± 0.5 **a**	0.61 ± 0.36 **a**
	Fertilized	12.5 ± 2.4 b	3.4 ± 0.8	0.3 ± 0 **b**	15.9 ± 3.2 **b**	0.7 ± 0.38 **b**
ANOVA	S	0.85,0.56 ns	2.56,0.19 ns	0.93,0.52 ns	1.16,0.44 ns	7.38,<0.001 ***
	F	17.2, .0014 **	3.75,0.13 ns	23.06,0.009 **	14.27,0.020 *	10.55,0.031 *

The main effect of fertilization was a drastic increase in available nitrate as compared to small or no effects on ammonium availability. It seems quite striking that in all sites the NH_4^+-N : NO_3^--N ratio decreased with fertilization despite the fact that the fertilizer was balanced (44% NO_3-N and 56% NH_4-N). Fertilization increased P availability slightly but consistently across sites, and values in control and fertilized plots were similar across sites except for Golden Downs where P availability was eight-fold greater than in other sites (Table D.2.1).

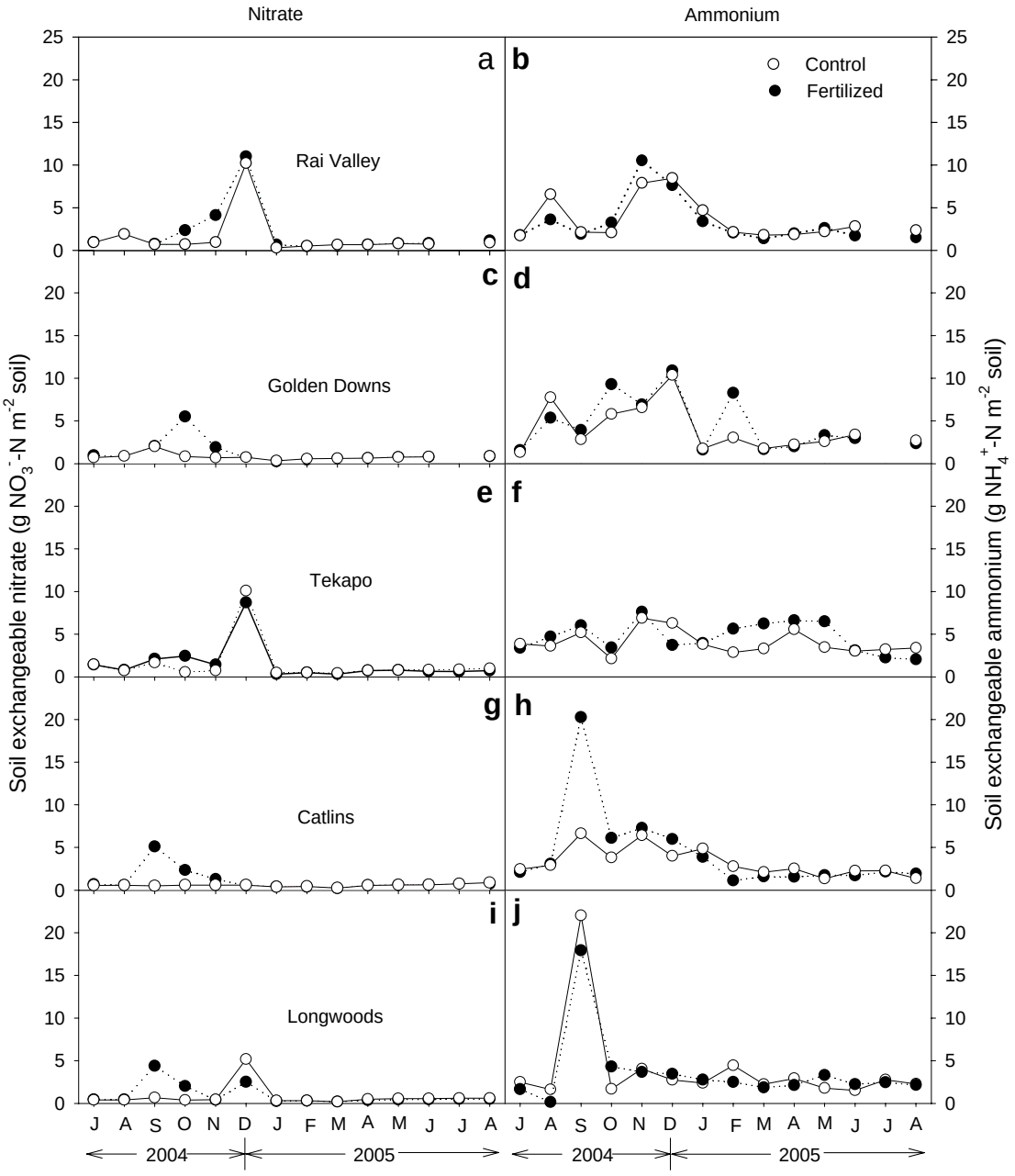


Figure D.2.2 Soil mineralization as measured by soil extracts (10 g fresh soil in 50 cm^3 KCl 2 M) for control (○) and fertilized plots (●) of *Pinus radiata* across a fertility gradient in the South Island of New Zealand. Sites are presented as rows while extractable NO_3^- and NH_4^+ are presented as the left and right column respectively. Exchangeable ions are expressed as grams of elemental N per square meter of soil to a depth of 30 cm.

Seasonal patterns of ion availability were also observed using soil extracts similarly to those observed using ion exchange membranes (Figure D.2.2). However, seasonal and fertilization effects on ion availability were more clearly marked using ion exchange membranes than soil extracts. This is probably associated with soil extracts measuring ion availability at one point in time whereas ion exchange membranes accumulated ions during a

time interval resulting in more stable values. Average monthly values of exchangeable nitrate, ammonium and phosphates as measured by soil extracts are presented in Table D.2.2.

Table D.2.2. Monthly averages of soil exchangeable ammonium, nitrate and phosphates as measured by soil extracts (10 g fresh soil in 50 ml 2M KCl) in control and fertilized plots of *Pinus radiata* in five sites in the South Island of New Zealand. Exchangeable ions are expressed as grams per square meter of soil to a depth of 30 cm. Main effects of sites (S) and fertilization (F) were assessed by analysis of variance. Significant differences are presented as *F* values, *P* values and *P* range: ns, non significant; *, significant at $P < 0.05$; **, significant at $P < 0.01$; ***, significant at $P < 0.001$. Different letters indicate significant differences at $P < 0.05$. Values are presented as means (± 1 SE) for sites and fertilization treatments.

Site	Plot	Exchangeable ions (soil extracts)				
		NO_3^-N g m^{-2}	NH_4^+-N g m^{-2}	NH_4^+-N / NO_3^-N	Total N g m^{-2}	P g m^{-2}
Rai Valley	Control	1.53	3.58	2.34	5.12	0.16
	Fertilized	1.94	3.32	1.71	5.27	0.16
	Mean	1.7 ± 0.2 **a**	3.5 ± 0.1	2 ± 0.3 **b**	5.2 ± 0.1	0.16 ± 0 **a**
Golden Downs	Control	0.80	4.01	4.99	4.81	0.16
	Fertilized	1.23	4.63	3.75	5.86	0.16
	Mean	1 ± 0.2 **bc**	4.3 ± 0.3	4.4 ± 0.6 **ab**	5.3 ± 0.5	0.16 ± 0 **a**
Tekapo	Control	1.49	4.04	2.72	5.53	0.14
	Fertilized	1.54	4.65	3.02	6.19	0.14
	Mean	1.5 ± 0 **ab**	4.3 ± 0.3	2.9 ± 0.2 **ab**	5.9 ± 0.3	0.14 ± 0 **a**
Catlins	Control	0.57	3.27	5.79	3.84	0.16
	Fertilized	1.05	4.31	4.11	5.36	0.16
	Mean	0.8 ± 0.2 **c**	3.8 ± 0.5	5 ± 0.8 **a**	4.6 ± 0.8	0.16 ± 0 **a**
Longwoods	Control	0.80	3.93	4.91	4.73	0.09
	Fertilized	0.95	3.61	3.79	4.57	0.07
	Mean	0.9 ± 0.1 **c**	3.8 ± 0.2	4.4 ± 0.6 **ab**	4.7 ± 0.1	0.08 ± 0.01 **b**
Fertilization	Control	1 ± 0.2 **a**	3.8 ± 0.1	4.2 ± 0.7	4.8 ± 0.3	0.14 ± 0.01
	Fertilized	1.3 ± 0.2 **b**	4.1 ± 0.3	3.3 ± 0.4	5.5 ± 0.3	0.14 ± 0.02
ANOVA	S	18.5,0.008 **	1.67,0.31 ns	10.4,0.020 *	2.39,0.20 ns	60,<0.001 ***
	F	12.63,0.024 *	1.59,0.28 ns	6.7,0.06 ns	4.55,0.10 ns	1.0,0.37 ns

Results using soil extracts confirm previous findings using ion exchange membranes in that the main effect of fertilization was to substantially increase the amount of available nitrate with only small and not uncommonly negative effect on ammonium availability. As a consequence, the NH_4^+-N : NO_3^--N ratio consistently decreased with fertilization except for Tekapo, the most fertile site, where fertilization did not have a major effect. Again total available N did not differ largely between sites suggesting that the NH_4^+-N : NO_3^--N ratio might be a stronger driver of fertility than total available N (Table 6.5).

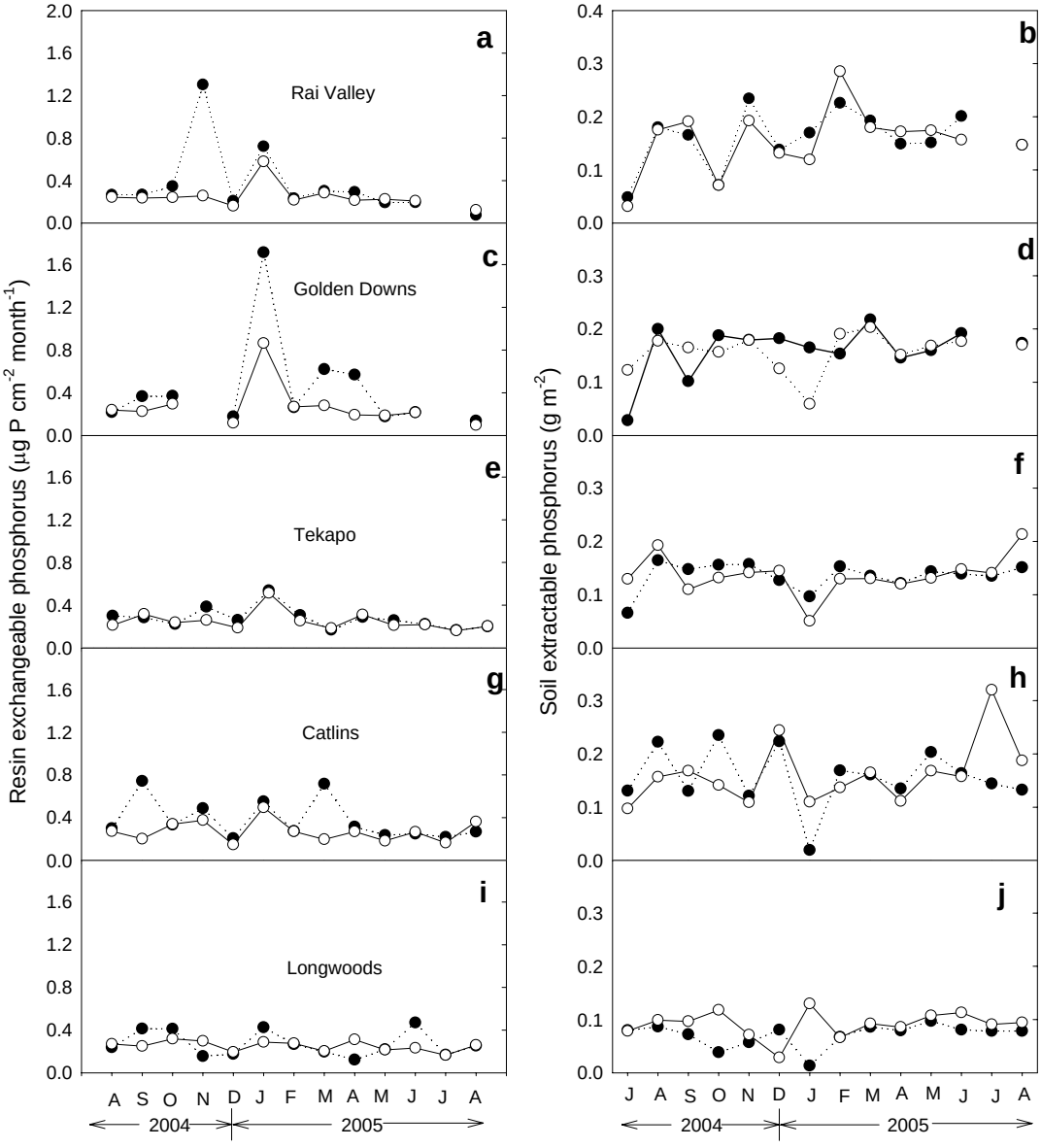


Figure D.2.3. Soil exchangeable phosphorus as measured by resin exchange membranes and soil extracts (10 g fresh soil in 50 cm^3 KCl 2 M) for control (○) and fertilized plots (●) of *Pinus radiata* in five sites in the South Island of New Zealand. Soil extractable phosphorus is shown as grams per square meter in the first 30 cm of the soil.

Figure D.2.3 shows that the effect of fertilization was relatively modest in the case of soil extractable phosphorus as compared to nitrogen, and not drastically different between sites except at Longwoods where phosphorus availability was lowest. The evidence presented so far suggests that nitrogen rather than phosphorus was the main nutritional limitation across all five sites. This is because the small differences in P availability between control and fertilized plots in all sites are unlikely to produce the massive growth responses to fertilization observed at Longwoods, Golden Downs and the Catlins.

D.3 THE RESPONSE OF SOIL RESPIRATION TO TEMPERATURE AND WATER AVAILABILITY

Monthly values of soil respiration values in control and fertilized plots across all five sites are presented in Figure D.3.1.

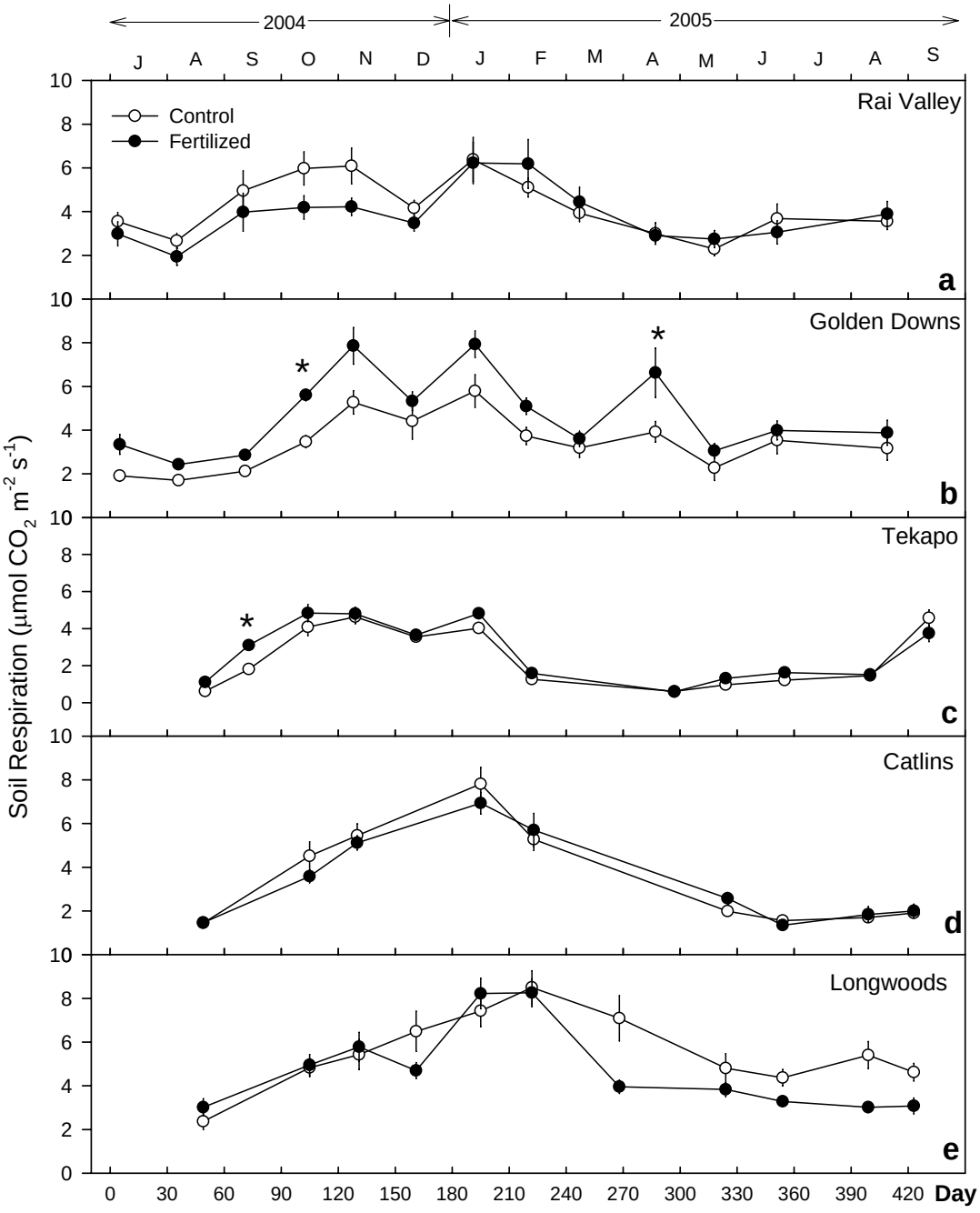


Figure D.3.1. Monthly values of soil respiration in control and fertilized plots of *Pinus radiata* in five sites in the South Island of New Zealand. Values are presented as means (± 1 SE, n = 9-12). Asterisks indicate significant differences at $P < 0.05$ between control and fertilized plots within the same month using a Tukey's test. Datum zero is 1st july 2004.

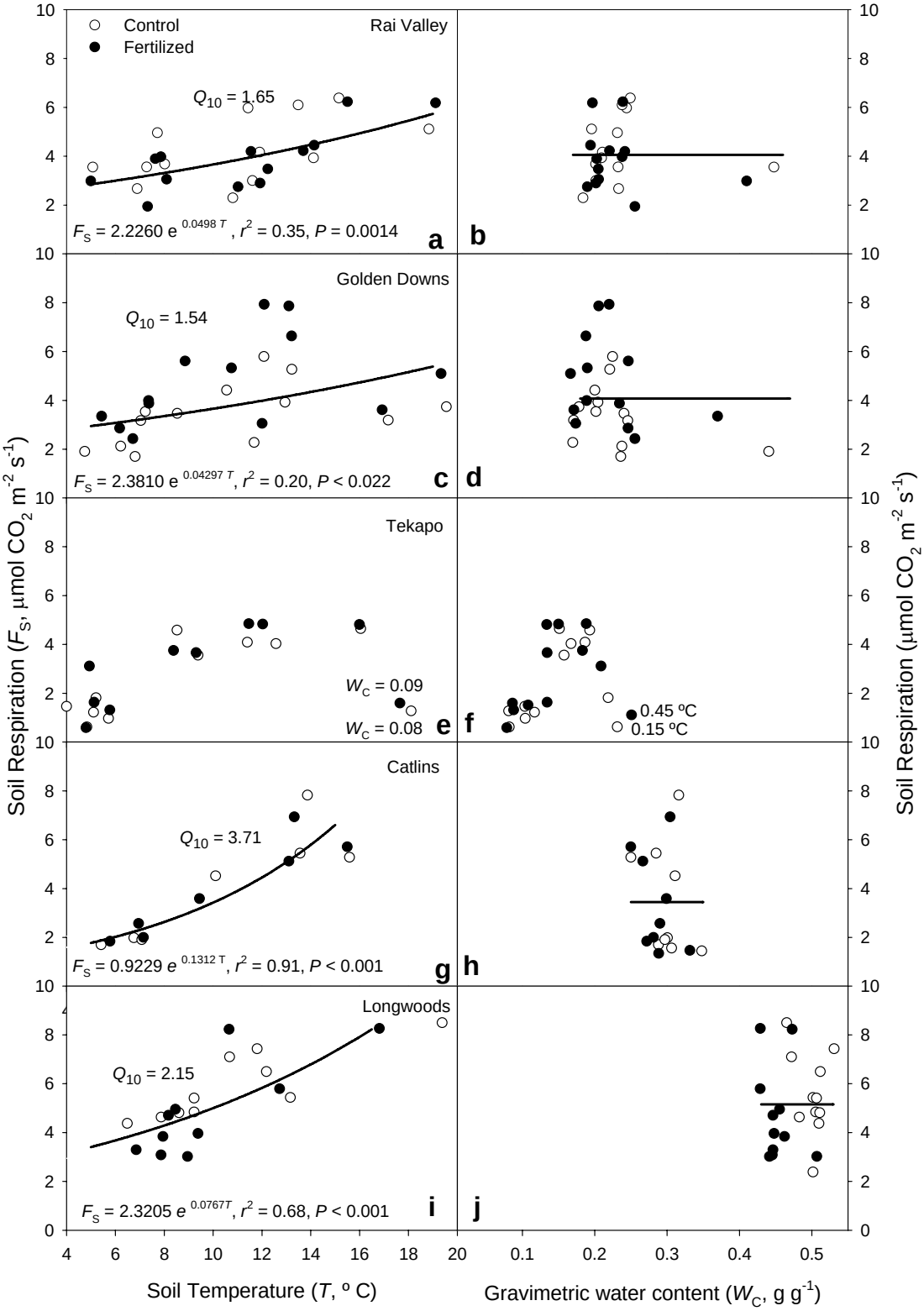


Figure D.3.2. The relationship between soil respiration rates, temperature and gravimetric water content in control and fertilized plots of *Pinus radiata* in five sites in the South Island of New Zealand.

Soil respiration followed a seasonal pattern that closely matched those of air temperature (Figure D.3.1, D.3.2), and additionally in the case of Tekapo, those of gravimetric water content (Figure D.3.3). In all sites soil respiration sharply increased in early spring, peaked in middle summer declining progressively in autumn to reach a minimum during winter. In Catlins and Longwoods there was only one peak in middle summer, while in other sites one in spring and one in the middle of summer. Differences in soil respiration between control and fertilized plots were non significant except for September 2004 in Tekapo and October 2004 and April 2005 in Golden Downs (Figure D.3.1) and were all in favour of fertilized plots.

Soil respiration rates scaled positively and significantly with temperature ($r^2 > 0.20$, $P < 0.022$) but not with gravimetric water content ($r^2 < 0.12$, $P > 0.09$). Slopes ($F_{1,14\text{-}22} < 0.48$, $P > 0.49$) and intercepts ($F_{1,14\text{-}22} < 0.98$, $P > 0.34$) of the linear relationships between (log) soil respiration rates and temperature ($\log_e y = a + bx$) were not significantly influenced by fertilization treatment (Figure D.3.2).

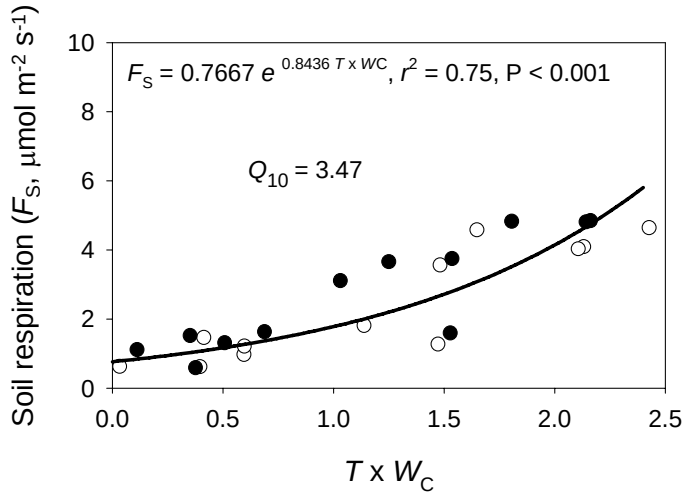


Figure D.3.3. The relationship between soil respiration rate and the product of soil temperature (T, °C) and gravimetric water content (W_C, g g^{-1}). The Q_{10}, which is the rate of increase in soil respiration with a 10° C increase in soil temperature, was calculated by replacing the average gravimetric water content ($W_C = 0.1476$) in the equation presented in this figure i.e. $F_S = 0.7667\ e^{0.1245\ T}$, and then $Q_{10} = e^{0.1245 \times 10} = 3.47$.

Gravimetric water content was lowest in Tekapo compared to other sites (Figure D.1.2f), and there was a clear interaction between soil temperature and gravimetric water content on soil respiration in this site. For instance in Figure D.3.2e, notice that two values of soil respiration measured at high temperatures were much lower than expected because their gravimetric water contents were very low (< 0.1 g g^{-1}). Similarly, in Figure D.3.2f, two measurements of soil respiration (different to the ones previously discussed in Figure D.3.1 d) taken at high gravimetric water content were lower than expected because soil temperatures

were very low (< 1 °C). This interaction was modelled by correlating (log) soil respiration rates with the product of temperature and gravimetric water content (Figure D.3.3). Slopes ($F_{1,20}$ = 0.06, P = 0.81) and intercepts ($F_{1,20}$ = 1.16, P = 0.29) of this linear relationship were not significantly influenced by fertilization treatment.

Values of Q_{10}, which corresponds to the rate of increase in soil respiration with a 10 °C increase in soil temperature, was lowest in Golden Downs (1.54) and Rai Valley (1.65), intermediate in Longwoods (2.15) and high in Tekapo (3.47) and Catlins (3.71). These equations and Q_{10} values were used to scale soil respiration for the year ending August 2005.

The proportion of total soil respiration represented by heterotrophic respiration was calculated as the slope in the linear relationship between soil respiration measured in deep and shallow collars with intercepts equal to zero (Figure D.3.4).

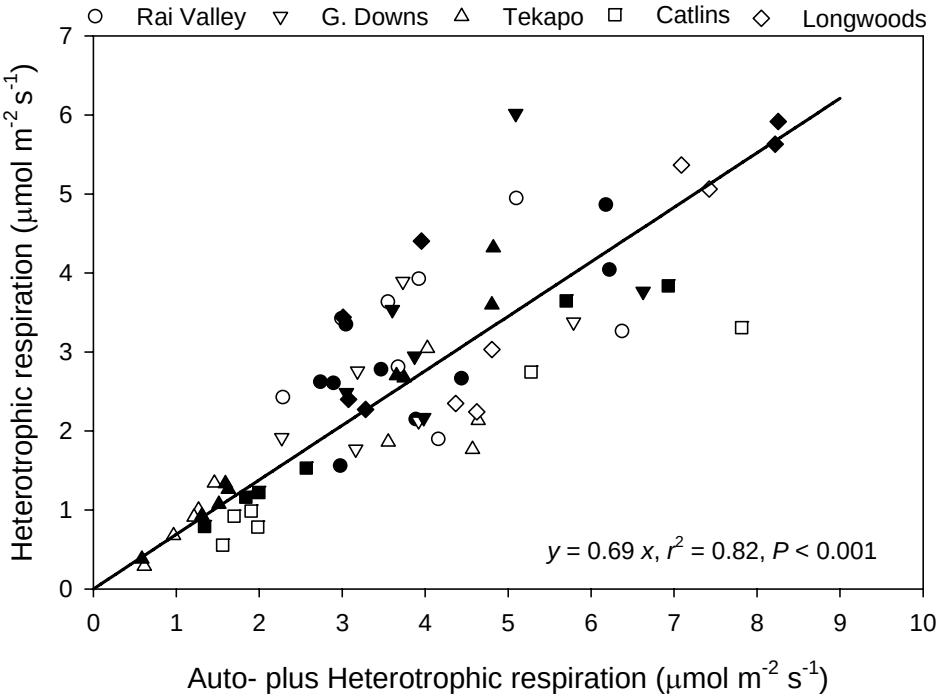


Figure D.3.4. The relationship between heterotrophic and auto- plus heterotrophic soil respiration in control and fertilized plots of *Pinus radiata* in five sites in the South Island of New Zealand. Control plots are represented by open symbols while fertilized plots by closed symbols.

Slopes ($F_{9,60}$ = 2.68, P = 0.011) of this linear relationships fitted without intercepts were significantly influenced by site and fertilization treatment (Table D.3.1). The proportion of total soil respiration (auto- plus heterotrophic, R_{A+H}) represented by heterotrophic (R_H) respiration significantly increased with fertilization in Tekapo and Catlins, tended to increase in G. Downs and Longwoods and slightly decrease in Rai Valley. Overall, values of R_H / R_{A+H} were lowest in Catlins and highest in Rai Valley.

Table D.3.1. The proportion of total soil respiration represented by heterotrophic respiration in control and fertilized plots of *Pinus radiata* in five sites in the South Island of New Zealand. This value was calculated as the slope of the linear relationship between soil respiration measured in deep collars to shallow collars. Significance of slopes is presented as *t* values and *P* range: ***: significant at $P < 0.001$.

Site	Plot	Slope	95% confidence interval	*t* value
Rai Valley	Control	0.78 ± 0.09 bc	(0.55 - 1)	8.19***
	Fertilized	0.73 ± 0.05 bc	(0.6 - 0.85)	13.77***
Golden Downs	Control	0.69 ± 0.08 ab	(0.48 - 0.91)	8.32***
	Fertilized	0.78 ± 0.11 ab	(0.49 - 1.07)	7.03***
Tekapo	Control	0.54 ± 0.06 ab	(0.41 - 0.67)	9.81***
	Fertilized	0.78 ± 0.03 c	(0.72 - 0.84)	30.03***
Catlins	Control	0.45 ± 0.02 a	(0.4 - 0.51)	20.26***
	Fertilized	0.59 ± 0.02 b	(0.55 - 0.64)	33.48***
Longwoods	Control	0.66 ± 0.05 bc	(0.53 - 0.78)	14.12***
	Fertilized	0.76 ± 0.07 bc	(0.59 - 0.93)	11.7***
Overall mean		0.69 ± 0.02	(0.64 - 0.73)	30.32***

Specific values of R_H / R_{A+H} by site were used to partitioned auto- from heterotrophic respiration. This was required in order to calculate net primary productivity (NPP) as gross-primary productivity (GPP) minus autotrophic respiration (R_A).

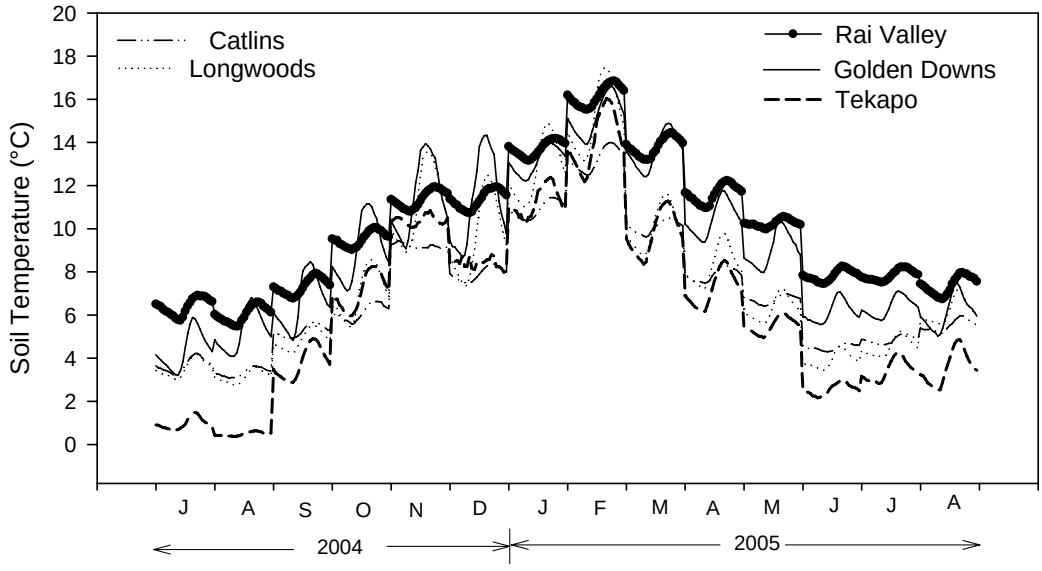


Figure D.3.5. Average monthly hourly soil temperatures across five sites in the South Island of New Zealand.

Soil temperatures resembled closely those of air temperature but their oscillation was smaller (Figure D.3.5). It can be seen than soil temperature was generally highest in Rai Valley and lowest in Tekapo with other sites lying in between. Peaks occurred in February 2005 in all sites.

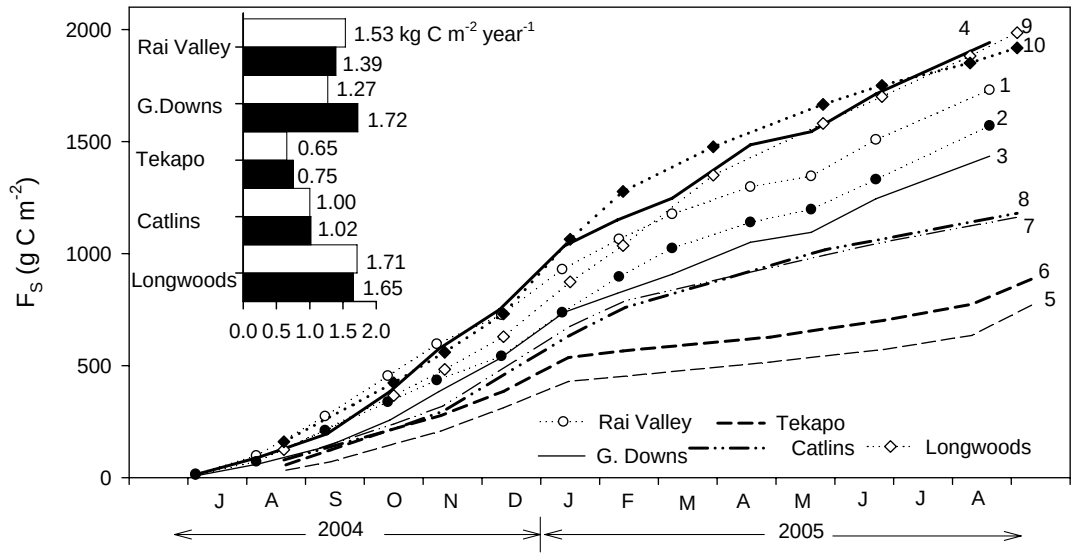


Figure D.3.6. Accumulated values of soil respiration (F_S) for control and fertilized plots of *Pinus radiata* in five sites in the South Island of New Zealand for the year ending August 2005. Graph insert corresponds to accumulated values of soil respiration for control (open bars) and fertilized (closed bars) plots for the year ending August 2005. Plot numbers are indicated at the right of each curve: 1-2, Rai Valley; 3-4, Golden Downs; 5-6, Tekapo; 7-8, Catlins; 9-10, Longwoods. Note that values of F_S for the control (9) and the fertilized (10) plot in Longwoods were the highest of all sites, explaining greater carbon allocation to roots.

Accumulated values of soil respiration for the year ending August 2005 were consistently highest for Longwoods and smallest for Tekapo (Figure D.3.6). It is quite striking that Longwoods being the less productive most unfertile site showed the highest soil respiration of all sites. It seems quite remarkable also that Rai Valley, the most productive and one of the most fertile sites together with Tekapo, showed smaller accumulated soil respiration than Longwoods.

D.4. CALCULATION OF GROSS-PRIMARY PRODUCTIVITY FRACTIONS

Total-below ground carbon allocation

Total C allocated belowground for root and mycorrhizae construction and respiration, and C released through root exudates and root turnover (TBCA), was calculated as,

$$TBCA = F_S + F_E - F_A + \Delta C_S + \Delta C_R + \Delta C_L$$

where F_S is the soil respiration C efflux, F_E is the C flux off the system by leaching or erosion, ΔC_S is change in C content in the mineral soil, ΔC_R is the change in C content of root biomass, and ΔC_L is the change in C content in the litter layer. Soil carbon was assumed not to change for the year ending August 2005.

Table D.4.1. Components of total below-ground carbon allocation (TBCA) in control and fertilized plots of *Pinus radiata* in five sites in the South Island of New Zealand.

Site	Plot	F_S (kg C m^{-2})	$-F_A$ (kg C m^{-2})	ΔC_R (kg C m^{-2})	ΔC_L (kg C m^{-2})	TBCA (kg C m^{-2})
Rai Valley	Control	1.54	-0.18	0.29	0.15	1.80
	Fertilized	1.39	-0.21	0.30	0.15	1.63
Golden Downs	Control	1.27	-0.11	0.26	0.07	1.49
	Fertilized	1.72	-0.15	0.31	0.40	2.28
Tekapo	Control	0.65	-0.11	0.25	0.28	1.07
	Fertilized	0.75	-0.14	0.20	0.31	1.12
Catlins	Control	1.00	-0.11	0.35	0.30	1.55
	Fertilized	1.02	-0.19	0.29	0.52	1.65
Longwoods	Control	1.71	-0.06	0.19	-0.09	1.76
	Fertilized	1.65	-0.18	0.43	0.22	2.12
Overall	Mean	1.27	-0.14	0.29	0.23	1.65

Soil respiration (F_S) represented between 61 to 97 % of TBCA (mean 77%), and this proportion was generally smaller in Tekapo and Catlins than other sites. In Longwoods, the less productive less fertile site, soil respiration represented 97% of TBCA in the control plot compared to 78% in the fertilized plot. This is similar to what was found in Chapter *Five* in that soil respiration represented a larger fraction of TBCA as fertility dropped.

The relationship between F_S and TBCA was highly significant ($r^2 = 0.79$, $P < 0.001$). Slopes ($F_{1,6} = 1.85$, $P = 0.22$) and intercepts ($F_{1,6} = 1.14$, $P < 0.001$) of this linear relationship were not influenced by fertilization treatment (Figure D.4.1).

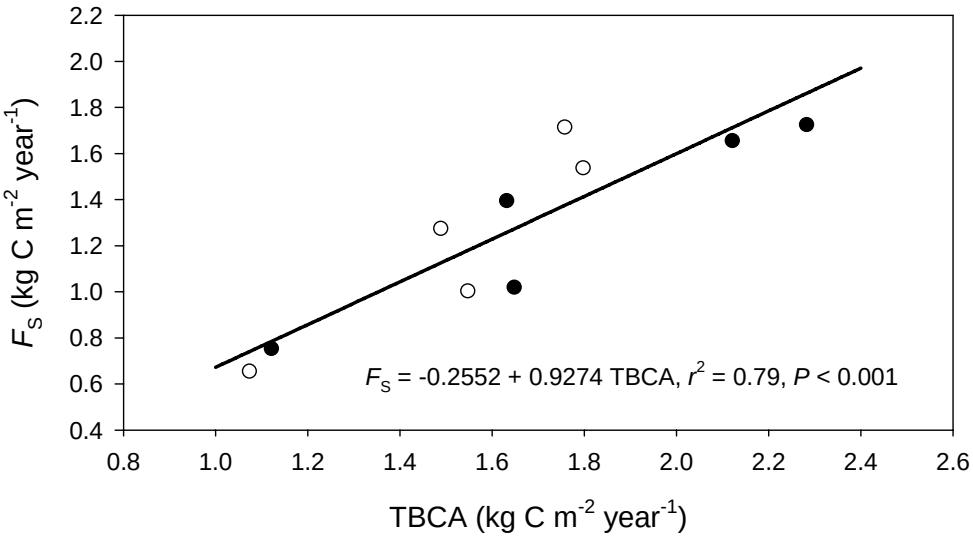


Figure D.4.1. The relationship between soil respiration integral (F_S) and total below-ground carbon allocation (TBCA) in control and fertilized plots of *Pinus radiata* in five sites in the South Island of New Zealand.

Above-ground net primary production

Above-ground net primary production was calculated for the year ending August 2005 for all ten plots as,

$$ANPP = F_A + F_W + \Delta C_C + \Delta C_w$$

where F_A is the carbon content of above-ground litterfall, F_W is the C content associated to tree mortality, ΔC_C is the change in C content of live foliage, and ΔCw is C content change in live branches, bark and wood, over a given period of time.

Table D.4.2. Components of above ground net primary production (ANPP) in control and fertilized plots of *Pinus radiata* in five sites in the South Island of New Zealand.

Site	Plot	F_A (kg C m^{-2})	ΔCc (kg C m^{-2})	ΔCw (kg C m^{-2})	ANPP (kg C m^{-2})
Rai Valley	Control	0.18	0.26	0.89	1.32
	Fertilized	0.21	0.23	0.82	1.26
Golden Downs	Control	0.11	0.24	0.81	1.15
	Fertilized	0.15	0.24	0.88	1.27
Tekapo	Control	0.11	0.27	0.50	0.88
	Fertilized	0.14	0.21	0.40	0.75
Catlins	Control	0.11	0.39	0.93	1.43
	Fertilized	0.19	0.28	0.81	1.28
Longwoods	Control	0.06	0.27	0.39	0.72
	Fertilized	0.18	0.34	0.77	1.28
Overall	Mean	0.14	0.27	0.72	1.13
		13%	24%	63%	

The main component of ANPP was by far wood (63%) followed by foliage (24%) and litterfall (13%) (Table D.4.2). ANPP was best correlated to wood production (r^2 = 0.96, P < 0.001) but poorly correlated to foliage or litterfall production. Slopes ($F_{1,6}$ =0, P = 0.98) and intercepts ($F_{1,6}$ = 0.02, P = 0.89) of the linear relationship between ΔC_W and ANPP were not influenced by fertilization treatment (Figure D.4.3).

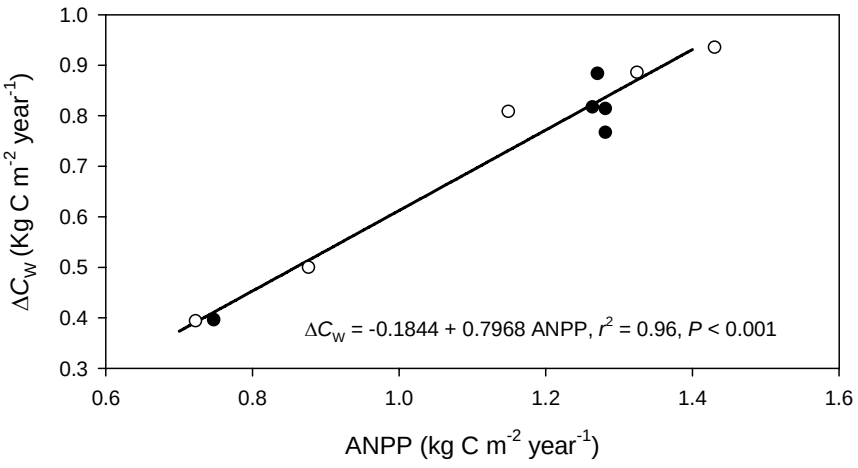


Figure D.4.3. The relationship between woody- and above-ground net primary productivity in control and fertilized plots of *Pinus radiata* in five sites in the South Island of New Zealand.

Above-ground plant respiration

Above-ground plant respiration, APR, was estimated as a sum of construction wood respiration (W_R), foliage construction (L_{RC}) and foliage maintenance respiration (L_{RM}),

$$APR = L_{RC} + L_{RM} + W_R$$

Foliage maintenance respiration was by far the main component of APR (82%), while foliage construction (6%) and wood construction (11%) represented a small proportion of overall APR. APR was highest in Rai Valley consistently with the highest above-ground production over the four years of growth (Table D.4.3).

Because L_{RM} depends on the LAI and dark respiration and APR is strongly determined by L_{RM}, we correlated both these variables against the LAI at the end of the fourth year of growth. Both L_{RM} and APR scaled positively and significantly with the LAI ($r^2 > 0.84$, $P < 0.001$). Slopes ($F_{1,6} < 1.17$, $P > 0.32$) and intercepts ($F_{1,6} < 1.21$, $P > 0.31$) of these linear relationships were not influenced by fertilization treatment (Figure D.4.4)

Table D.4.3. Components of above ground plant respiration (APR) in control and fertilized plots of *Pinus radiata* in five sites in the South Island of New Zealand.

Site	Plot	L_{RC} (kg C m^{-2})	L_{RM} (kg C m^{-2})	W_R (kg C m^{-2})	APR (kg C m^{-2})
Rai Valley	Control	0.11	2.20	0.22	2.53
	Fertilized	0.11	1.93	0.21	2.25
Golden Downs	Control	0.08	1.06	0.20	1.35
	Fertilized	0.10	1.65	0.22	1.96
Tekapo	Control	0.09	1.14	0.13	1.35
	Fertilized	0.09	1.35	0.10	1.53
Catlins	Control	0.12	1.13	0.23	1.48
	Fertilized	0.12	1.42	0.20	1.74
Longwoods	Control	0.08	0.54	0.10	0.73
	Fertilized	0.13	0.68	0.19	1.00
Overall	Mean	0.10	1.31	0.18	1.59
		6%	82%	11%	100%

Foliage respiration rates in the dark (R_d) were measured in control and fertilized plots of *Pinus radiata* in four sites in the South Island of New Zealand. Values of R_d were not measured in Golden Downs, and were estimated based on equations of night respiration rates (R_d^*) against foliage nitrogen concentration developed in the greenhouse ($R_d^* = 0.0616\ N_p +$

0.1639, r^2 = 0.07; see also Chapter 5, Figure 5.5) and foliage nitrogen concentrations measured in the field (N_p, %). Estimates of R_d were not widely different from actual measurements, and therefore estimates were preferred in all cases to use only one approach for the whole analysis (Table D.4.4).

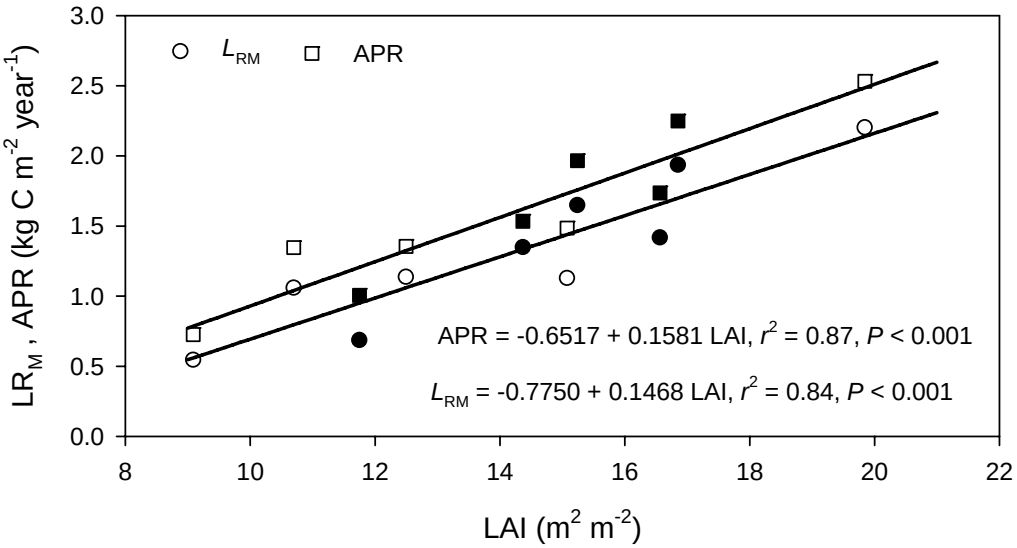


Figure D.4.4. The relationship between foliage maintenance respiration (L_{RM}) and total above ground plant respiration (APR) against the leaf area index in control (open symbols) and fertilized (closed symbols) plots of *Pinus radiata* in five sites in the South Island of New Zealand.

Table D.4.4. Foliage respiration rates in the dark (R_d) in control and fertilized plots of *Pinus radiata* in four sites in the South Island of New Zealand. Values of R_d were not measured in Golden Downs. Nitrogen concentration of the foliage (N_p) and estimated values of R_d (R_d^*) using an equation developed in the greenhouse under high controlled conditions did not show to be widely different from measured values (R_d^* = 0.0616 N_p + 0.1639, r^2 = 0.07; see also Chapter 5, Figure 5.5).

Site	Plot	n	R_d (μmol m^{-2} s^{-1})	N_p %	R_d^* (μmol m^{-2} s^{-1})
Rai Valley	Control	7	0.19 ± 0.02	1.32	0.25
	Fertilized	7	0.28 ± 0.03	1.44	0.25
Golden Downs	Control			1.19	0.24
	Fertilized			1.28	0.24
Tekapo	Control	8	0.28 ± 0.03	1.41	0.25
	Fertilized	8	0.27 ± 0.03	1.29	0.24
Catlins	Control	5	0.19 ± 0.02	1.37	0.25
	Fertilized	5	0.14 ± 0.01	1.48	0.25
Longwoods	Control	6	0.21 ± 0.02	1.47	0.25
	Fertilized	6	0.14 ± 0.02	1.25	0.24
Overall	Mean	52	0.22 ± 0.011		0.25

Because LAI was the best explanatory variable for GPP, their relationship is presented in Figure D.4.5. Values of GPP significantly increased with LAI (r^2 = 0.60, P = 0.009), with slopes ($F_{1,6}$ = 0.10, P = 0.77) and intercepts ($F_{1,6}$ = 0.14, P =0.72) of this linear relationship not influenced by fertilization treatment.

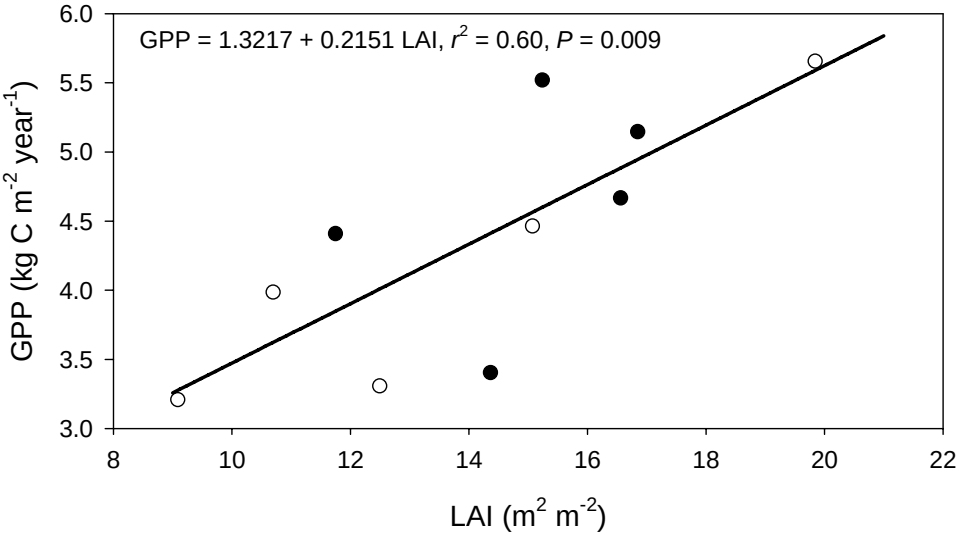


Figure D.4.5. The relationship between GPP and LAI for control (open symbols) and fertilized (closed symbols) plots of *Pinus radiata* in five sites in the South island of New Zealand. Leaf area is expressed on an hemi-surface area.

Gross (GPP) and net C fluxes (NPP, NEP) across sites and fertilization treatments

Table D.4.5. Relevant carbon assimilation variables for control and fertilized plots of *Pinus radiata* in five sites in the South Island of New Zealand. Gross primary production (GPP) integrates net canopy photosynthesis over a time interval. Net ecosystem exchange is the difference between GPP and both auto- and heterotrophic respiration. Net primary productivity (NPP) is GPP minus autotrophic respiration. Main effects of sites (S), fertilization (F) were assessed by analysis of variance. Significant differences are presented as *F* values, *P* values and *P* range: ns, non significant; *, significant at $P < 0.05$; **, significant at $P < 0.01$; ***, significant at $P < 0.001$. Different letters indicate significant differences at $P < 0.05$. Values are presented as means (± 1SE) for sites and fertilization treatments.

Site	Plot	GPP	NEP	NPP	NPP/GPP
		kg C m^{-2} $year^{-1}$	kg C m^{-2} $year^{-1}$	kg C m^{-2} $year^{-1}$	
Rai Valley	Control	5.65	1.59	2.78	0.49
	Fertilized	5.14	1.50	2.52	0.49
	Mean	5.4 ± 0.26	1.55 ± 0.05	2.65 ± 0.13	0.49 ± 0
Golden Downs	Control	3.98	1.36	2.24	0.56
	Fertilized	5.52	1.83	3.17	0.58
	Mean	4.75± 0.77	1.6± 0.24	2.71± 0.47	0.57± 0.01
Tekapo	Control	3.30	1.30	1.65	0.50
	Fertilized	3.40	1.12	1.70	0.50
	Mean	3.35± 0.05	1.21± 0.09	1.68± 0.03	0.50± 0
Catlins	Control	4.46	1.98	2.43	0.54
	Fertilized	4.67	1.91	2.51	0.54
	Mean	4.57± 0.11	1.95± 0.04	2.47± 0.04	0.54± 0
Longwoods	Control	3.21	0.77	1.73	0.54
	Fertilized	4.41	1.75	3.01	0.68
	Mean	3.81± 0.6	1.26± 0.49	2.37± 0.64	0.61± 0.07
Fertilization	Control	4.12± 0.45	1.4± 0.2	2.17± 0.21	0.53± 0.01
	Fertilized	4.63± 0.36	1.62± 0.14	2.58± 0.26	0.56± 0.03
ANOVA	S	3.65	1.43	1.59	2.66
		0.12	0.37	0.33	0.18
		ns	ns	ns	ns
	F	1.82	1.01	2.02	1.38
		0.24	0.37	0.23	0.31
		ns	ns	ns	ns

Table D.4.6. Major fractions of gross primary productivity (GPP) in control and fertilized plots of *Pinus radiata* in five sites in the South Island of New Zealand. Major GPP fractions are: above-ground net primary productivity (ANPP), above-ground plant respiration (APR) and total belowground carbon allocation (TBCA). Main effects of sites (S) and fertilization treatments (F) were assessed by analysis of variance. Significant differences are presented as *F* values, *P* values and *P* range: ns, non significant; *, significant at $P < 0.05$; **, significant at $P < 0.01$; ***, significant at $P < 0.001$. Different letters indicate significant differences at $P < 0.05$. Values are presented as means (± 1SE) for sites and fertilization treatments.

Site	Plot	ANPP	APR	TBCA	ANPP/GPP	APR/GPP	TBCA/GPP
		kg C m^{-2} $year^{-1}$	kg C m^{-2} $year^{-1}$	Kg C m^{-2} $year^{-1}$			
Rai Valley	Control	1.32	2.53	1.80	0.23	0.45	0.32
	Fertilized	1.26	2.25	1.63	0.25	0.44	0.32
	Mean	1.29 ± 0.03	2.39 ± 0.14 **a**	1.72 ± 0.09	0.24 ± 0.01	0.45 ± 0.01 **a**	0.32 ± 0 **b**
Golden Downs	Control	1.15	1.35	1.49	0.29	0.34	0.37
	Fertilized	1.27	1.96	2.28	0.23	0.36	0.41
	Mean	1.21 ± 0.06	1.66 ± 0.31 **ab**	1.89 ± 0.4	0.26 ± 0.03	0.35 ± 0.01 **b**	0.39 ± 0.02 **ab**
Tekapo	Control	0.88	1.35	1.07	0.27	0.41	0.32
	Fertilized	0.75	1.53	1.12	0.22	0.45	0.33
	Mean	0.82 ± 0.07	1.44 ± 0.09 **ab**	1.1 ± 0.03	0.25 ± 0.03	0.43 ± 0.02 **a**	0.33 ± 0.01 **b**
Catlins	Control	1.43	1.48	1.55	0.32	0.33	0.35
	Fertilized	1.28	1.74	1.65	0.27	0.37	0.35
	Mean	1.36 ± 0.08	1.61 ± 0.13 **ab**	1.6 ± 0.05	0.3 ± 0.03	0.35 ± 0.02 **b**	0.35 ± 0 **b**
Longwoods	Control	0.72	0.73	1.76	0.23	0.23	0.55
	Fertilized	1.28	1.00	2.12	0.29	0.23	0.48
	Mean	1 ± 0.28	0.87 ± 0.14 **b**	1.94 ± 0.18	0.26 ± 0.03	0.23 ± 0 **c**	0.52 ± 0.04 **a**
Fertilization	Control	1.1 ± 0.13	1.49 ± 0.29	1.53 ± 0.13	0.27 ± 0.02	0.35 ± 0.04	0.38 ± 0.04
	Fertilized	1.17 ± 0.1	1.7 ± 0.21	1.76 ± 0.2	0.25 ± 0.01	0.37 ± 0.04	0.38 ± 0.03
ANOVA	S	2.28 0.22 ns	11.72 0.018 *	3.36 0.13 ns	0.65 0.65 ns	56.19 <0.001 ***	15.86 0.010 *
	F	0.27 0.63 ns	2.13 0.22 ns	1.89 0.24 ns	0.45 0.54 ns	3.12 0.15 ns	0.05 0.84 ns

D.5. STATE VARIABLES, DRY MATTER PARTITIONING AND NUTRIENT CONTENT

Treatment influences on plot characteristics

Basal area, plant and component dry masses and the leaf area index were influenced by site and fertilization treatment (Table D.5.1). In control plots, these plot characteristics were largest at Rai Valley and lowest at Longwoods, and these sites coincided with the smallest and largest responses to fertilization of all sites, respectively. Relative growth responses to fertilization were small at Tekapo and Rai Valley, while at Golden Downs and Catlins, growth responses were intermediate between Longwoods and Rai Valley.

Table D.5.1. Basal area, biomass and leaf area index on a hemi-surface leaf area basis (LAI) in control and fertilized mini-plots of four-years old *Pinus radiata* in five sites in the South Island of New Zealand. Main effects of sites (S) and fertilization (F) were assessed by analysis of variance. Significant differences are presented as *F* values, *P* values and *P* range: ns, non significant; *, significant at $P < 0.05$; **, significant at $P < 0.01$; ***, significant at $P < 0.001$. Different letters indicate significant differences at $P < 0.05$. Values are presented as means (± 1SE) for sites and fertilization treatments.

Site	Plot	Basal area ($cm^2\ m^{-2}$)	Roots ($kg\ m^{-2}$)	Stems ($kg\ m^{-2}$)	Branches ($kg\ m^{-2}$)	Foliage ($kg\ m^{-2}$)	Total ($kg\ m^{-2}$)	LAI ($m^2\ m^{-2}$)
Rai Valley	Control	89.9	1.5	5.9	1.1	1.9	10.5	19.8
	Fertilized	86.4	1.8	5.3	1	1.7	9.9	16.9
	Mean	88.2 ± 1.8	1.7 ± 0.2	5.6 ± 0.3 **a**	1.1 ± 0.1	1.8 ± 0.1	10.2 ± 0.3 **a**	18.4 ± 1.5
Golden Downs	Control	58.1	1.1	3.2	0.8	1.1	6.2	10.7
	Fertilized	92.6	1.5	5.3	1.2	1.6	9.6	15.2
	Mean	75.4 ± 17.3	1.3 ± 0.2	4.3 ± 1.1 **ab**	1 ± 0.2	1.4 ± 0.3	7.9 ± 1.7 **ab**	13 ± 2.3
Tekapo	Control	59.5	1.1	2.3	0.7	1.4	5.5	12.5
	Fertilized	64.3	1.3	2.7	0.7	1.6	6.4	14.4
	Mean	61.9 ± 2.4	1.2 ± 0.1	2.5 ± 0.2 **ab**	0.7 ± 0	1.5 ± 0.1	6 ± 0.5 **ab**	13.4 ± 0.9
Catlins	Control	82.4	1.4	3.6	0.8	1.7	7.5	15.1
	Fertilized	92.8	1.5	4.7	1.1	1.9	9.2	16.6
	Mean	87.6 ± 5.2	1.5 ± 0.1	4.2 ± 0.6 **ab**	1 ± 0.2	1.8 ± 0.1	8.4 ± 0.9 **ab**	15.8 ± 0.7
Longwoods	Control	40.5	0.6	1.1	0.5	1	3.2	9.1
	Fertilized	64.4	1.3	2.2	0.8	1.3	5.6	11.7
	Mean	52.5 ± 12	1 ± 0.4	1.7 ± 0.6 **b**	0.7 ± 0.2	1.2 ± 0.2	4.4 ± 1.2 **b**	10.4 ± 1.3
Fertilization	Control	66.1 ± 8.9	1.1 ± 0.2 **a**	3.2 ± 0.8	0.8 ± 0.1	1.4 ± 0.2	6.6 ± 1.2	13.4 ± 1.9
	Fertilized	80.1 ± 6.5	1.5 ± 0.1 **b**	4 ± 0.7	1 ± 0.1	1.6 ± 0.1	8.1 ± 0.9	15 ± 0.9
ANOVA	S	4.31, 0.09 ns	5.23,0.07 ns	9.76,0.02 *	2.83,0.17 ns	4.97,0.07 ns	8.72,0.03 *	4.68,0.08 ns
	F	4.26,0.11 ns	10.91,0.03 *	3.37,0.14 ns	3.45,0.14 ns	3.08,0.15 ns	5.28,0.08 ns	1.47,0.29 ns

All plot variables tended to increase with fertilization and were greater at Rai Valley than at Longwoods. Total tree and stem mass differed significantly between sites ($F_{4,4} > 8.72$, $P < 0.03$), whereas root mass significantly increased with fertilization ($F_{1,4} = 10.9$, $P = 0.03$). All other plot characteristics conformed to plant mass but were not significantly influenced by site or fertilization. A fertility rating, calculated as the ratio of any variable in control to fertilized plots, was highest for Rai Valley (1.04-1.17), progressively decreasing at Tekapo (0.86-0.93), Catlins (0.77-0.89), Golden Downs (0.63-0.70), and being generally lowest at Longwoods (0.57-0.77).

Tree mass and leaf area were strongly correlated with basal area ($r^2 > 0.79$) (Figure D.5.1). Slopes ($F_{1,6} < 1.90$, $P > 0.22$) and intercepts ($F_{1,6} < 1.37$, $P > 0.28$) of these linear relationships were not influenced by fertilization treatment. Because basal area well explains plant mass and leaf area development, the seasonal trend in basal area for the year ending August 2005 is presented in Figure D.5.2. This is relevant because this is the time interval for which the carbon balance was determined.

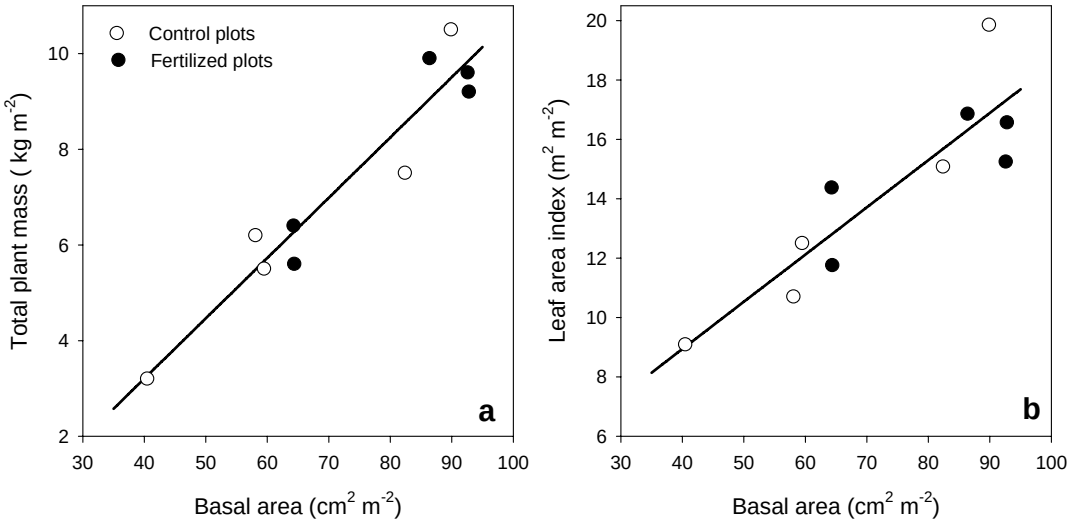


Figure D.5.1. The relationship between tree mass (W_T), leaf area index (LAI) and basal (B_A) area in control and fertilized mini-plots of four-years old *Pinus radiata* cultivated in five sites in the South Island of New Zealand. Slopes and intercepts of the W_T / B_A and LAI / B_A linear relationships were not influenced by fertilization treatment. $W_T = -1.835 + 0.126\ B_A$, $r^2 = 0.91$, $P < 0.001$; $LAI = 2.580 + 0.159\ B_A$, $r^2 = 0.79$, $P < 0.001$.

Basal area increment during the fourth year of growth steadily increased during spring-summer, decreasing progressively during autumn-winter (Figure D.5.2). Trajectories of basal area maintained their ranking during the fourth compared to the previous three years of growth, indicating that trends observed in basal area and presumably carbon assimilation and allocation during this year conformed to the patterns of growth observed during the three previous years. However, basal area growth rates during the fourth year were generally greater than the mean annual basal area increment over the entire mini-rotation. Figure D.5.2

(b) shows that provided that fertilization was applied Rai Valley, Golden Dows and Catlins realized similar potential, while Tekapo and particularly Longwoods lagged behind.

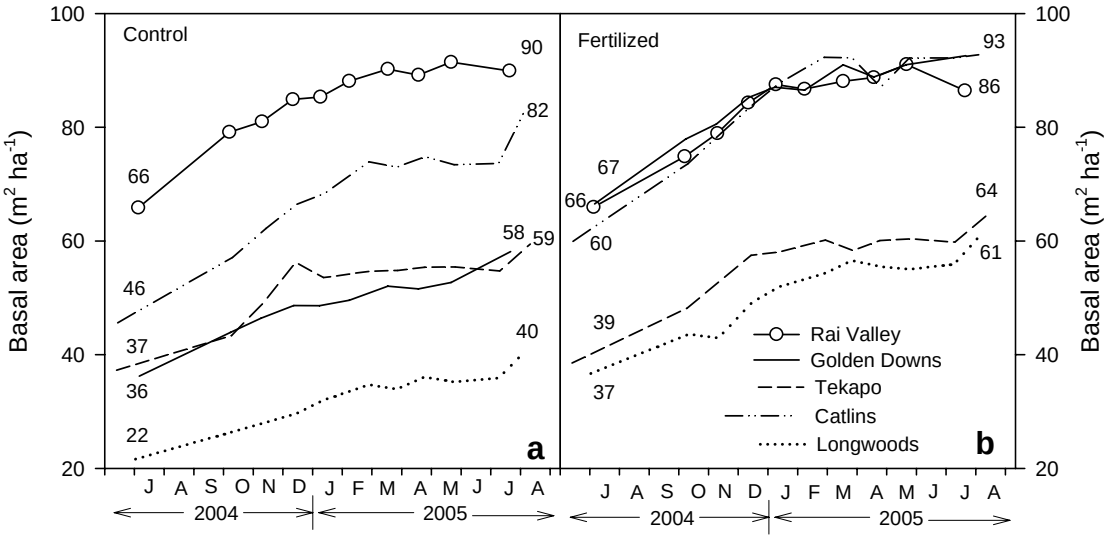


Figure D.5.2. Basal area increment for the fourth year of growth in **a** control and **b** fertilized mini-plots of *Pinus radiata* cultivated in five sites in the South Island of New Zealand. Initial and final basal area for the year ending August 2005 are presented at the extremes of each line.

Treatment influences on plant nutrient content

Carbon concentration was remarkably stable within plant tissues being on average (± 1 SE, n = 69) 51.1 ± 0.2 % (range 45-54%) independently of plot ($F_{9,53}$ =1.06, P = 0.40). However, carbon concentration slightly but significantly decreased ($F_{6,53}$ = 41.4, P < 0.001) in foliage compared to wood components being the lowest in fine roots (data not shown). Because carbon concentration was so stable, the relationship between carbon content and biomass was almost exact (Figure D.5.3).

Nitrogen and phosphorus plot content, calculated as the sum of tissue plant mass multiplied by tissue nutrient concentration, significantly increased with plant mass (r^2 > 0.45, P < 0.033). Slopes ($F_{1,9}$ < 0.03, P > 0.87) and intercepts ($F_{1,9}$ < 0.03, P > 0.86) of these linear relationships were not influenced by fertilization treatment (Figure D.5.4). Fitted lines were used as a reference of comparison between sites and fertilization treatments, so that plot values above the fitted line were less nutrient limited than those below the fitted line (Figure D.5.4).

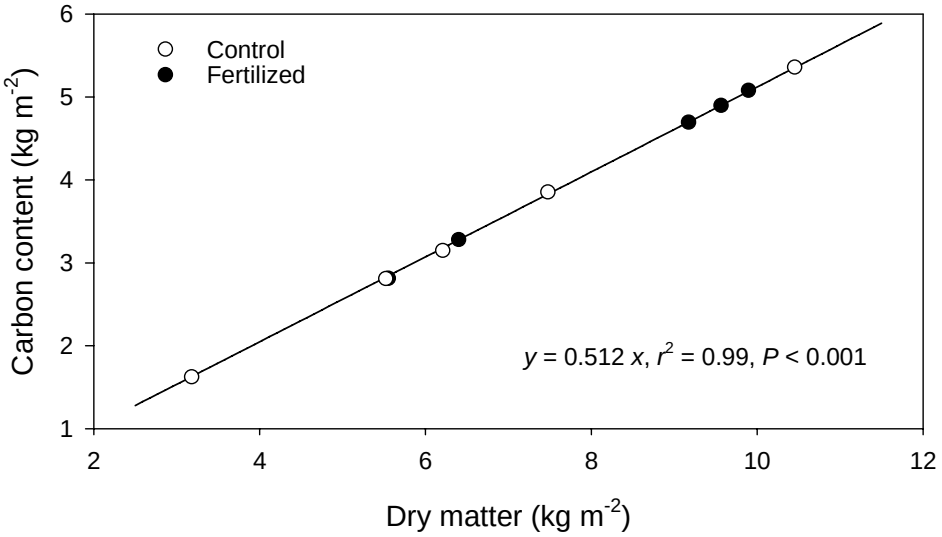


Figure D.5.3. The relationship between carbon content and total dry matter accumulated over four years in control and fertilized plots of *Pinus radiata* in five sites in the South Island of New Zealand.

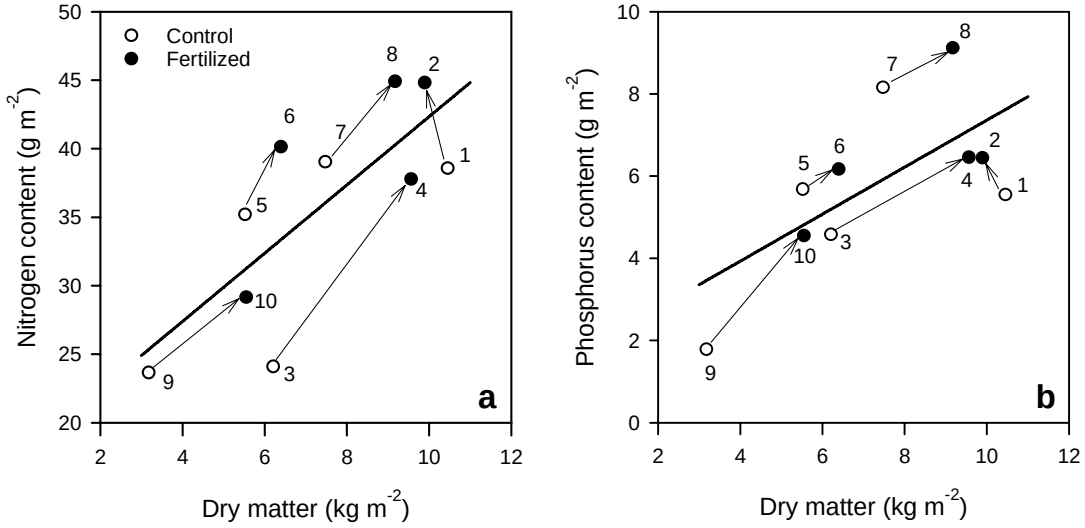


Figure D.5.4. The relationships between **a** nitrogen and **b** phosphorus content against total plant mass (W_T) of *Pinus radiata* cultivated for four years in control and fertilized plots in five sites in the South Island of New Zealand. Slopes and intercepts of these linear relationships were not influenced by fertilization treatment. For nitrogen content (N_K): $N_K = 17.428 + 2.491\ W_T$, $r^2 = 0.59$, $P = 0.009$. For phosphorus content (P_K): $P_K = 1.641 + 0.572\ W_T$, $r^2 = 0.45$, $P = 0.033$. Numbers besides dots indicate plot numbers: 1-2 Rai Valley, 3-4 Golden Downs, 5-6 Tekapo, 7-8 Catlins and 9-10 Longwoods. Arrows indicate likely effects of fertilization on nutrient content within the same site.

Nitrogen (K_N) and phosphorus (K_P) contents were highest at Catlins and lowest at Longwoods. Values of K_N and K_P were below the fitted lines at Longwoods and Golden Downs and their arrow lengths, an indication of the effect of fertilization, were the longest (Figure D.5.4), indicating that fertilization responses in uptake were the largest at these sites. In contrast, values of K_N and K_P were above the regression line at Tekapo and Catlins, and

arrow lengths shortest at Tekapo, suggesting this site to be the less nutrient limited. At Rai Valley dry mass was greater in the control than the fertilized plot, but the fertilized plot exhibited greater N and P content, suggesting that uptake and dry mass production were not necessarily matched in these plots.

In Chapter *Two* it was suggested that a ratio of 10:1 on a mass basis (23 mole basis) might be useful for separating nitrogen ($N_m : P_m \leq 10$ g g^{-1}) from phosphorus ($N_m : P_m > 10$ g g^{-1}) deficiencies in photosynthesis studies, and this has been previously proposed by several authors for growth studies (Reich and Schoettle 1988, Marschner 1995, Aerts and Chapin 2000). The relationship between N and P content for all ten plots is presented in Figure D.5.5, with a dotted line representing a slope of 10:1. It can be seen that nine out of ten plots are below the 10:1 slope line, suggesting that these plots were N rather than P limited. The control plot at Longwoods (plot 9) is slightly above the 10:1 line which might suggest that this plot was at the transition between N to P limitations (Figure D.5.5).

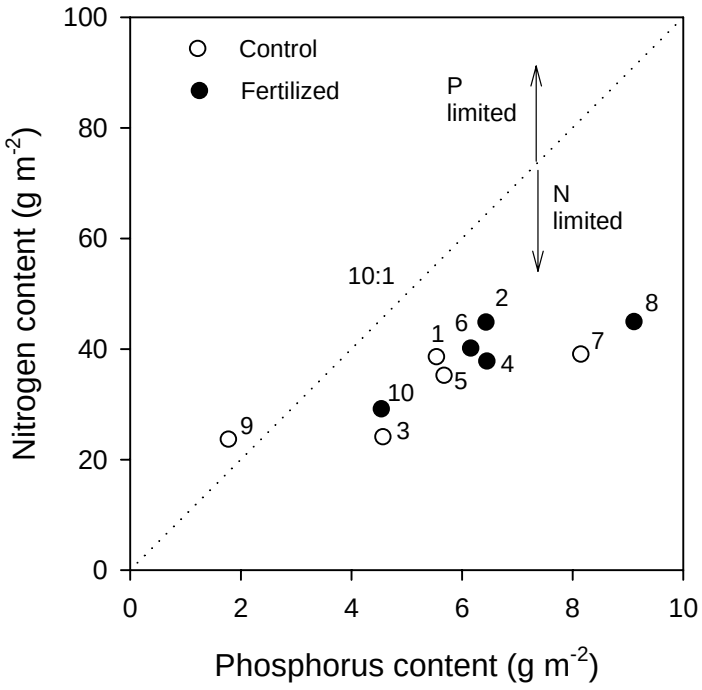


Figure D.5.5. The relationship between nitrogen and phosphorus content in control and fertilized plots of four-year old *Pinus radiata* in five sites in the South Island of New Zealand. Dotted line has a slope 10:1 argued to separate nitrogen (N : P < 10) from phosphorus (N : P > 10 g g^{-1}) deficiencies. Plot numbers are indicated besides symbols: 1-2 Rai Valley, 3-4 Golden Downs, 5-6 Tekapo, 7-8 Catlins, 9-10 Longwoods.

Treatment influences on tree characteristics

Ground-line diameter, tree height, height to diameter ratio and tree mass for all sites and fertilization treatments are presented in Table D.5.2. All these tree characteristics were strongly influenced by site ($F_{4,75} > 5.06$, $P < 0.001$), and all these variables except for the height to diameter ratio were also significantly influenced by fertilization ($F_{1,75} > 9.92$, $P < 0.002$). The site by fertilization interaction was insignificant for all variables ($F_{4.75} < 2.31$, $P > 0.07$) except for tree height where the interaction was marginally significant ($F_{4,75} = 2.78$, $P = 0.03$). Significance in this analysis of variance might be overestimated because trees were not true experimental replicates. Nevertheless the analysis was performed to provide guidance on the relative importance of sites compared to fertilization.

The main effects of site were greater than the main effects of fertilization. Plant mass was on average 2.2 times greater in Rai Valley than Longwoods, whereas 1.3 times greater in fertilized than control plots. In control plots, trees were largest in Rai Valley and smallest in Longwoods, and these sites coincided with the smallest and largest response to fertilization, respectively. Growth responses to fertilization were also small in Tekapo. In Golden Downs and Catlins, fertilization responses were intermediate between Longwoods and Rai Valley (Tekapo). The height to diameter ratio, a measurement of slenderness, was not significantly influenced by fertilization treatment, but was greater in Rai Valley and Golden Downs, decreasing progressively in Catlins, Tekapo and Longwoods (Table D.5.2).

Table D.5.2. Tree characteristics of *Pinus radiata* cultivated for four years in five sites in the South Island of New Zealand. Values are presented as means (± 1 SE) for each plot, n = 7-9. Main effects of sites (S), fertilization (F) and their interaction were assessed by analysis of variance. Significant differences are presented as F values, P values and P range: ns, non significant; *, significant at $P < 0.05$; **, significant at $P < 0.01$; ***, significant at $P < 0.001$. Different letters indicate significant differences at $P < 0.05$. Significance might be overestimated because trees are not true replicates.

Site	Plot	Trees per plot	ground-line diameter (mm)	Tree Height (mm)	Height: Diam Ratio	Plant mass (kg)
Rai Valley	Control	9	53.1 ± 2.2 a	434 ± 17 a	8.2 ± 0.2 a	2.61 ± 0.24 a
	Fertilized	8	53.8 ± 3.2 a	437 ± 17 a	8.2 ± 0.2 a	2.69 ± 0.36 a
	Mean		**53.4 ± 1.9 a**	**436 ± 12 a**	**8.2 ± 0.2 a**	**2.65 ± 0.21 a**
Golden Downs	Control	8	44.9 ± 3.2 a	395 ± 20 a	8.9 ± 0.3 a	1.75 ± 0.29 a
	Fertilized	9	53.9 ± 2.4 b	422 ± 17 a	7.9 ± 0.4 a	2.39 ± 0.23 a
	Mean		**49.6 ± 2.2 ab**	**409 ± 13 a**	**8.4 ± 0.3 a**	**2.09 ± 0.2 ab**
Tekapo	Control	9	41.8 ± 4.2 a	248 ± 15 a	6.1 ± 0.3 a	1.38 ± 0.29 a
	Fertilized	8	47.4 ± 2.5 a	272 ± 8 a	5.8 ± 0.2 a	1.76 ± 0.18 a
	Mean		**44.5 ± 2.5 bc**	**259 ± 9 c**	**6 ± 0.2 bc**	**1.56 ± 0.18 bc**
Catlins	Control	9	50.4 ± 3.3 a	322 ± 16 a	6.5 ± 0.3 a	1.87 ± 0.25 a
	Fertilized	9	53.9 ± 2.4 a	371 ± 7 b	7.0 ± 0.2 a	2.29 ± 0.19 a
	Mean		**52.2 ± 2 ab**	**346 ± 10 b**	**6.7 ± 0.2 b**	**2.08 ± 0.16 ab**
Longwoods	Control	9	34.7 ± 3.2 a	176 ± 12 a	5.2 ± 0.2 a	0.8 ± 0.17 a
	Fertilized	7	49.4 ± 2.9 b	271 ± 8 b	5.6 ± 0.4 a	1.72 ± 0.16 b
	Mean		**41.1 ± 2.8 c**	**217 ± 14 d**	**5.4 ± 0.2 c**	**1.2 ± 0.16 c**
Overall	Control	44	45 ± 1.7 a	313 ± 16 a	6.9 ± 0.2 a	1.68 ± 0.14 a
	Fertilized	41	51.8 ± 1.2 b	359 ± 12 b	7.0 ± 0.2 a	2.19 ± 0.12 b
ANOVA	S		5.06,<0.001 ***	77.16,<0.001 ***	44.71,<0.001 ***	9.19,<0.001 ***
	F		12.17,<0.001 ***	18.66,<0.001 ***	0.16,0.69 Ns	9.92,0.002 **
	S × F		1.54, 0.20 ns	2.78, 0.03 *	2.31,0.07 Ns	0.80,0.53 ns

The fractions of total plant mass partitioned to foliage, wood and roots are presented in Table D.5.3. On average, plant mass was partitioned mainly to branches and stems (59%) and the difference similarly apportioned between roots (19%) and foliage (22%). Differences in component mass fractions were relatively small but significantly different among sites. The wood fraction was generally higher in Rai Valley and Golden Downs at the expense of the root and foliage mass fractions. Fertilization treatment did not influence the proportion of plant mass partitioned to roots, wood and foliage, except in Longwoods where the control plot exhibited a larger foliage fraction (0.31) than the fertilized plot (0.23).

Table D.5.3. Comparison of root, wood and foliage mass fractions and total tree mass in control and fertilized plots of *Pinus radiata* cultivated for four years in five sites in the South Island of New Zealand. Values are presented as means (± 1 SE) for each plot. Significance is presented as *F* values and *P* range: *, $P < 0.05$, ***, $P < 0.001$. Multiple comparisons were carried out using the Tukey-Kramer test. Different letters indicate significant differences between plots at $P < 0.05$. (§): Tukey's grouping did not show differences among sites.

Site	Plot	Root:Total	Wood:Total	Foliage:Total	Total (g)
Rai Valley	Control	0.15 ± 0.01	0.66 ± 0.02	0.19 ± 0.01	2.61 ± 0.24 a
	Fertilized	0.18 ± 0.01	0.64 ± 0.02	0.18 ± 0.01	2.69 ± 0.36 a
		0.16 ± 0.01	0.65 ± 0.01 **a**	0.18 ± 0.01 **c**	2.65 ± 0.21 **a**
Golden Downs	Control	0.18 ± 0.01	0.64 ± 0.01	0.17 ± 0.01	1.75 ± 0.29 a
	Fertilized	0.16 ± 0.01	0.67 ± 0.02	0.16 ± 0.01	2.39 ± 0.23 a
		0.17 ± 0.01	0.66 ± 0.01 **a**	0.17 ± 0.01 **c**	2.09 ± 0.2 **ab**
Tekapo	Control	0.23 ± 0.03	0.52 ± 0.03	0.25 ± 0.01	1.38 ± 0.29 a
	Fertilized	0.20 ± 0.03	0.55 ± 0.02	0.26 ± 0.01	1.76 ± 0.18 a
		0.21 ± 0.02	0.53 ± 0.02 **b**	0.26 ± 0.01 **a**	1.56 ± 0.18 **bc**
Catlins	Control	0.19 ± 0.01	0.58 ± 0.01	0.22 ± 0.01	1.87 ± 0.25 a
	Fertilized	0.17 ± 0.01	0.63 ± 0.01	0.20 ± 0.01	2.29 ± 0.19 a
		0.18 ± 0.01	0.61 ± 0.01 **a**	0.21 ± 0.01 **b**	2.08 ± 0.16 **ab**
Longwoods	Control	0.20 ± 0.02	0.50 ± 0.01	0.31 ± 0.02 **a**	0.8 ± 0.17 a
	Fertilized	0.24 ± 0.01	0.53 ± 0.01	0.23 ± 0.01 **b**	1.72 ± 0.16 b
		0.21 ± 0.01	0.51 ± 0.01 **b**	0.28 ± 0.01 **a**	1.2 ± 0.16 **c**
Fertilization	Control	0.19 ± 0.01	0.58 ± 0.01	0.23 ± 0.01	1.68 ± 0.14 a
	Fertilized	0.19 ± 0.01	0.61 ± 0.01	0.21 ± 0.01	2.19 ± 0.12 b
		2.62	25.29	35.67	9.19
ANOVA	S	0.04	<0.001	<0.001	<0.001
		* (§)	***	***	***
		0	3.53	7.81	9.92
	F	0.97	0.064	0.007	0.002
		ns	ns	**	**
		2.16	0.98	2.50	0.80
	S × F	0.08	0.42	0.049	0.53
		ns	ns	*	ns

Average tree mass varied over three-fold and differed significantly across plots ($F_{9,75} = 8.5$, $P < 0.001$). Because plant mass may confound the interpretation of component mass fractions, a more accurate interpretation of dry matter partitioning is presented in Table D.5.4 using allometric analysis. The following generalized linear model was fitted between a given component (y) and total plant mass (x),

$$\log_e y = b_0 + b_1 \log_e x$$

Analysis of covariance was used to test whether slopes and intercepts of this linear relationship were significantly influenced by site and fertilization treatment.

Table D.5.4. Influence of fertilization treatment and site on the alometric relationship $\log_e y = b_0 + b_1 \log_e x$ between selected plant components (y) against total plant mass (x) of four-years old *Pinus radiata*. Significance of total plant mass (covariate), and slopes and intercepts of allometric relationships as influenced by site and fertilization treatment are presented as F values and P range: ns, non-significant; ***, significant at $P < 0.001$. The treatment influence is expressed as the percentage change in the ratio y:x compared to either the arithmetic (conventional approach, $y{:}x^C$) or the allometric average (allometric approach, $y{:}x^A$), the latter calculated using the following equations: $W_f = 0.853\ W_T^{0.816}$, $r^2 = 0.96$, $P < 0.001$; $W_w = 0.2149\ W_T^{1.1372}$, $r^2 = 0.99$, $P < 0.001$; $W_R = 1.5605\ W_T^{0.7139}$, $r^2 = 0.95$, $P < 0.001$. As an example a change in the ratio y:x of -2% means that actual value was 2% less than expected average.

Site	Plot	Root:Total		Wood:Total		Foliage:Total	
		$y{:}x^A$ (%)	$y{:}x^C$ (%)	$y{:}x^A$ (%)	$y{:}x^C$ (%)	$y{:}x^A$ (%)	$y{:}x^C$ (%)
Rai Valley	Control	-2%	-4%	3%	7%	-1%	-3%
	Fertilized	2%	-1%	0%	5%	-2%	-4%
Golden Downs	Control	0%	-1%	4%	5%	-4%	-5%
	Fertilized	0%	-2%	4%	8%	-4%	-5%
Tekapo	Control	3%	4%	-6%	-7%	3%	3%
	Fertilized	1%	1%	-5%	-5%	4%	4%
Catlins	Control	1%	0%	-2%	-1%	1%	1%
	Fertilized	0%	-2%	0%	3%	0%	-1%
Longwoods	Control	-3%	1%	-3%	-9%	6%	9%
	Fertilized	5%	5%	-7%	-6%	2%	2%
ANCOVA							
Covariate		54.4^{***}		1771.3^{***}		497.4^{***}	
Slopes		0.64^{ns}		1.95^{ns}		1.11^{ns}	
Intercepts		0.59^{ns}		1.72^{ns}		1.01^{ns}	

The log-linear relationships between foliage, wood and root biomass against total plant mass were highly significant ($F_{1,65} > 54$, $P < 0.001$), and slopes ($F_{9,65} < 1.72$, $P > 0.10$) and intercepts ($F_{9,65} < 1.95$, $P > 0.06$) of these log-linear relationships were not influenced by site or fertilization treatment (Table D.5.3). The fraction of total plant mass represented by foliage and roots decreased with plant size, while the proportion partitioned to stems and branches remained largely constant (Figure D.5.6).

Compared to arithmetic averages (Conventional analysis), component mass fractions across sites and fertilization treatments did not change by more than 9%. Compared to the allometric average (Allometric analysis), component mass fractions did not differ by more than 7%, and changes in the y:x ratio were consistently lower using the allometric than the conventional approach. These small differences suggest that component mass fractions were not influenced by site or fertilization treatment.

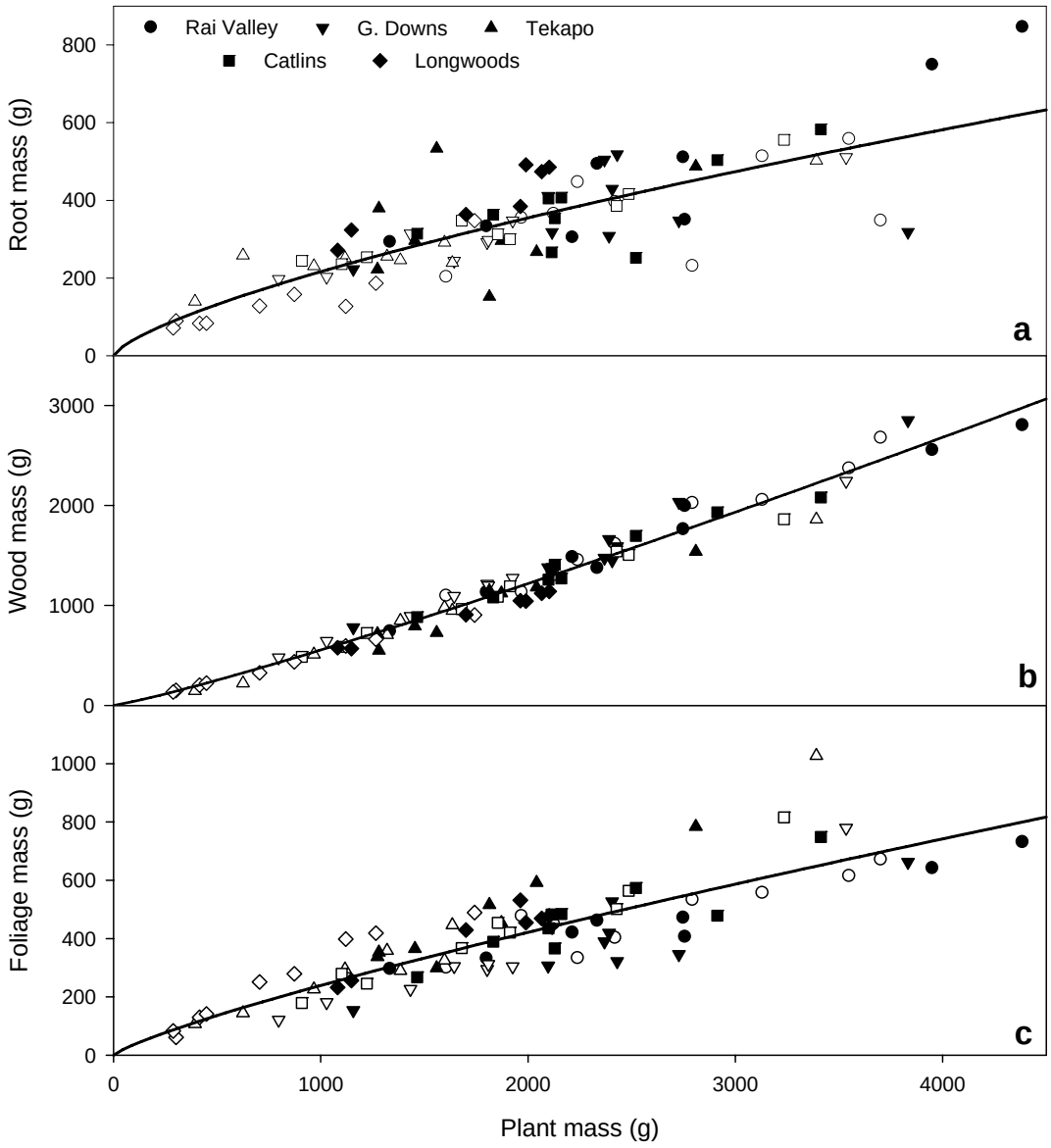


Figure D.5.6. Allometric relationship $\log_e y = b_0 + b_1 \log_e x$ between selected plant components (y) against total plant mass (x) of four-years old *Pinus radiata*. Component plant mass scaled with total plant mass independent of site and fertilization treatment. Allometric equations fitted were: $W_f = 0.853\ W_T^{\ 0.816}$, $r^2 = 0.96$, $P < 0.001$; $W_w = 0.2149\ W_T^{\ 1.1372}$, $r^2 = 0.99$, $P < 0.001$; $W_R = 1.5605\ W_T^{\ 0.7139}$, $r^2 = 0.95$, $P < 0.001$. Open symbols indicate control plots, while closed symbols fertilized plots.

D.6. TREE PHENOLOGY AND LEAF AREA TO MASS RATIO

To measure needle growth, a branch was randomly selected in five trees per plot. The starting point of measurement was marked at the base of the leading bud during the winter 2004, and then fascicle length measured monthly at random in selected shoots from bud break in spring until fascicle expansion stopped the following winter. Trajectories of needle expansion (l) against the number of days after bud break (t) were fitted to individual trees using the von Bertalanffy equation ([1]Richards 1959),

$$l = l_{\mathrm{m}} \, (1 - \mathrm{e}^{-\mathrm{b}_0 \, t})^{\mathrm{b}_1}$$

where l is the mean needle length, l_{m} is the maximum needle length, and b_0 and b_1 are parameters to be fitted (Watt *et al*. 2003). Maximum rate of needle growth at the point of inflexion (l_{i}) and time when this ocurrs (t_{i}) were determined by numerical approximation using excel heuristic tools. Five fascicles per age-class per plot were destructively sampled monthly to determine fascicle size and the leaf area to mass ratio. Changes in fascicle mass over the growing season were regressed against the number of days after a reference starting date.

Fascicle expansion started in early spring (day 99, reference day is July 1st 2004 equal to day zero) and this date was not influenced by sites or fertilization treatments ($F_{9,47} = 1.20$, $P = 0.32$) (Table D.5.1). The time at which maximum needle growth occurred differed among sites but was not influenced by fertilization treatment ($F_{9,47} = 4.49$, $P < 0.001$). Maximum fascicle growth was attained earlier in Rai Valley, and progressively later in Golden Downs, Tekapo, Catlins and Longwoods, and this was probably associated with the global trend of temperature to decrease with latitude. The maximum growth rate did not differ significantly between sites and fertilization treatments ($F_{9,47} = 0.95$, $P = 0.49$), but values were slightly higher in Rai Valley than in other sites. By early autumn (day 299) fascicles reached 95% of their maximum length (t_{95}) and this date was not influenced by site or fertilization treatment ($F_{9,47} = 1.80$, $P = 0.09$).

[1] Richards, F.J. 1959. A flexible growth function for empirical use. Journal of Experimental Botany. 10 (29): 290-300.

Table D.6.1. Maximum fascicle length, l_m, maximum fascicle length growth at inflexion point, l_i, time at inflexion point , t_i, and time required for leaf expansion to reach 95% of maximum length, t_{95}, of current year needles in control and fertilized plots of *Pinus radiata* cultivated for four years in five sites in the South Island of New Zealand. Values are presented as means ± 1 standard error from 5-6 trees. Significance of plot on fascicle growth variables was determined with a one-way analysis of variance, and shown as *F* values and *P* range: ns, non-significant, ***, significant at $P < 0.001$. Different letters indicate significant differences at $P < 0.05$. Day zero was referenced to 1st July 1st 2004.

Site	Plot	l_m (mm)	l_i (mm day^{-1})	t_i (day)	t_{95} (day)
Rai Valley	Control	55 ± 3 bcd	0.98 ± 0.35 a	120 ± 7 bc	264 ± 10 a
	Fertilized	51 ± 2 cd	1.11 ± 0.28 a	104 ± 1 c	288 ± 21 a
Golden Downs	Control	32 ± 7 d	0.69 ± 0.19 a	121 ± 8 abc	282 ± 15 a
	Fertilized	65 ± 5 bc	0.66 ± 0.11 a	140 ± 9 abc	290 ± 22 a
Tekapo	Control	85 ± 2 ab	0.72 ± 0.04 a	138 ± 6 abc	301 ± 7 a
	Fertilized	84 ± 4 ab	0.7 ± 0.05 a	137 ± 7 abc	296 ± 13 a
Catlins	Control	79 ± 9 abc	0.73 ± 0.17 a	161 ± 12 ab	310 ± 9 a
	Fertilized	72 ± 13 abc	0.61 ± 0.1 a	162 ± 14 ab	311 ± 10 a
Longwoods	Control	84 ± 4 ab	0.64 ± 0.06 a	166 ± 15 ab	319 ± 8 a
	Fertilized	99 ± 5 a	0.72 ± 0.06 a	162 ± 10 ab	314 ± 5 a
Overall mean		72 ± 3	0.74 ± 0.05	143 ± 4	299 ± 4
One-Way ANOVA	Plot	9.85***	1.20ns	4.49***	1.80ns

Average fascicle mass (w_f) and the leaf area to mass ratio (M) in 1-year and older foliage was not influenced by site or fertilization treatment ($F_{9,40} < 1.56$, $P > 0.16$) (Table D.6.2.). However current year foliage exhibited distinct values of w_f and M across sites but not fertilization treatments ($F_{9,40} > 4.87$, $P < 0.001$).

A more accurate interpretation of the relationship between w_f and M was carried out by analysis of covariance (Figure D.6.1). Slopes ($F_{9,80} = 1.16$, $P = 0.33$) and intercepts ($F_{9,80} = 1.09$, $P = 0.38$) of the log-linear relationship between M and w_f were not significantly influenced by site or fertilization treatment, and only intercepts ($F_{1,96} = 3.91$, $P = 0.051$) but not slopes ($F_{1,96} = 2.97$, $P = 0.08$) were marginally influenced by foliage age class.

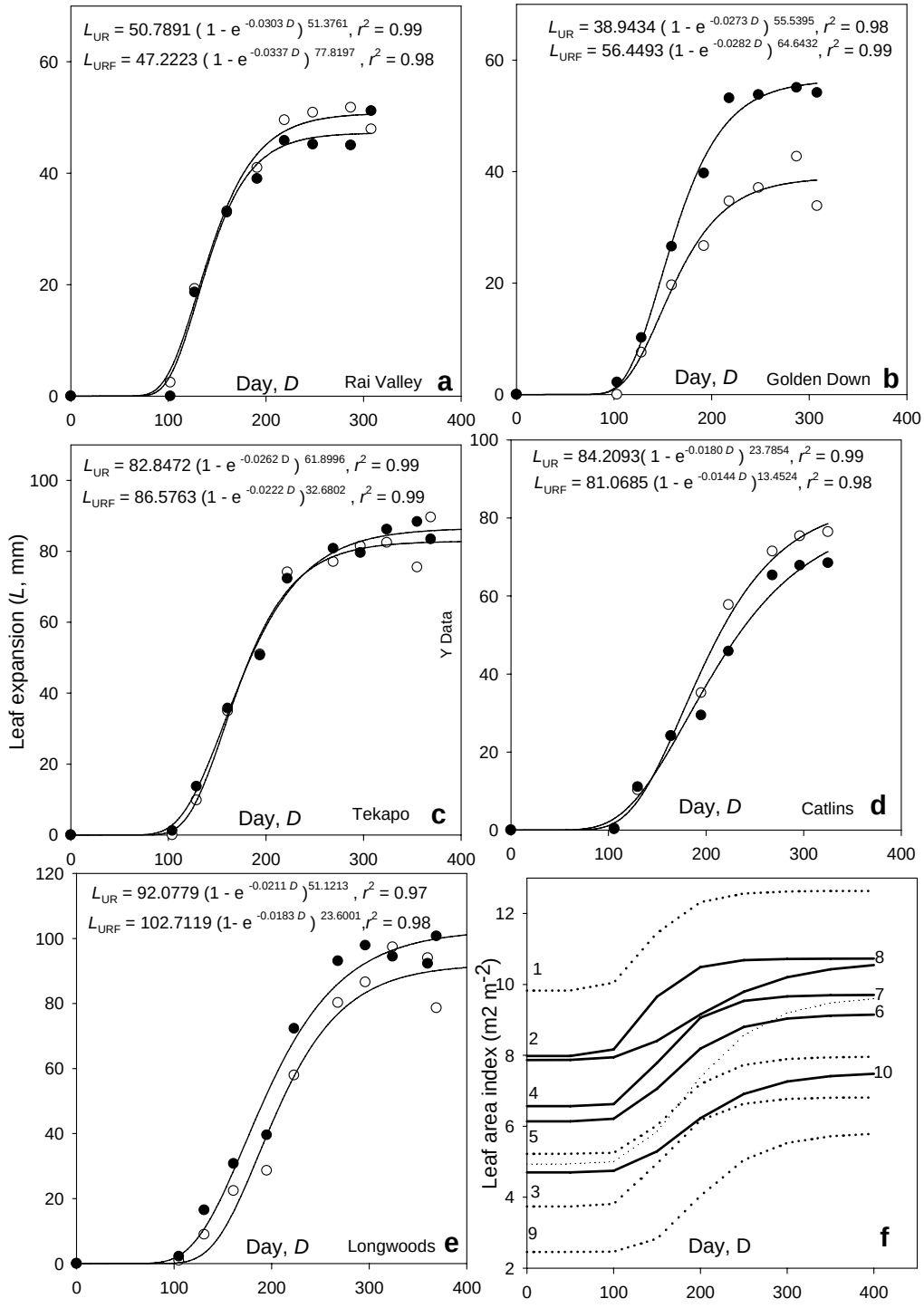


Figure D.6.1. Non-destructive measurements of leaf phenology in control and fertilized plots of *Pinus radiata* in five sites with contrasting climate and soil fertility in the South Island of New Zealand. Equations a-e were used to scale the leaf area index (LAI) from August 2004 to August 2005 (f). Monthly values of LAI were used to scale foliage maintenance respiration.

Table D.6.2. Leaf area to mass ratio, *M*, and average mass per fascicle, w_f, of current- and 1-year and older needles of *Pinus radiata* cultivated in control and fertilized plots in five sites in the South Island of New Zealand. Significance of plot on fascicle growth variables was determined with a one-way analysis of variance, and shown as *F* values and *P* range: ns, non-significant, ***, significant at $P < 0.001$. Different letters indicate significant differences at $P < 0.05$.

Site	Plot	Current *M* (m^2 kg^{-1})	Older *M* (m^2 kg^{-1})	Current w_f (mg)	Older w_f (mg)
Rai Valley	Control	22.9 ± 1.0 ab	18.3 ± 0.9 a	24.4 ± 2.5 c	63.3 ± 4.2 a
	Fertilized	24.1 ± 1.3 a	17.6 ± 0.3 a	29.6 ± 3.7 bc	62.4 ± 5.5 a
Golden Downs	Control	21.1 ± 0.8 abcd	18.3 ± 2.4 a	29.2 ± 3.4 bc	56 ± 11.3 a
	Fertilized	22.8 ± 0.7 abc	17.4 ± 0.8 a	27.2 ± 2.1 bc	51.6 ± 5 a
Tekapo	Control	19.1 ± 1 cde	15.7 ± 0.7 a	39.6 ± 4.7 abc	46.8 ± 3.2 a
	Fertilized	18.5 ± 0.4 de	17.4 ± 0.3 a	42 ± 2 abc	38.4 ± 2.6 a
Catlins	Control	19.7 ± 0.7 bcde	16.5 ± 0.4 a	45.2 ± 3 ab	55.2 ± 3.3 a
	Fertilized	19.2 ± 0.5 bcde	17.5 ± 0.8 a	48.6 ± 3.4 a	53.2 ± 9.3 a
Longwoods	Control	19.2 ± 2.1 e	17.3 ± 1.1 a	43.8 ± 7.8 ab	45.1 ± 8 a
	Fertilized	20.1 ± 0.6 bcde	17.7 ± 1 a	44.5 ± 4.3 ab	56.4 ± 2.2 a
Overall mean		20.7 ± 0.4	17.4 ± 0.3	37.4 ± 1.7	52.8 ± 2.1
One-Way ANOVA	Plot	7.25***	0.54ns	4.87***	1.56ns

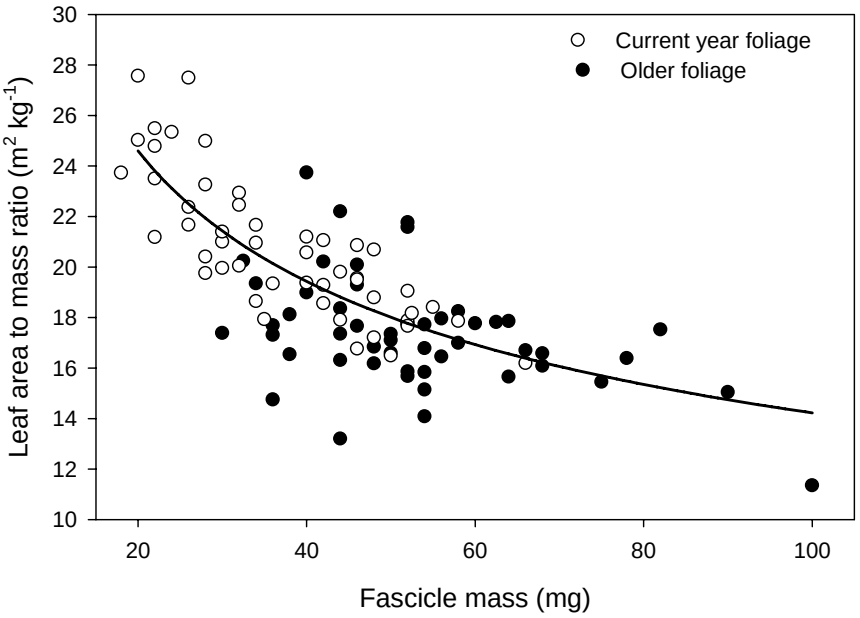


Figure D.6.2. The relationship between the leaf area to mass ratio and average mass per fascicle. The relationship was not significantly influenced by site or fertilization treatment, and only marginally affected by foliage age-class. Equation is: $M = 68.102\ w_f^{-0.340}$, $r^2 = 0.57$, $P < 0.001$.

D.7. CHLOROPHYLL FLUORESCENCE AS AN INDEX OF PHOTOSYNTHETIC PERFORMANCE

The maximum photochemical efficiency of PSII (F_v/F_m), measured when all PSII reaction centre are open in dark-adapted leaves, was significantly influenced by site and fertilization treatment ($F_{9,1329} = 20.9$, $P < 0.001$), time of measurement ($F_{8,1329} = 59.9$, $P < 0.001$) and their interaction ($F_{62,1329} = 12.5$, $P < 0.001$). Values of F_v / F_m for middle months of every season are presented in Table D.7.1.

Table D.7.1. Maximum photochemical efficiency (F_v / F_m) of *Pinus radiata* cultivated in control and fertilized plots in five sites in the South Island of New Zealand. Values are presented as means (± 1 SE, n = 9-15). Significance was determined with a one-way analysis of variance for middle months within each season, and shown as F values and P range: ns, non-significant, ***, significant at $P < 0.001$. Different letters indicate significant differences at $P < 0.05$.

Site	Plot	F_v / F_m			
		Spring	Summer	Autumn	Winter
		(October)	(January)	(May)	(August)
Rai Valley	Control	824 ± 5 *abc*	844 ± 2 *abc*	856 ± 2 *a*	850 ± 4 *ab*
	Fertilized	829 ± 4 *abc*	837 ± 2 *bc*	855 ± 3 *a*	852 ± 3 *a*
Golden Downs	Control	833 ± 3 *abc*	838 ± 2 *bc*	852 ± 3 *a*	833 ± 4 *abc*
	Fertilized	841 ± 3 *a*	838 ± 2 *bc*	855 ± 3 *a*	842 ± 4 *abc*
Tekapo	Control	830 ± 6 *abc*	850 ± 2 *a*	847 ± 4 *ab*	839 ± 7 *abc*
	Fertilized	840 ± 5 *ab*	847 ± 3 *ab*	842 ± 2 *ab*	840 ± 6 *abc*
Catlins	Control	809 ± 14 *c*	844 ± 2 *abc*	848 ± 2 *ab*	835 ± 5 *abc*
	Fertilized	837 ± 7 *abc*	836 ± 2 *bc*	847 ± 3 *ab*	829 ± 4 *bc*
Longwoods	Control	812 ± 6 *bc*	834 ± 4 *c*	834 ± 5 *b*	821 ± 6 *c*
	Fertilized	829 ± 4 *abc*	842 ± 2 *abc*	849 ± 1 *a*	832 ± 3 *abc*
Overall mean		828 ± 2	841 ± 1	849 ± 1	837 ± 2
One-Way ANOVA	Plot	3.6***	4.6***	5.0***	3.7***

Maximum photochemical efficiency (F_v / F_m) changed seasonally increasing from spring to summer, generally reaching a maximum in autumn and then declining during winter. Across sites, Rai Valley generally exhibited higher F_v / F_m values than Longwoods, and the difference became more acute during autumn and winter. Fertilization tended to slightly increase F_v / F_m, and differences between control and fertilized plots were largest and only significant in Longwoods compared to other sites. The merit of this parameter is to be independent of temperature, making possible comparisons across sites and seasons.

APPENDIX E

E.1. Complementary information to Chapter *Four*

E.2. Complementary information to Chapter *Five*

E.1. Complementary information to Chapter Four

Fascicle size and number

Table E.1.1. Average mass per fascicle for one-year old (old) and current-year foliage (new), and total number of fascicles per plant, across nutrient treatments and clones. Nutrient treatments comprised two nitrogen supply regimes (N_0=1.43 and N_1=7.14 mM) and two phosphorus supply regimes (P_0=0.084 and P_1=0.420 mM). Values are presented as means (± 1 SE) for each treatment and clone. Significance of main effects of clones (C) and nutrient treatments (T) or the interaction between clones and treatments (C x T) are shown as *F* values, *P* values and *P*-range: ns, non significant; **, significant at $P < 0.01$; ***, significant at $P < 0.001$. Separation of means was determined by a Tukey test. Different letters within treatments or clones indicate that means were significantly different at $P < 0.05$.

	Mass per fascicle (mg)			Number of fascicles per plant Month 24
	Old Month 18+24	New Month 18	New Month 24	
Treatments				
N_0P_0	45 ± 3 a	37 ± 4 a	55 ± 6 a	746 ± 51 a
N_0P_1	50 ± 2ab	44 ± 4 a	64 ± 7ab	715 ± 33 a
N_1P_0	59 ± 4 b	53 ± 6ab	58 ± 5 a	1234 ± 94 b
N_1P_1	80 ± 4 c	70 ± 7 b	81 ± 8 b	2322 ± 147 c
Clones				
A	75 ± 4 c	66 ± 8 b	84 ± 7 b	858 ± 105 a
B	60 ± 4 b	53 ± 6ab	72 ± 6 b	1374 ± 202 b
C	51 ± 4ab	45 ± 4 a	56 ± 5 a	1277 ± 131 b
D	46 ± 3 a	40 ± 4 a	46 ± 5 a	1520 ± 173 b
Mean	58 ± 2	51 ± 3	64 ± 3	1255 ± 81
ANOVA				
T	33.15, <0.001 ***	9.87, <0.001 ***	6.77, 0.0013 **	73.01,<0.001 ***
C	21.60, <0.001 ***	6.47, 0.002 **	17.21,<0.001 ***	15.71,<0.001 ***
C × T	1.13, 0.35 ns	1.37, 0.24 ns	1.55,0.18 ns	1.50, 0.20 ns

Treatment influences on sites of N storage

Nitrogen was primarily stored in foliage (49%), roots (39%) and to a lesser extent in stems (12%) at the end of the first year of growth (Table E.1.2). All these fractions were significantly influenced by nutrient treatment ($F_{3,30} > 4.47$, $P < 0.011$) but not clone ($F_{3,27} < 2.87$, $P > 0.055$) or their interaction ($F_{9,27} < 1.77$, $P > 0.12$).

Table E.1.2. Partitioning of plant mass and N content among foliage, wood and roots across nutrient treatments at the end of the first year of growth. Nutrient treatments comprised two nitrogen supply regimes (N_0=1.43 and N_1=7.14 mM) and two phosphorus supply regimes (P_0=0.084 and P_1=0.420 mM). Values are presented as means (± 1 SE) for each treatment and plant component. Significance of main effects of clones (C) and nutrient treatments (T) or the interaction between clones and treatments (C × T) are shown as *F* values, *P* values and *P*-range (ns: non significant, *: significant at $P < 0.05$, **: significant at $P < 0.01$, ***: significant at $P < 0.001$). Separation of means was determined by a Tukey test. Different letters within treatments or clones indicate that means were significantly different at $P < 0.05$. §: anova indicated significance but not Tukey's grouping.

	Plant mass			N content		
	Foliage (%)	Wood (%)	Root (%)	Foliage (%)	Wood (%)	Root (%)
Treatment						
N_0P_0	32.9 ± 1.2ab	19 ± 1.4 a	48.1 ± 1.9 b	45.7 ± 1.3a	9.2 ± 0.7 a	45.1 ± 1.7b
N_0P_1	31.1 ± 1.2 a	23.2 ± 1.4ab	45.8 ± 2.1ab	43.8 ± 1.7a	12.4 ± 1.1ab	43.8 ± 2.4b
N_1P_0	37.4 ± 1.0 b	24.7 ± 1.3 b	37.8 ± 1.5 a	54.9 ± 1.1b	12.3 ± 0.8ab	32.8 ± 1.1a
N_1P_1	35.4 ± 0.9ab	26.8 ± 1.1 b	37.8 ± 1.6 a	51.9 ± 1.3b	14.3 ± 1.2 b	33.8 ± 2a
Mean	34.1 ± 0.7	23.2 ± 0.8	42.7 ± 1.1	48.9 ± 1	11.9 ± 0.5	39.2 ± 1.2
ANOVA						
T	5.82,0.0033 **	3.98,0.018 *	6.13,0.026 *	16.42,<0.001 ***	4.47,0.011 **	10.64,<0.001 ***
C	0.81,0.50 ns	1.74,0.182 ns	1.48,0.24 ns	3.40,0.032 ns§	2.53,0.078 ns	2.87,0.055 ns
C × T	0.62,0.77 ns	0.24,0.98 ns	0.21,0.99 ns	1.77,0.120 ns	0.46,0.88 ns	0.89,0.54 ns

Increased nitrogen availability significantly increased the N fraction stored in the foliage from 0.45 (± 0.01 SE) in low-N supply regimes (average N_0P_0 and N_0P_1) to 0.54 (± 0.01 SE) in high-N supply regimes (average N_1P_0 and N_1P_1), as a result of both higher foliar N concentrations (data not shown) and greater biomass allocation to foliage in the high N supply regimes. In contrast, the fraction of N stored in roots was significantly greater in the low-N regimes (0.44 ± 0.01 SE) compared to high-N regimes (0.33 ± 0.01 SE). This occurred as increased biomass partitioned to roots in the low-N regimes that more than offset higher root N concentrations in the high-N supply regimes (data not shown). Similarly to foliage, the proportion of N in stems increased with N and P supply as a result of greater carbon partitioning to stems and higher N concentrations in the high-N supply regimes compared to low N supply treatments (Table E.1.2).

Treatment influences on tissue ^{15}N concentration

One-year old plants were enriched with ^{15}N at levels that were 5.6-7.6 times higher than those of ambient air. At the end of the first year of growth the proportions of tissue ^{15}N in relation to $^{15}N + {}^{14}N$ (Atom % ^{15}N) were about 2 % in low-N supply regimes (N_0P_0 and N_0P_1) compared to about 2.8 % in high-N supply regimes (N_1P_0 and N_1P_1). These values were considerably higher than Atom % ^{15}N 0.3663033 in ambient air and Atom % ^{15}N applied as NH_4NO_3 in nutrient solution during the second year of growth (0.3664899 %). Enrichment values were stable across clones and within treatments. At the plant level, Atom % ^{15}N in foliage, stems and roots at month 12 were similar (Figure E.1.1, averages on top of graphs), suggesting that an even ^{15}N enrichment of tissues was achieved during the first year of growth. This is relevant as tissues need to be evenly saturated in ^{15}N in remobilization studies before the start of the second growing season when ^{15}N will be depleted from old tissues to enrich new tissues.

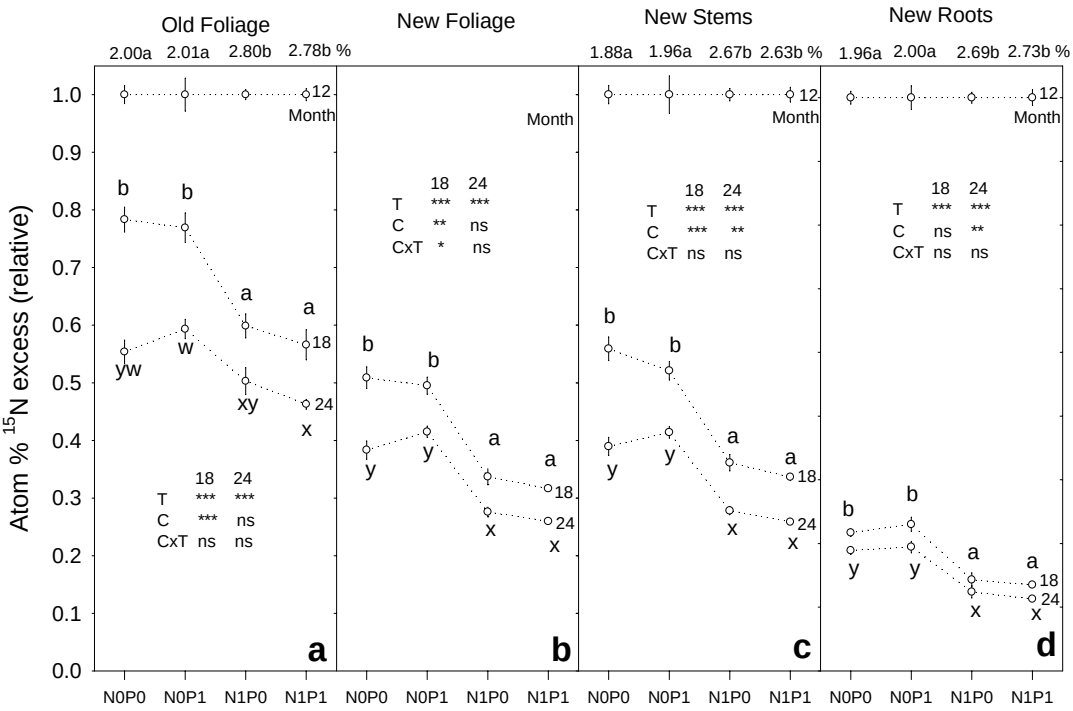


Figure E.1.1. Atom % ^{15}N excess (relative) in plant tissues at months 12, 18 and 24. Absolute values (month 12) are presented at the top of each graph for each nutrient treatment, while fractions of the maximum values are plotted for months 12, 18 and 24. Atom % ^{15}N values presented here were calculated as excess over background level provided by chemical source (NH_4NO_3) in nutrient solution applied during the second year (0.3664899 atom% ^{15}N, $\delta^{15}N$ = 0.51 ‰). Tissue Atom % ^{15}N at the end of the first year was significantly influenced by the main effects of nutrient treatment ($F_{3,30} > 91.5$, $P < 0.001$) and clones ($F_{3,30} > 3.14$, $P < 0.04$) but not by their interaction ($F_{9,30} < 0.75$, $P > 0.66$). Differences in ^{15}N labeling between clones were small (not shown). Significance of main effects of nutrient treatments (T) and clones (C) or their interaction (C×T) are presented as P range : ns, non significant; *, significant at $P < 0.05$; **, significant at $P < 0.01$; ***, significant at $P < 0.001$. Separation of means was determined by a Tukey test. Different letters indicate significant differences at $P < 0.05$ (month 18 *a* to *b*, month 24 *x* to *w*).

Atom % ^{15}N in new foliage and stems was significantly higher than Atom % ^{15}N in new roots at month 18 and 24, and collectively significantly higher than the background level, indicating that remobilization was directed mainly to new foliage and stems but also considerably to roots. Also the small separation between treatment lines at months 18 and 24 in new roots (Figure E.1.1d), indicates that almost all N was remobilized to roots between months 12-18 (95-97%), while the wider line separation between nutrient treatments for new foliage and stems at months 18 and 24 in Figure E.1.1 b,c shows that some N was mobilized to new foliage (8-20%) and new stems (10-28%) during months 18-24. This may be explained as priorities of N investment on a scale: new roots > new foliage > new stems.

Atom % ^{15}N in new tissues created during the second year were always greater than background level provided by the source of nitrogen in nutrient solution (NH_4NO_3, Atom % ^{15}N 0.3664899) and lower than Atom % ^{15}N in labeled tissues during the first year of growth (Figure E.1.1 b,c,d), indicating that remobilization took place. Atom % ^{15}N in old foliage substantially decreased from month 12 to month 18 and 24 (Figure E.1.1a), and that difference was greater in the high-N compared to the low-N supply regimes. If we assume this difference to be an index of N remobilization, then 54% of old foliage N was remobilized at month 24 in the high-nutrient supply regime compared to 45% in the low-nutrient supply regime. Also a greater proportion of N was remobilized between months 12 and 18, compared to months 18 to 24, suggesting a seasonal effect additional to a nutrient availability effect on N remobilization.

Tissue Atom % ^{15}N can be considered only as an indication of remobilization, because differences in growth, allocation and uptake can drastically change N remobilization to different components. For instance from Figure E.1.1 b,c,d (but not a) we may get the wrong impression that N remobilization was greater in the low-nutrient supply regime than at higher N and P addition rates, while a whole ^{15}N budget will show the opposite (Table 4.4, Chapter Four).

E.2. Complementary information to Chapter *Five*

Allometric equations used to predict biomass and leaf area based on plant collar diameter (d, mm) and plant height, (h, cm)

Time	Component	Equation
Start	Total dry mass (W_T, g)	$W_T = 0.007252\ d^{1.0611}\ h^{1.5129}$, $n = 40$, $r^2 = 0.90$, $P < 0.001$
	Foliage mass (W_F, g)	$W_F = 0.02272\ d^{0.668819}$, Clone A;
		$W_F = 0.002607\ d^{1.909418}$, Clone B;
		$W_F = 0.001776\ d^{2.450549}$, Clone C;
		$W_F = 0.00101\ d^{3.034653}$, Clone D;
		$n = 40$, $r^2 = 0.65$, $P < 0.001$.
	Wood mass(W_W, g)	$W_W = 0.0033543\ d^{1.154494}$, Clone A;
		$W_W = 0.043889\ d^{1.26619}$, Clone B;
		$W_W = 0.023996\ d^{1.837821}$, Clone C;
		$W_W = 0.029088\ d^{1.861876}$, Clone D;
		$n = 40$, $r^2 = 0.90$, $P < 0.001$.
	Root mass(W_R, g)	$W_R = 0.139878\ d^{1.413007}$, Clone A;
		$W_R = 0.493966\ d^{0.359756}$, Clone B;
		$W_R = 0.065465\ d^{2.507481}$, Clone C;
		$W_R = 0.149096\ d^{1.939382}$, Clone D;
		$n = 40$, $r^2 = 0.80$, $P < 0.001$.
	Leaf area (L, m^2)	$L = 47.2556 \times 10^{-5}\ d^{0.658819}$, Clone A;
		$L = 5.4227 \times 10^{-5}\ d^{1.909418}$, Clone B;
		$L = 3.6951 \times 10^{-5}\ d^{2.450549}$, Clone C;
		$L = 2.1017 \times 10^{-5}\ d^{3.03465}$, Clone D;
		$n = 40$, $r^2 = 0.65$, $P < 0.001$.
End	Leaf area (L, m^2)	$L = 0.0982\ d^{1.6234}$, $n = 24$, $r^2 = 0.95$, $P < 0.001$, N_0P_0;
		$L = 0.0227\ d^{1.3428}$, $n = 24$, $r^2 = 0.98$, $P < 0.001$, N_0P_1;
		$L = 0.00584\ d^{2.0022}$, $n = 24$, $r^2 = 0.97$, $P < 0.001$, N_1P_0;
		$L = 0.0223\ d^{1.5386}$, $n = 24$, $r^2 = 0.97$, $P < 0.001$, N_1P_1.

APPENDIX F

FITTING THE NUTRITIONAL MODIFIER (F_N) TO CONTROL AND FERTILIZED MINI-PLOTS OF PINUS RADIATA AT FIVE SITES IN THE SOUTH ISLAND OF NEW ZEALAND

Values of a fertility rating (F_N) were fitted to actual values of gross-primary productivity (GPP) calculated for control and fertilized mini-plots of *Pinus radiata* at five sites in the South Island of New Zealand. Values of F_N were fitted using algorithms of 3-PG, climatic and water balance data available for each site.

The following equation, based on 3-PG, was used to calculate gross primary productivity (P_G):

$$P_G = \varepsilon \Sigma Q_a \min \{F_\theta, F_D\} F_T F_N \quad \text{(F.1)}$$

where ε is the canopy quantum efficiency (default: 0.055 mol CO_2 mol^{-1} quanta), Q_a is absorbed photosynthetically active radiation and F_i are the modifying factors reducing the effectiveness of a unit of Q_a as a result of soil water deficit (θ), the vapor pressure deficit of the air (D), temperature (T) and fertility (N) (Landsberg and Hingston 1996, Landsberg and Waring 1997). The modifiers are dimensionless with values between zero (no growth) and unity (no environmental constraints). Because both soil water and air vapour pressure deficit affect stomatal conductance, only the most limiting of these two factors, F_θ or F_D, is included in the calculation. The resulting value of Q_a may be interpreted as utilizable radiation by plants.

Values of Q_a were calculated from solar radiation (Q), leaf area index (L) and Beer's Law ($Q_a = Q(1\text{-}e^{-kL})$, where k is the light-extinction coefficient (k = 0.5 used in this study). The following equations were used for the modifying factors (from 3-PG):

Vapour-pressure deficit modifier

$$F_D = e^{-0.05\,D}$$

where D is average vapour pressure deficit (kPa).

Soil water deficit modifier

$F_\theta = 1 / (1 + (1-r_\theta)/c_\theta)^{n\theta}$

where c_θ and the power n_θ take different values for different soil types and r_θ is the soil available water. Volumetric water content was measured monthly in all plots (θ_i) and the moisture ratio was calculated as fractional available water (θ_a): $\theta_a = (\theta_i - \theta_{min})/(\theta_{max} - \theta_{min})$, where θ_{min} and θ_{max} are minimum and maximum volumetric water content.

Temperature Modifier

$$F_T = \left(\frac{\overline{T} - T_{min}}{T_{opt} - T_{min}} \right) \left(\frac{T_{max} - \overline{T}}{T_{max} - T_{opt}} \right)^{\frac{T_{max} - T_{opt}}{T_{opt} - T_{min}}}$$

Where T_{min}, T_{opt} and T_{max} are minimum, optimum and maximum temperatures for growth, and T_{mean} is the average temperature for each month.

Main parameter values used in the analysis are presented in Table F.1.

Table F.1. Description and source of 3-PG parameters used in the fitting of the fertility rating (F_N) to control and fertilized mini-plots of *Pinus radiata* at five sites in the South Island of New Zealand.

Meaning/comments	Parameter Source	*P.radiata*	Units
Temperature and frost modifier (F_T)			
Minimum temperature for growth	P. radiata Default	0	°C
Optimum temperature for growth	P. radiata Default	20	°C
Maximum temperature for growth	P. radiata Default	32	°C
Soil water modifier (F_θ)			
Moisture ratio deficit which gives $F_\theta = 0.5$	P. radiata Default	0.7	-
Power of moisture ratio deficit in F_θ	P. radiata Default	9	-
Conductance			
Defines stomatal response to VPD	P. radiata Default	0.05	-
Canopy structure and processes			
Extinction coefficient for absorption of PAR by canopy	Default 3-PG	0.5	-
Canopy quantum efficiency	Appendix A	0.065	molC molPAR^{-1}
Conversion factors			
Conversion of solar radiation to PAR	Default 3-PG	2.3	mol MJ^{-1}

Modelling GPP across fertilization treatments and sites

Extinction coefficient (k)	0.5	
Canopy quantum efficiency	0.065	molC mol^{-1} PAR
Conversion of solar radiation to PAR	2.3	mol MJ^{-1}
Tmin	0	°C
Topt	20	°C
Tmax	32	°C
VPD constant	-0.05	
Moisture ratio deficit for $f_q = 0.5$	0.7	
Power of moisture ratio deficit	9	

Site	Plot Number: Control	Fertilized
Rai Valley	1	2
Golden Downs	3	4
Tekapo	5	6
Catlins	7	8
Longwoods	9	10

Plot	θmax	θmin
1	0.248	0.072
2	0.254	0.072
3	0.282	0.072
4	0.293	0.072
5	0.213	0.072
6	0.231	0.072
7	0.275	0.072
8	0.262	0.072
9	0.292	0.072
10	0.258	0.072

		Year	2004					2005						
		Month	A	S	O	N	D	J	F	M	A	M	J	J
			2	3	4	5	6	7	8	9	10	11	12	13
Solar radiation (MJ)	Plot	1	303	400	477	650	603	637	536	493	381	244	190	186
		2	303	400	477	650	603	637	536	493	381	244	190	186
		3	320	418	533	685	786	831	621	539	397	246	190	191
		4	320	418	533	685	786	831	621	539	397	246	190	191
		5	226	346	475	575	605	658	481	387	315	233	157	182
		6	226	346	475	575	605	658	481	387	315	233	157	182
		7	304	365	483	495	453	496	431	345	359	189	126	156
		8	304	365	483	495	453	496	431	345	359	189	126	156
		9	214	317	486	517	538	560	450	378	310	160	97	137
		10	214	317	486	517	538	560	450	378	310	160	97	137
LAI (m^2 m^{-2})	Plot	1	9.29	9.29	9.39	10.14	11.22	11.97	12.35	12.52	12.59	12.62	12.63	12.63
		2	7.86	7.86	7.92	8.59	9.60	10.25	10.55	10.66	10.70	10.72	10.72	10.73
		3	4.01	4.01	4.03	4.34	5.11	5.87	6.35	6.60	6.72	6.77	6.79	6.81
		4	6.83	6.83	6.85	7.14	7.95	8.75	9.25	9.50	9.62	9.67	9.69	9.70
		5	5.04	5.04	5.05	5.24	5.90	6.72	7.31	7.64	7.81	7.89	7.93	7.95
		6	6.85	6.85	6.87	7.07	7.55	8.11	8.54	8.82	8.98	9.06	9.11	9.13
		7	5.33	5.33	5.36	5.57	6.16	7.01	7.84	8.49	8.94	9.23	9.41	9.52
		8	7.44	7.44	7.49	7.68	8.07	8.58	9.10	9.55	9.89	10.15	10.32	10.44
		9	2.76	2.76	2.76	2.81	3.10	3.72	4.42	4.97	5.34	5.55	5.68	5.74
		10	3.59	3.59	3.62	3.84	4.42	5.21	5.96	6.54	6.93	7.17	7.33	7.42
Absorbed radiation (MJ)	Plot	1	300	396	473	646	601	635	535	492	380	243	189	186
		2	297	392	468	641	598	633	533	491	379	242	189	185
		3	277	361	462	606	725	787	595	519	383	238	184	184
		4	310	404	516	665	771	821	615	534	394	244	189	189
		5	208	318	437	533	574	635	469	379	309	229	154	179
		6	218	335	459	559	591	647	474	383	312	231	155	181
		7	283	340	450	464	432	481	422	340	355	187	125	155
		8	297	357	472	484	445	489	426	342	357	188	126	155
		9	160	237	364	390	424	473	400	347	288	150	91	129
		10	178	264	406	441	478	519	427	364	300	156	94	134
Mean Temperature (°C)	Plot	1	9.5	10.6	12.7	14.5	14.2	17.4	19.3	17.4	14.2	12.8	9.6	9.7
		2	9.5	10.6	12.7	14.5	14.2	17.4	19.3	17.4	14.2	12.8	9.6	9.7
		3	8.8	10.5	13.4	15.5	15.1	18.3	19.2	17.9	13.8	12.4	9.3	10.0
		4	8.8	10.5	13.4	15.5	15.1	18.3	19.2	17.9	13.8	12.4	9.3	10.0
		5	3.3	8.3	11.0	14.4	12.0	17.9	19.3	14.8	12.1	8.6	4.0	6.0
		6	3.3	8.3	11.0	14.4	12.0	17.9	19.3	14.8	12.1	8.6	4.0	6.0
		7	5.0	8.6	10.0	13.4	10.0	15.5	16.6	13.4	10.8	8.7	6.2	7.2
		8	5.0	8.6	10.0	13.4	10.0	15.5	16.6	13.4	10.8	8.7	6.2	7.2
		9	3.4	6.2	7.5	10.2	7.7	12.4	13.7	11.0	8.8	7.0	4.9	5.6
		10	3.4	6.2	7.5	10.2	7.7	12.4	13.7	11.0	8.8	7.0	4.9	5.6
VPD (kPa)	Plot	1	0.35	0.44	0.50	0.58	0.59	0.72	0.81	0.73	0.67	0.52	0.42	0.41
		2	0.35	0.44	0.50	0.58	0.59	0.72	0.81	0.73	0.67	0.52	0.42	0.41
		3	0.30	0.38	0.48	0.69	0.65	0.91	1.02	0.89	0.65	0.50	0.36	0.36
		4	0.30	0.38	0.48	0.69	0.65	0.91	1.02	0.89	0.65	0.50	0.36	0.36
		5	0.25	0.46	0.53	0.76	0.62	0.92	1.06	0.73	0.67	0.43	0.26	0.30
		6	0.25	0.46	0.53	0.76	0.62	0.92	1.06	0.73	0.67	0.43	0.26	0.30
		7	0.23	0.36	0.44	0.61	0.34	0.66	0.75	0.50	0.49	0.37	0.28	0.33
		8	0.23	0.36	0.44	0.61	0.34	0.66	0.75	0.50	0.49	0.37	0.28	0.33
		9	0.20	0.27	0.39	0.47	0.31	0.54	0.58	0.39	0.42	0.25	0.21	0.22
		10	0.20	0.27	0.39	0.47	0.31	0.54	0.58	0.39	0.42	0.25	0.21	0.22

		Year	2004					2005						
		Month	A	S	O	N	D	J	F	M	A	M	J	J
			2	3	4	5	6	7	8	9	10	11	12	13
θ (m^3 m^{-3})	Plot	1	0.23	0.23	0.24	0.24	0.21	0.25	0.19	0.21	0.20	0.18	0.20	0.23
		2	0.25	0.24	0.24	0.22	0.20	0.24	0.20	0.19	0.20	0.19	0.20	0.20
		3	0.27	0.27	0.28	0.25	0.23	0.26	0.20	0.20	0.23	0.19	0.23	0.28
		4	0.29	0.28	0.28	0.24	0.22	0.25	0.19	0.20	0.22	0.20	0.22	0.27
		5	0.21	0.20	0.17	0.14	0.15	0.15	0.07	0.08	0.08	0.10	0.11	0.10
		6	0.23	0.19	0.17	0.12	0.12	0.14	0.08	0.08	0.07	0.08	0.12	0.10
		7	0.28	0.26	0.25	0.23	0.25	0.25	0.20	0.23	0.21	0.24	0.24	0.23
		8	0.26	0.26	0.24	0.21	0.25	0.24	0.20	0.22	0.21	0.23	0.23	0.22
		9	0.24	0.29	0.24	0.24	0.24	0.25	0.22	0.22	0.24	0.24	0.24	0.24
		10	0.24	0.26	0.22	0.20	0.21	0.22	0.20	0.21	0.20	0.22	0.21	0.21
fractional θ	Plot	1	0.91	0.90	0.97	0.94	0.78	1.00	0.70	0.77	0.73	0.63	0.73	0.91
		2	1.04	0.94	0.96	0.84	0.75	0.94	0.70	0.69	0.73	0.66	0.75	0.74
		3	1.13	1.14	1.16	1.03	0.90	1.05	0.75	0.70	0.92	0.70	0.91	1.19
		4	1.26	1.20	1.20	0.93	0.83	1.02	0.68	0.71	0.81	0.72	0.82	1.12
		5	0.80	0.73	0.57	0.38	0.41	0.47	0.01	0.04	0.02	0.13	0.20	0.13
		6	0.90	0.68	0.58	0.29	0.29	0.37	0.04	0.03	0.00	0.05	0.29	0.15
		7	1.16	1.08	0.99	0.87	1.00	1.01	0.72	0.89	0.76	0.94	0.97	0.89
		8	1.08	1.04	0.94	0.79	1.04	0.96	0.71	0.82	0.76	0.90	0.89	0.81
		9	0.94	1.25	0.95	0.94	0.97	1.02	0.84	0.86	0.95	0.96	0.96	0.95
		10	0.95	1.06	0.81	0.74	0.79	0.86	0.74	0.79	0.73	0.83	0.79	0.78
Temperature Modifier	Plot	1	0.69	0.75	0.85	0.91	0.90	0.98	1.00	0.98	0.90	0.85	0.70	0.71
		2	0.69	0.75	0.85	0.91	0.90	0.98	1.00	0.98	0.90	0.85	0.70	0.71
		3	0.66	0.74	0.87	0.94	0.93	0.99	1.00	0.99	0.89	0.83	0.68	0.72
		4	0.66	0.74	0.87	0.94	0.93	0.99	1.00	0.99	0.89	0.83	0.68	0.72
		5	0.28	0.63	0.77	0.90	0.82	0.99	1.00	0.92	0.82	0.64	0.33	0.48
		6	0.28	0.63	0.77	0.90	0.82	0.99	1.00	0.92	0.82	0.64	0.33	0.48
		7	0.40	0.64	0.72	0.87	0.72	0.94	0.96	0.87	0.76	0.65	0.49	0.56
		8	0.40	0.64	0.72	0.87	0.72	0.94	0.96	0.87	0.76	0.65	0.49	0.56
		9	0.29	0.49	0.57	0.73	0.59	0.83	0.88	0.77	0.65	0.55	0.40	0.45
		10	0.29	0.49	0.57	0.73	0.59	0.83	0.88	0.77	0.65	0.55	0.40	0.45
VPD Modifier	Plot	1	0.98	0.95	0.95	0.96	0.96	0.95	0.97	0.97	0.96	0.97	0.96	0.96
		2	0.98	0.94	0.94	0.95	0.96	0.95	0.96	0.97	0.95	0.97	0.96	0.94
		3	0.99	0.94	0.94	0.95	0.96	0.95	0.97	0.97	0.96	0.96	0.96	0.95
		4	0.99	0.96	0.97	0.98	0.98	0.98	1.00	1.00	1.00	0.99	0.99	0.99
		5	0.99	0.97	0.97	0.99	0.99	0.98	1.00	1.00	1.00	1.00	0.99	0.99
		6	0.99	0.95	0.95	0.96	0.95	0.95	0.96	0.96	0.96	0.95	0.95	0.96
		7	0.99	0.95	0.95	0.96	0.95	0.95	0.96	0.96	0.96	0.96	0.96	0.96
		8	0.99	0.94	0.95	0.95	0.95	0.95	0.96	0.96	0.95	0.95	0.95	0.95
		9	0.99	0.95	0.96	0.96	0.96	0.96	0.96	0.96	0.96	0.96	0.96	0.96
		10	0.99	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Water modifier	Plot	1	1.000	1.000	1.000	1.000	1.000	1.000	0.999	1.000	1.000	0.997	1.000	1.000
		2	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.999	1.000	0.999	1.000	1.000
		3	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.999	1.000	1.000
		4	1.000	1.000	1.000	1.000	1.000	1.000	0.999	1.000	1.000	1.000	1.000	1.000
		5	1.000	1.000	0.987	0.749	0.834	0.918	0.044	0.056	0.045	0.128	0.233	0.124
		6	1.000	0.999	0.989	0.466	0.474	0.731	0.056	0.052	0.039	0.060	0.472	0.155
		7	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
		8	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
		9	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
		10	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
Nutritional Modifier	Plot	1	0.73	0.73	0.73	0.73	0.73	0.73	0.73	0.73	0.73	0.73	0.73	0.73
		2	0.67	0.67	0.67	0.67	0.67	0.67	0.67	0.67	0.67	0.67	0.67	0.67
		3	0.48	0.48	0.48	0.48	0.48	0.48	0.48	0.48	0.48	0.48	0.48	0.48
		4	0.62	0.62	0.62	0.62	0.62	0.62	0.62	0.62	0.62	0.62	0.62	0.62
		5	0.92	0.92	0.92	0.92	0.92	0.92	0.92	0.92	0.92	0.92	0.92	0.92
		6	1.17	1.17	1.17	1.17	1.17	1.17	1.17	1.17	1.17	1.17	1.17	1.17
		7	0.85	0.85	0.85	0.85	0.85	0.85	0.85	0.85	0.85	0.85	0.85	0.85
		8	0.87	0.87	0.87	0.87	0.87	0.87	0.87	0.87	0.87	0.87	0.87	0.87
		9	0.81	0.81	0.81	0.81	0.81	0.81	0.81	0.81	0.81	0.81	0.81	0.81
		10	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99

265.00

	Year	2004					2005						
	Month	A	S	O	N	D	J	F	M	A	M	J	J
		2	3	4	5	6	7	8	9	10	11	12	13
GPP (g C m^{-2}) uncorrected	Plot 1	539	711	849	1160	1079	1140	960	884	683	436	340	333
	2	533	704	841	1151	1074	1136	957	881	681	435	339	332
	3	498	649	830	1089	1302	1413	1068	932	688	427	330	331
	4	556	725	926	1195	1385	1473	1104	960	707	438	339	339
	5	373	571	784	958	1030	1141	841	680	555	410	276	321
	6	392	601	825	1003	1062	1161	852	687	560	414	278	324
	7	508	610	808	834	776	863	758	610	637	336	225	278
	8	533	640	847	869	799	878	765	614	640	338	225	279
	9	287	426	653	701	760	849	719	623	517	270	164	232
	10	320	474	729	792	859	931	766	653	538	280	170	240

GPP corrected	Plot 1	268	371	500	738	682	778	676	611	432	262	167	166
	2	244	334	451	668	621	709	619	560	392	240	152	149
	3	156	220	330	473	561	645	500	430	284	166	105	109
	4	222	321	484	679	775	879	679	582	387	224	141	149
	5	93	319	539	598	646	952	34	32	19	31	20	17
	6	124	415	702	493	478	976	55	38	21	19	51	28
	7	172	315	468	590	449	653	596	431	395	176	89	126
	8	185	336	504	627	476	679	614	445	404	180	91	129
	9	67	161	294	400	349	552	498	376	265	115	51	82
	10	91	230	415	570	498	767	669	498	347	151	67	107

	GPP uncorr.	GPP measured (1)	GPP corr. (2)	Diff. (2)-(1)	F_N	soil C:N	soil N
Plot 1	9114	5650	5650.0	0.0	0.73	18.6	0.23
2	9065	5140	5140.0	0.0	0.67	21.9	0.23
3	9556	3980	3980.0	0.0	0.48	24.6	0.25
4	10147	5520	5520.0	0.0	0.62	24.5	0.23
5	7940	3300	3300.0	0.0	0.92	13.9	0.29
6	8159	3400	3400.0	0.0	1.17	14.6	0.28
7	7245	4460	4460.0	0.0	0.85	22.3	0.31
8	7428	4670	4670.0	0.0	0.87	19.3	0.34
9	6201	3210	3210.0	0.0	0.81	31.3	0.85
10	6753	4410	4410.0	0.0	0.99	30.5	0.80