Lincoln University
Te Whare Wānaka o Aoraki
CHRISTCHURCH•NEW ZEALAND

Lincoln University Digital Thesis

Copyright Statement

The digital copy of this thesis is protected by the Copyright Act 1994 (New Zealand).

This thesis may be consulted by you, provided you comply with the provisions of the Act and the following conditions of use:

- you will use the copy only for the purposes of research or private study
- you will recognise the author's right to be identified as the author of the thesis and due acknowledgement will be made to the author where appropriate
- you will obtain the author's permission before publishing any material from the thesis.

The Interaction of New Zealand Native Plants with Nitrogen in Canterbury's Agricultural Landscapes

---

A thesis
submitted in partial fulfilment
of the requirements for the Degree of
Doctor of Philosophy

at
Lincoln University
by
Hannah Mayford Franklin

Lincoln University
2014

Abstract of a thesis submitted in partial fulfilment of the requirements for the Degree of Doctor of Philosophy.

The Interaction of New Zealand Native Plants with Nitrogen in Canterbury's Agricultural Landscapes

by

Hannah Mayford Franklin

Less than 0.5 % native vegetation cover remains in the productive Canterbury Plains region of New Zealand. Incorporating native plants into agricultural landscapes could provide numerous benefits including shelter, supplementary stock fodder, production of essential oils or honey, wildlife-corridors, and protection of waterways. New Zealand's native species are adapted to environments where nitrogen (N) occurs at low concentrations. Such environments are in stark contrast to New Zealand's agricultural landscapes, where high inputs of fertilisers and animal effluents have elevated soil N. There is a lack of knowledge on how native species will interact with N in agricultural environments. Potentially, native species may alter nitrate leaching to receiving waters and emissions of nitrous oxide (N_2O), a potent greenhouse gas. This research aims to investigate the interaction with soil N of selected native species and their rhizospheres to gain an understanding of species-specific differences and potential effects on N fluxes.

The native species investigated were typical of those used in restoration projects. Perennial ryegrass (*Lolium perenne*), an introduced species that dominates New Zealand pasturelands, was used as a control. I studied rhizosphere soil and foliar N status at two planted restoration sites. Plant growth and uptake in response to agriculturally elevated N levels were investigated in greenhouse pot trials. A field experiment explored the effect of *Kunzea robusta* (kānuka) on N_2O fluxes from soil. Finally, farm-scale N uptake and reduction in N losses were modelled for various native planting scenarios.

At the restoration sites New Zealand native species *Austroderia richardii* (toetoe), *Phormium tenax* (flax), *Cordyline australis* (cabbage tree), *Coprosma robusta* (karamu), *K. robusta*, *Olearia paniculata* (akeake) and *Pittosporum tenuifolium* (black matipo) had similar foliar N concentrations to *L. perenne*. While native species with winter leaf loss, *Plagianthus regius* (ribbonwood) and *Sophora*

microphylla (kōwhai), had higher foliar N than these other species. There was significant inter-species variation in rhizosphere soil mineral N concentrations among native species, with *A. richardii* and *P. regius* having higher nitrate status than *L. perenne*. Pot trials revealed that while native species tolerate high N-loading (up to 1600 kg ha^{-1}), there was negligible growth response. Increased soil N concentrations resulted in increased foliar N in native plants, of which the high-biomass-producing monocotyledons assimilated the most. Nevertheless, foliar N concentrations were higher for *L. perenne* receiving N and farm-scale calculations showed *L. perenne* to extract more soil N than the native species. *K. robusta* reduced N_2O emissions following effluent application by 80 % relative to control soil, which emitted significant amounts.

Modelling revealed that incorporating native species into agricultural landscapes reduced the N loading per hectare due to the reduced area of fertilised and grazed soil. The native monocotyledons, in particular *P. tenax* and *Carex virgata* (pukio), have greater potential to reduce nitrate leaching than the woody species and are the most suitable for receiving effluent, whereas *K. robusta* in farm paddocks may mitigate N_2O emissions following urine deposition by sheltering stock. Further work could involve lysimetry to quantify simultaneously the effects of native species on nitrate leaching and N_2O emissions. These findings provide a first step towards targeted native planting strategies in sustainable agricultural management.

Keywords: New Zealand native plant species, rhizosphere, vegetation loss, nitrogen, nitrate leaching, nitrous oxide, agriculture, *Lolium perenne,* nutrient uptake, dairy farming

Acknowledgements

Firstly, I would like to express my sincere thanks to my supervisors Professor Nicholas Dickinson and Associate Professor Brett Robinson for their continual support, encouragement and guidance thought my PhD research. Thank you for all your reading and helpful comments during the writing process. You were both a big part of my decision to begin my PhD and it has been my pleasure to work with you both.

The technicians in the Soil and Physical Sciences Department at Lincoln University have provided outstanding service in their assistance with laboratory equipment and the timely analysis of samples. I would particularly like to thank Roger Cresswell, Roger McLenaghen, Qian Liang, Manjula Premaratne, Vicky Zhang, Lynne Clucas, Roger Atkinson, Leanne Hassall, Roger Atkinson and Trevor Hendry.

I am also indebted to the staff at the Lincoln University Nursery Greenhouse Complex, Brent Richards and Leona Meachen, without whom extensive glasshouse trials would not have been possible.

Thank you also to Amal Torky, Emma Gambles, Chrissy Gibson, Gail Harkers and Robyn Wilson for administrative assistance and making my time at Lincoln University so easy.

I am grateful for the advice of Professor Leo Condron at the outset of this project. I would like to thank Carol Smith for her assistance with soil profile descriptions, as well as Jim Moir, Nimlesh Balaine and Professor Timothy Clough for their advice when setting up experiments.

It was a pleasure to work with Cyril Enault from Agrocampus-Ouest in France during his internship placement at Lincoln University and Roshean Woods during her time as an honours student. I greatly appreciate your contributions to Chapter 5 and 6 respectively, as well as your company and enthusiasm during experimental work.

I also wish to acknowledge staff and students within the department who have willingly assisted me with field, glasshouse and laboratory work and whose company has made my thesis time so enjoyable. A special thanks to Youngnam Kim, Tao Zhong, Paula Greer, Juergen Esperschuetz and Obed Nedjo Lense for working long hours in the greenhouse helping me harvest experimental plants and for so much digging in the field. I am also grateful for the help of Jason Hahner, Quam-are Kim, Dharini Paramashivam and Rebecca Dollery. Without you, the PhD would have been impossible.

I was lucky to receive many scholarships during my PhD. Thank you to the Lincoln University Doctoral Scholarship 2012, Gordon Williams Postgraduate Scholarship in Ecology 2012 and William Gao Postgraduate Scholarship for Excellence 2014 for stipend assistance. In addition, the New Zealand Federation of Graduate Women Canterbury Branch Conference Presentation Travel Award 2013 and the Claude McCarthy Fellowship for assistance with conference travel. I am thankful to have received research funding from Lincoln University and generous contributions from Pioneer Brand Products (Genetic Technologies Ltd) and South Island Dairy Event.

To my most amazing proof-readers, Sarah Dawson and Anna Henderson, yet again I cannot express how grateful I am for your help and for finding all the errors that I never see. A final thanks is needed to my family, partner, flatmates and friends for their support, encouragement and keeping me sane during the past three years.

New Zealand native species in a planted area at the Lincoln University Dairy Farm.

Table of Contents

List of Tables

List of Figures

List of Plates

List of plant species and glossary of abbreviations

List of plant species investigated (Chapter 3 provides further information)

Latin species name	Abbreviated	Common Māori and English names
Austroderia richardii	*A. richardii*	toetoe
Carex virgata	*C. virgata*	pukio, swamp sedge
Coprosma robusta	*C. robusta*	karamu
Cordyline australis	*C. australis*	tī kōuka, cabbage tree
Kunzea ericoides [1]	*K. ericoides*	kānuka, white tea tree
Kunzea robusta [1]	*K. robusta*	kānuka, white tea tree
Leptospermum scoparium	*L. scoparium*	mānuka, red tea tree
Lolium perenne [2]	*L. perenne*	perennial ryegrass
Olearia paniculata	*O. paniculata*	akiraho, golden akeake
Phormium tenax	*P. tenax*	harakeke, New Zealand flax,
Pittosporum tenuifolium	*P. tenuifolium*	kōhūhū, black matipo
Plagianthus regius	*P. regius*	manatu, lowland ribbonwood
Sophora microphylla	*S. microphylla*	kowhai, small-leaved kowhai

[1] Recent revisions of the *Kunzea* genus have distributed those previously described as *Kunzea ericoides* into several species (most literature records use this name). *Kunzea robusta*, the most widespread member of the genus in New Zealand, is the species studied in this thesis.

[2] *Lolium perenne*, a common pasture grass species, is not native to New Zealand but is included in this thesis an experimental reference.

List of abbreviations commonly used

Term	Abbreviation
Nitrogen	N
Nitrate	NO_3^-
Ammonium	NH_4^+
Nitrous oxide	N_2O
Dairy shed effluent	DSE
Phosphorus	P
Carbon	C

Chapter 1

Introduction

1.1 General Introduction

As a consequence of more than 80 million years and at least 2,000 km of evolutionary and geographic isolation, more than 80% of New Zealand's native biota is endemic and the country is recognised as a world biodiversity hotspot (Mittermeier et al. 1999). Despite relatively recent human colonisation in a global context, by both Polynesians (from c. 800 years ago) and Europeans (c. 200 years ago), deforestation has been extensive in New Zealand (McGlone and Wilmshurst 1999). Presently, roughly 20 % of the land area has native forest cover, compared to an original estimate of 75 % (Atkinson and Cameron 1993). In lowland productive agricultural regions, native vegetation loss has been particularly prevalent. Despite increased environmental awareness, loss of indigenous cover has continued (Walker et al. 2006). For example less than 0.5 % indigenous cover remains in the Canterbury Plains region (Thompson et al. 2003), which has been described as depauperate of native vegetation (Meurk 2008).

Approximately one third of New Zealand's land area consists of grazed pastures, with dairy products the country's biggest export commodity (Ministry for the Environment 2007). Virtually all plants used in pastoral, horticultural and silvicultural land use systems in New Zealand are introduced species (Meurk and Swaffield 2000). Ryegrass-clover mixtures dominate pasture for grazing mammals, and plantation forests consist of northern hemisphere conifers. In the Canterbury Plains region, the predominant land cover is perennial ryegrass (*Lolium perenne*) (Charlton and Stewart 1999). Up until the last decade a vast majority of the plants used in parks, gardens, hedgerows and shelterbelts were also introduced species (Meurk and Swaffield 2000).

A growing awareness of the poor health of New Zealand's water bodies and interest in increasing biodiversity in agricultural landscapes during the past two decades has seen New Zealand plants replace exotic trees and shrubs on farms, adjacent to waterways, on set-aside areas and marginal land (Meurk and Swaffield 2000, Meurk and Hall 2006). In the Canterbury region, large scale conversion to dairy farming has necessitated the removal of tall exotic trees (such as *Salix* and *Populus* spp.), to allow installation of centre-pivot irrigators (Meurk 2008). This has provided an opportunity to re-introduce lower stature native plants to the landscape, providing improved habitats for native fauna (Meurk and Swaffield 2000). The replacement of exotic trees on riparian margins with native plants is encouraged nowadays to enhance habitat quality for native freshwater species (Environment

Canterbury 2011). Native species offer a range of ecosystem services (reviewed in Section 2.6) of value to agriculture such as increased crop pollination and pest control, as well as improving the public perception of the agricultural sector and cultural identity of these landscapes (Sandhu et al. 2008).

Extensive use of nitrogen (N) fertilisers and land application of recycled dairy shed effluent (DSE) has been necessary to maintain high productivity of introduced agricultural plants that are adapted to more fertile soils (Di and Cameron 2002b). The amount of N applied to land is increasing, in line with a trend towards more intensive farming, particularly dairy farming (Ministry for the Environment 2007). This has elevated soil N loading above historical levels (Schipper et al. 2007). Leaching of nitrate (NO_3^-), the most mobile form of N from agricultural soils is of concern due to the potential adverse impacts on water quality and human health. New Zealand Ministry of Health (2008) guidelines recommend that NO_3^--N concentrations in drinking water should be below 11.3 mg L^{-1} to be suitable for human consumption. However, this limit is frequently exceeded in Canterbury, particularly in shallow groundwater wells (Ministry for the Environment 2009). Elevated N concentrations in surface water can also cause eutrophication, which can endanger aquatic health (Carpenter et al. 1998). Nitrogen losses from soil also occur as nitrous oxide (N_2O), a potent greenhouse gas produced largely by soil biological denitrification (de Klein et al. 2001). Furthermore, loss of soil N represents a significant economic loss for the farmer. In light of recent media attention on declining water quality in New Zealand, it is in the interest of the country's agriculture and tourism based economy, to protect the "100 % Pure New Zealand" branding of the country by ensuring steps are taken to mitigate the adverse effects of N losses from agricultural soils.

The intensification of agricultural land use in New Zealand has led to further loss of native vegetation cover. Vegetation disturbance in terrestrial systems alters nutrient uptake by plants and can change the biochemistry of N cycling (Jobbágy and Jackson 2004), potentially amplifying N transport to water bodies (Reeves et al. 2004). Native vegetation loss, particularly in riparian zones, has exacerbated the impacts of agricultural N on New Zealand's water quality (Davies-Colley and Wilcock 2004). Plants can influence N fluxes in rhizosphere (root-zone) soil through nutrient uptake, litter fall, rhizodeposition (changes to soil physico-chemistry) and interactions with soil microorganisms (Pinton et al. 2007, Bardgett and Wardle 2010). Like many temperate forest systems, natural forest in New Zealand is thought to be N limited (Vitousek and Howarth 1991).

The present work explores whether NO_3^- mobility varies in soils beneath native species. Differences in plant root morphology and physiology, as well as variable nutrient requirements, create inter-species differences in rhizosphere effects on nutrient cycling (Richardson et al. 2009). The quantity and quality of resources returned to soil (via rhizodeposition and litter fall) by different plant

species can affect rhizosphere soil physico-chemistry and biota (Bardgett and Wardle 2010). Research shows that New Zealand native plants have variable rooting profiles which are deeper and more extensive than those of pasture grass species (Marden et al. 2005), potentially interacting with soil N in different ways. Variation in canopy cover between New Zealand native species may influence rhizosphere soil N conditions, through differential rates of rain through fall and subsequent NO_3^- leaching. However, investigations of soil N associated with particular native species are rare (Davis 2014). Available data report low concentrations of NO_3^--N in rhizosphere soil water of mature native forest (Davis 1990) and shrubland (Ross et al. 2009). This is supported by typically low total N concentrations in stream water draining catchments dominated by native forests compared with other vegetation types (Davis 2014). In addition, estimated N losses as gaseous N_2O emissions are low from native forest and shrubland in New Zealand (Saggar et al. 2008) and reflect low concentrations of available N in the rhizosphere soil. The recent study of Hahner et al. (2014) constitutes the only research regarding native plants in which species-specific interactions with soil N have been examined comparatively. They found NO_3^--N concentrations in rhizosphere soil water varied between native species planted in a re-vegetated area retired from dairy farming (Hahner et al. 2014).

There is a lack of consensus over the growth response of native plants to high N levels. Naturally fertile soils are of limited extent in New Zealand (Molloy 1998, Leathwick et al. 2003). The primary rocks are low in essential plant nutrients (McLaren and Cameron 1996) and many soils have been strongly leached or weathered (Molloy 1998, Wardle 2002). Many native species are potentially adapted to these low fertility soils. Examples are *Leptospermum scoparium* and *Kunzea* spp. which rapidly colonise infertile sites (Porteous 1993, Wardle 2002, Stephens et al. 2005) and a range of native grass species which have traits associated with low N availability (Craine and Lee 2003). It has been suggested that native species are poorly adapted to being productive at higher nutrient supply, typical of agricultural soils (Craine and Lee 2003); the present work investigates this hypothesis. Limited research has been conducted into the nutrient requirements, limits and growth of native plants. Of the few published studies, some suggest that fertilising soil produces negligible increases in the growth and survival of native seedlings (Ogle 1996, Pratt 1999, Douglas et al. 2007). While in others, the growth of native species was improved through fertiliser use (Hawkins and Sweet 1989, Langer et al. 1999).

In addition to investigating the growth response to N, the present study seeks to understand whether New Zealand native species accumulate N from high fertility soils into their plant tissues. Slow-growing plants that are adapted to low fertility typically have long-lived leaves with lower N concentrations than those adapted to fertile sites (Aerts and Chapin 2000). Native species potentially

have lower foliar N concentrations than introduced plants which are adapted to more fertile soil (Craine and Lee 2003), and foliar N concentrations are known to vary between native species in unfertilised conditions (Lambert et al. 1989c, Wardle 2002, Bellingham et al. 2013). Attempts have been made to estimate total N uptake into plant biomass, finding greater N storage in native podocarp-broadleaf forest than in *Chionochloa* spp. grasslands, due to differences in biomass production (Wardle 2002). Within-species variation in foliar N uptake in response to soil fertility has been reported for native forest species (Adams 1976, Wardle 2002), yet is unknown for native species typically used in agricultural plantings or for the high soil N concentrations likely experienced in these situations.

The present work also investigates the effect of a native shrub on N_2O emissions from rhizosphere soil, *Kunzea robusta* was a focus species for this experiment. Species-specific plant compounds have the potential to inhibit soil bacteria and in turn alter soil N cycling. The essential oils of *L. scoparium* and *Kunzea* spp. contain antibacterial compounds (Lis-Balchin et al. 1995, Porter and Wilkins 1999, Maddocks-Jennings et al. 2005) similar to those known to be responsible for low nitrification rates in soils internationally under several exotic species (Ward et al. 1997, Haile et al. 2006). Antibacterial agents may transfer to soil through rhizodeposition from roots or leaf fall followed by degradation (Prosser et al. 2014). Recent studies have investigated the potential for *L. scoparium* and *Kunzea* spp. to alter the soil N cycle through inputs of specific compounds (Ross et al. 2009, Prosser et al. 2014). In laboratory experiments, water extracts from *L. scoparium* roots and leaves inhibited the growth of pathogens found in biosolids (Prosser et al. 2014) and low rates of N_2O production from *L. scoparium-Kunzea* scrubland have been observed in the field (Ross et al. 2009, Price et al. 2010, Hedley et al. 2013). However, previous studies neither provide a direct comparison between rhizosphere and bulk soil, nor investigate the response to N loading typical of agricultural soils.

The findings of this research will be used to elucidate whether there is sufficient evidence to implement native plants as an integral component of N management in intensive agricultural landscapes, in particular dairy farming systems.

1.2 Aim and Objectives

This work aims to investigate the interaction with soil N of selected native species and their rhizospheres to gain an understanding of species-specific differences and potential effects on N fluxes.

The research programme had the following objectives:

Objective 1: To determine the variation in foliar N and rhizosphere soil N of New Zealand native species, as distinct from *L. perenne* (Chapter 4).

Objective 2: To quantify the growth and uptake response of selected native species and *Lolium perenne* to high levels of added N in controlled conditions (Chapter 5).

Objective 3: To evaluate the potential of New Zealand native species *Kunzea robusta* (kānuka) to alter the soil N cycle and reduce N_2O emissions following N loading from DSE (Chapter 6).

Objective 4: To calculate potential farm-scale N uptake possible through planting native species and model losses for various native planting scenarios, drawing on the findings of Objectives 1-3 (Chapter 7).

These objectives were pursued through a combination of field and greenhouse studies. Native species selected for investigation were those occurring naturally in riparian or other marginal lowland environments (Marden et al. 2005), as commonly used in agricultural borders and restoration planting in the Canterbury region.

1.3 Chapter descriptions

Chapter 2: Background

This chapter provides a detailed review of literature relating to the N cycle in the soil-plant environment, the pathways through which N is lost from soils, factors affecting these losses and the resulting adverse effects. The various ways in which plants interact with soil N are discussed, as well as species-specific effects. Finally, the ecosystem services context of the work is reviewed with respect to New Zealand native plants.

Chapter 3: Species selection, description

This chapter firstly provides a description of the choice of species for this research, including details of each species' morphology (above- and below-ground) and ecology (Section A). Secondly, an overview of literature knowledge of variation in native plant root systems is presented, in addition to information gathered during a preliminary field investigation of native rhizosphere soil profiles at the principal field research site (Section B).

Chapter 4: Rhizosphere and foliar nitrogen status of New Zealand native plants

This chapter addresses Objective 1. Foliar N content and soil total N and N speciation in the rhizosphere of New Zealand native species and *L. perenne*, were studied at two planted restoration sites established on soil of contrasting type and N status.

Chapter 5: Response of New Zealand native plants to agriculturally elevated levels of soil nitrogen

This chapter addresses Objective 2. The growth and uptake response of New Zealand native species to elevated N levels was investigated in two greenhouse pot trials. A low-fertility silt loam soil was used as a growth medium. The first trial studied the response of five native species, *L. perenne* and unvegetated soil to a range of N levels (200-1600 kg N ha^{-1}). The second trial investigated whether increasing the pH and phosphorus content of the silt loam soil would modify the response to added N.

Chapter 6: Suppression of nitrous oxide production by *Kunzea robusta* (kānuka)

To address Objective 3, *K. robusta* was selected as a study species due to its known antimicrobial properties. A field experiment was conducted to assess if this species was capable of modifying the soil N cycle and reducing N_2O emissions following the application of DSE to the soil surface.

Chapter 7: Upscaling and practical application of the findings

This chapter addresses Objective 4, by upsacling the plant growth and uptake response (Chapter 5) to calculate potential farm-scale N uptake possible through planting selected native species and *L. perenne*. Nitrogen losses under various on-farm planting scenarios are discussed in light of the findings of Chapters 4-6.

Chapter 8: Conclusions

The main conclusions of this research are synthesised in this chapter with respect to the four objectives of the study. A summary of the applications of the research and recommendations for future study are presented, prior to a closing statement.

Appendices

Appendix A (Soil profile descriptions) provides the full soil profile descriptions discussed in Chapter 3, while Appendix B (Supplementary information to Chapter 5) provides additional data to accompany Chapter 5.

1.4 General layout

The scientific and common names of plant species investigated in this thesis are listed preceding Chapter 1 (page xiii). Species are referred to by their full scientific name on first mention in each chapter and in abbreviated form thereafter. Abbreviations for common terms are introduced in brackets following their first mention in each chapter and a list of abbreviated terms is also provided (page xii). The three empirical chapters of this thesis (Chapters 4–6) are written with the intention that they will be published (likely in a reduced form) as stand-alone manuscripts. Due to this, there is some repetition between these chapters. However, every attempt has been made to keep this to a minimum.

Chapter 2

Background

2.1 Introduction

Vegetation clearance and conversion of around one third of New Zealand's land area to grazed pastures has involved application of nitrogen (N) in the form of fertilisers and dairy shed effluent (DSE). This has led to elevated soil N stocks as well as N losses from agricultural landscapes. Problems with N leaching losses from agricultural land are widespread (Di and Cameron 2002b, Larned et al. 2004) and can lead to surface and ground water contamination, water body eutrophication and threats to aquatic and human health (Carpenter et al. 1998). Additional N losses from soil can occur as nitrous oxide (N_2O), a potent greenhouse gas, produced by largely by biological denitrification (de Klein et al. 2001). This is a greenhouse gas of particular concern in New Zealand. Loss of mature vegetation communities in terrestrial systems may have exacerbated the impacts of agricultural N by reducing uptake by plants and changing the flux of N in soil (Jobbágy and Jackson 2004).

This literature review describes the relevant components of the N cycle, the pathways through which N is lost from the soil-plant system, factors affecting these losses and the resulting adverse effects. The various ways in which plants interact with soil N, and species-specific effects are discussed, followed by an overview of ecosystem services in the context of New Zealand native plants.

2.2 The nitrogen cycle

2.2.1 Nitrogen distribution and sources in agricultural soils

The lithosphere (crust and upper mantle) contains as much as 98 % of the earth's total N, in igneous rocks and minerals (Haynes et al. 1986). The remaining N is found predominately in gaseous form in the atmosphere, with a smaller amount of N dissolved within the hydrosphere (earth-water and atmospheric moisture) (Haynes et al. 1986). Soils typically contain 0.1 to 0.5 % of the earth's total N in the top 0.15 m (McLaren and Cameron 1996). Soil N is present in three main forms (Figure 2.1): (i) mineral N in soil solution (ammonium (NH_4^+), nitrate(NO_3^-) and nitrite (NO_2^-)), (ii) NH_4^+ ions held by clay minerals, and (iii) organic compounds in plant tissue, soil organisms, detritus and soil humus (McLaren and Cameron 1996). The vast majority of soil N (over 94 %) is in the form of organic compounds and therefore is unavailable to plants. Organic forms of N become available to plants

when released by soil processes (decomposition and mineralisation), and thus, plants rely on continuous transformation of organic N to mineral N for survival.

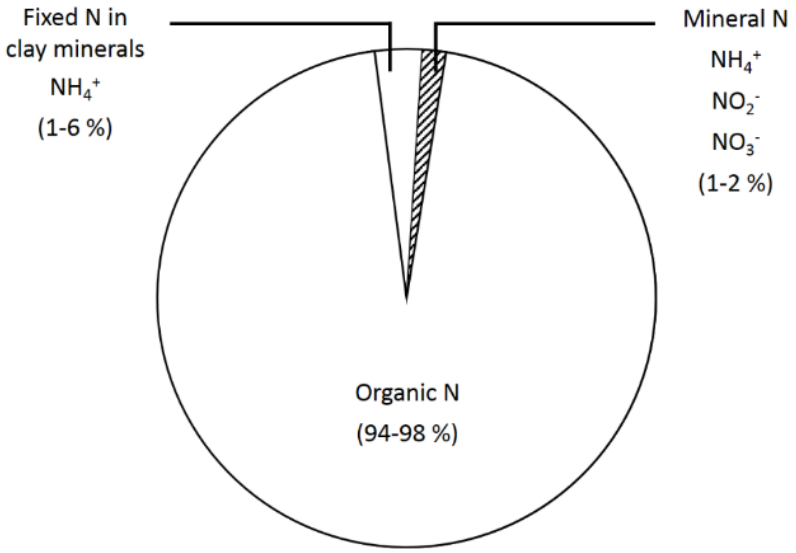


Figure 2.1 The proportion of total soil N that is present as fixed N in clay minerals, mineral N and organic N (adapted from McLaren and Cameron 1996).

Human activities have doubled the rate of N entering the land-based N cycle (Vitousek et al. 1997). The use of N fertilisers has increased agricultural production worldwide over the last few decades. Naturally fertile soils are of limited extent in New Zealand (Molloy 1998, Leathwick et al. 2003). The primary rocks are low in essential plant nutrients (McLaren and Cameron 1996) and many have been strongly leached or weathered (Molloy 1998, Wardle 2002). Extensive use of N and phosphorus fertilisers has been necessary to maintain high productivity of introduced agricultural plant species, which are typically adapted to soils that are more fertile. The amount of N fertiliser used in New Zealand has increased by about 10 times since 1985 (Ministry for the Environment 2007). In New Zealand, livestock manures and effluents are also recycled and applied to land (Longhurst et al. 2000), contributing about 5 times as much N to land as fertilisers (Ministry for the Environment 2007). The amount of livestock manure applied to land has steadily increased, in line with the recent trend towards more intensive farming, particularly dairy farming. Grazing animals also add substantial amounts of N to soils, through dung and urine (Haynes and Williams 1993). Following urine deposition, the N loading under a urine patch can be equivalent to 1000 kg N ha^{-1} (Di and Cameron 2012). Additional N input occurs through biological fixation in legume based pasture or leguminous crops. Nitrogen fixing clover is incorporated into many pastures in New Zealand (Ledgard et al. 2001).

Compared with other countries N deposited through rainwater is low in New Zealand (Parfitt et al. 2006).

2.2.2 Transformations of the soil-plant nitrogen cycle

The N cycle represents important processes in the soil, plant and atmospheric system, whereby N is transformed from one form to another by a variety of processes (Figure 2.2). Nitrogen cycling in soil involves five microbial processes: N fixation, mineralisation (decay), nitrification, immobilisation and denitrification (McLaren and Cameron 1996).

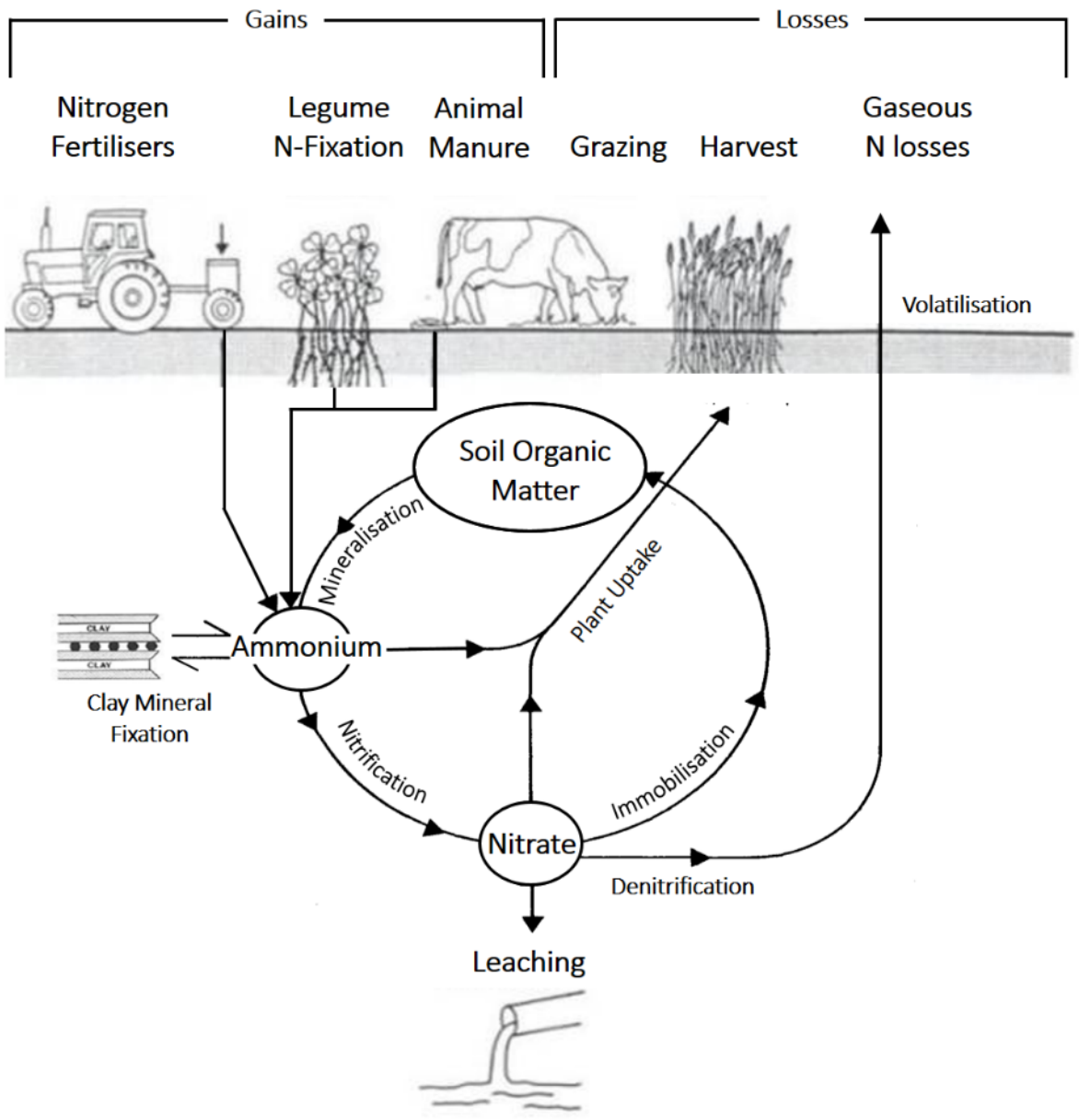


Figure 2.2 The soil-plant N cycle (adapted from McLaren and Cameron 1996).

In the case of *N fixation*, atmospheric nitrogen (N_2) is reduced to NH_4^+ by the activity of prokaryotes utilising nitrogenase, which is then available for plant and microbial growth (McLaren and Cameron 1996). Free-living organisms, (such as *Azotobacter* spp.) and those that live mutually with plants (such as *Rhizobium*) are responsible for fixing N.

Mineralisation involves the conversion of organic forms of N (present in soil or added in animal manures) into plant-available, inorganic or mineral forms, through the activity of a range of soil microorganisms (McLaren and Cameron 1996). During mineralisation, first heterotrophic microorganisms hydrolyse large organic N compounds (proteins) into simple N compounds (amines and amino acids). Then, through the process of ammonification, microorganisms convert amines and amino acids into NH_4^+ (Equation 2.1). This process is also known as urea hydrolysis and involves the urease enzyme in soil. Ammonium formed through ammonification (or added through ammonium fertilisers) is then available for plant uptake, nitrification, immobilisation, ammonium fixation or loss through volatilisation to ammonia (NH_3).

Equation 2.1 $$CO(NH_2)_2 \rightarrow 2NH_4^+ + 2OH^- + CO_2$$

The biological conversion of NH_4^+ to NO_3^- is known as *nitrification* and is carried out by autotrophic bacteria and archaea in aerobic conditions (DeLuca et al. 2009). The nitrification reaction is a two-step process in which the NH_4^+ ions are first oxidised into nitrite (NO_2^-) (Equation 2.1) and then to NO_3^- (Equation 2.3) (McLaren and Cameron 1996). Nitrite is toxic to plants, but since the rate of conversion of NO_2^- to NO_3^- is faster than the conversion of NH_4^+ to NO_2^-, it is unlikely to accumulate in soil. The nitrification process produces hydrogen ions (H^+), decreasing soil pH. Ammonification produces hydroxide ions (OH^-), although more H^+ ions are produced per unit of N, and urine and urea fertilizer ultimately acidify the soil. In low oxygen concentrations nitric oxide (NO) and N_2O can be formed as by-products of NH_4^+ oxidation (Kim and Hollocher 1983).

Equation 2.2 $$2NH_4^+ + 3O_2 \rightarrow 2NO_2^- + 2H_2O + 4H^+ + \text{energy}$$

Equation 2.3 $$2NO_2^- + 3O_2 \rightarrow 2NO_3^- + \text{energy}$$

In contrast to mineralisation is the process of *immobilisation*, the microbial process in which inorganic N forms are incorporated back into organic forms within the soil (McLaren and Cameron 1996). The addition of carbon (C) rich substances (C:N ratio above 30) to soil promotes immobilisation and reduces N availability to plants. Immobilisation and mineralisation are about equal when plant material containing less C is added (C:N ratio of 20–30). Mineralisation exceeds immobilisation when the C:N ratio of the decomposing materials is less than 20.

Gaseous loss of N from the soil N cycle results from *denitrification*, the process by which NO_3^- or NO_2^- is reduced to dinitrogen gas (N_2), of which both nitric oxide (NO) and N_2O are obligate intermediates (Equation 2.4) (McLaren and Cameron 1996). This occurs in the absence of oxygen and is carried out by heterotrophic facultative anaerobes (which can use both O_2 and NO_2^- or NO_3^- as electron acceptor). The four enzymes involved are: nitrate reductase, nitrite reductase, nitric oxide reductase and nitrous oxide reductase (van Zwieten et al. 2009, Uchida 2010).

Equation 2.4 $$NO_3^- \rightarrow NO_2^- \rightarrow NO \rightarrow N_2O \rightarrow N_2$$

Several chemical processes additionally form part of the soil N cycle. Ammonium fixation is when NH_4^+ ions are absorbed on inorganic and organic soil particles by cation exchange reactions and fixed in the interlayers of clay minerals (McLaren and Cameron 1996). In contrast, NO_3^- leaching occurs as NO_3^- is very weakly absorbed onto soil particles and moves with soil water (McLaren and Cameron 1996). Large amounts of N are lost from the soil N cycle as NO_3^- leaching into ground and surface waters. Finally, ammonium volatilisation is the dissociation of NH_4^+ ions into gaseous NH_3 released from the soil, this occurs when the soil pH is high (>7.5) (Bolan et al. 2004).

2.3 Factors affecting nitrogen losses from soils

As land use intensity increases, N inputs to the soil system increase in order to maintain high agricultural productivity (McLaren and Cameron 1996). Plants are often not able to take up all of the added N and a large amount is incorporated into soil organic matter, leached into ground and surface waters, or lost to the atmosphere as gases (Figure 2.2). These losses of N from the soil/plant system not only create adverse impacts on the environment but also effectively reduce soil fertility, plant yield and economic gains (Cameron et al. 2013) (reviewed in Section 2.4). A range of soil properties, climatic and management factors govern N losses from soils.

2.3.1 Nitrate leaching

Soil organic N is mineralised in significant amounts and is then available for uptake by plants (Di and Cameron 2002b). However, the concentration of NH_4^+ in soils is typically low, as it is easily converted to NO_3^-. The electrical charge on soils affects NO_3^- leaching, as soils with greater negative charge are more likely to repel the NO_3^- anions into soil solution. Most agricultural soils in the temperate region are negatively charged, therefore NO_3^- is the dominant type of N leached in New Zealand (Di and Cameron 2002b). As water drains through soil, leaching N losses occur. The amount of NO_3^- built up in soil compared to that required by plants and the rate and volume of drainage determine the amount leached. Thus, additionally to N inputs, NO_3^- leaching is affected by the rate of nitrification in soil, soil texture and structure, climate and management practices. Factors affecting NO_3^- leaching in temperate agricultural soils have been reviewed by Di and Cameron (2002b) and Cameron et al. (2013).

The rate of nitrification

Conditions that promote nitrification increase the amount of NO_3^- available for leaching. As nitrification is carried out by a specific group of autotrophic bacteria, its rate is sensitive to changes in soil conditions (McLaren and Cameron 1996). The optimum pH for nitrifying bacteria is thought to be 4.5 to 7.5 (Haynes et al. 1986). In acidic soils, nutrient deficiencies and/or Al toxicity may inhibit nitrification, while in conditions greater than pH 7.5 toxic levels of NH_3 may be present (Haynes et al. 1986). Nitrifying bacteria perform optimally at temperatures between 25 and 30 °C, with minimal nitrification occurring below 5 °C (Haynes et al. 1986). Nitrification rate is slow at high soil moisture potentials due to reduced oxygen availability (Haynes et al. 1986). While cultivation typically increases nitrification due to increased soil aeration (McLaren and Cameron 1996). The amount of NH_4^+ in soils controls nitrification activity, plants compete with nitrifying bacteria for NH_4^+ and can reduce its availability. Importantly, P deficiency can limit the rate of nitrification in older soils under climax vegetation (Haynes et al. 1986). Autotrophic nitrifying bacteria are also considered among the most sensitive groups of soil organisms to soil applied pesticides. Some chemicals have been specifically developed to affect them and reduce the rate of nitrification, termed nitrification inhibiters. Dicyandiamide (DCD), commercially developed as “Eco-N”, inhibits the growth and activity of ammonium-oxidising bacteria, slowing down the rate of nitrification and keeping the N in the NH_4^+ form which is adsorbed onto the soil exchange surfaces and is available for plant uptake (Amberger 1989, Di et al. 2009, Di et al. 2010a).

Soil properties

Nitrate is water soluble and therefore highly mobile in soil; NO_3^- leaching is primarily controlled by soil moisture and drainage rates. When soil moisture exceeds field capacity, soil macropores are unable to store water and drainage through the profile occurs. Soil properties can alter NO_3^- leaching by affecting rates of solute convection, diffusion and dispersion in soil (McLaren and Cameron 1996). Nitrate leaching losses are typically less from fine than coarse textured soils due to slower drainage rates, whereas coarse-textured soils generally have lower water holding capacity and reach field capacity quickly. Additionally, soil depth, profile layering (e.g. clay plan) and stone content all affect the ability of soil to store water and, in turn, the mobility of dissolved substance (McLaren and Cameron 1996). These factors combine with broader patterns in hydrological processes, determined by topography and climate, to control NO_3^- leaching across landscapes (Correll 1997). On flat land much leaching is vertical, while on hillsides or sloped riparian banks overland and lateral subsurface flow also occur (Correll 1997).

Soil organic matter increases the formation of stable soil aggregates (Bronick and Lal 2005). Soil with high aggregate stability has increased capacity to retain water, decreasing NO_3^- leaching. Intensive farming tends to reduce soil aggregate structure, through intensive irrigation, tillage and stock pugging (Williams and Petticrew 2009). Plants, through their addition of organic matter and root systems, microbes and soil macro-fauna, also contribute to the development of aggregates. Although the increased porosity of well-structured soil may make nutrients more mobile, overall a well-structured soil enhances water/nutrient uptake and microbial biodiversity, potentially improving the quality of leached water (Bronick and Lal 2005).

Soil macropores, formed by root channels, earthworm channels, fissures and voids, also affect NO_3^- leaching in soils (Silva et al. 2000). The size, continuity and impermeable linings of these channels often results in rapid flow of solutes through the soil profile (McLaren and Cameron 1996). In some soils, macropores can be continuous to great depth and the dominant mechanism of solute transport. However, in loamy and clay soils, macropores may be much less extensive, with flow routed to surface water via shallow drainage systems (Jarvis 2007). Undisturbed sites may have less rapid solute transport due to the increased organic matter content and less compaction, resulting in a finer soil structure. The rooting characteristics of crop species can influence macropore flow, for example those with a strong taproot can increase infiltration rates. Tillage practices, chemical and organic fertiliser use and drainage systems can also alter macropore flow (Jarvis 2007).

Climate

Climate and seasonal conditions have a strong effect on NO_3^- leaching losses. Rainfall, temperature and humidity impact on soil water content and movement of solutes in soil (McLaren and Cameron 1996). When rainfall exceeds evapotranspiration (autumn-winter), high amounts of drainage occur and NO_3^- leaching losses usually increase (Di and Cameron 2002b). A large proportion of mobile N is leached in wetter months than during the rest of the year (Di et al. 1999). Slope can affect the amount of rainfall transferred as overland flow, as well as the movement of water in subsurface layers. The connection of soil to groundwater is also important when considering solute movement as it impacts on soil hydraulics (Correll 2005). The hydraulic connectivity of the soil to ground and surface waters can vary significantly, even within a stream catchment area, changing the way that N moves through the soil (Dahm et al. 1998).

Management

The amount of N applied as fertilisers or livestock effluents affects the amount of NO_3^- leached, with N more prone to leaching when applied in excess of the requirements of the plants grown in the soil (Di and Cameron 2002b). Studies suggest that in order to meet drinking water standards in farm drainage water, urea fertiliser application rate to grazed ryegrass/clover pasture should not exceed 200 kg N ha^{-1} yr^{-1} (Di and Cameron 2000). The potential for NO_3^- leaching is higher in grazed than mown pasture (Silva et al. 1999), as between 60 and 90 % of N ingested by grazing animals is returned to the soil as urine and dung (Haynes and Williams 1993). Some urine N is lost through volatilization, but much is nitrified, resulting in high NO_3^--N concentrations (up to 120 mg L^{-1}) in drainage water under urine patches (Silva et al. 1999). Urine patches typically cover 20-30 % of the grazed paddock and can significantly raise total NO_3^- leaching losses, particularly if fertiliser is applied on top these areas (Di and Cameron 2002b). For cut pasture a higher urea application rate of 400 kg N ha^{-1} yr^{-1} is possible (Di and Cameron 2000). Splitting the annual application rates into a number of smaller applications (not exceeding 50 kg N ha^{-1}) to match the pasture N demand can lower NO_3^- leaching (Di et al. 1998a, Silva et al. 1999). Land application of most effluents and manures have lower short-term NO_3^- leaching losses than fertiliser due to their high organic N content (Di and Cameron 2002b). Following long-term effluent application, organic N may build up in soil. Increased microbial activities stimulated by the organic C and other effluent derived nutrients may increase mineralisation and NO_3^- losses (Zaman et al. 1999).

Land use systems clearly have a major impact on the amount of NO_3^- leaching, likely through differences in N application rate, form of N (fertiliser or effluent/manure), N returns to the soil, plant species grown and soil management practices. After reviewing the literature regarding NO_3^- leaching

in temperate ecosystems, Di and Cameron (2002b) concluded the potential for NO_3^- leaching in different land use systems would follow the order: forest < cut grassland < grazed pastures < arable cropping < vegetable cultivation. Post-harvest management in cropping systems also has a significant effect on NO_3^- leaching. Research in temperate cropping systems has shown that the use of cover crops after harvesting can reduce NO_3^- leaching compared to bare fallow. In New Zealand, leaching loss under a ryegrass cover crop was found to be 92% less than from bare fallow soil (McLenaghen et al. 1996).

2.3.2 Gaseous emissions - nitrous oxide

In addition to NO_3^- leaching, N is lost from the soil/plant system through NH_3 volatilisation and denitrification (as described in Section 2.2.2). Gaseous emissions of N from soil are undesirable as they represent a loss of soil fertility and a threat to the wider environment (Cameron et al. 2013). Factors affecting gaseous N losses from New Zealand soils have been reviewed by Bolan et al. (2004), Saggar et al. (2008) and Cameron et al. (2013).

Ammonium volatilisation is a chemical reaction that occurs under alkaline conditions when urine or urea-based fertilisers are deposited onto soil (Bolan et al. 2004). Losses of NH_3 can represent up to 46 % of N deposited in urine patches (Bolan et al. 2004) and 65 % of fertiliser applied N (Cameron et al. 2013). Most NH_3 that is volatilised is returned to the earth's surface causing acidification and eutrophicaton (Cameron et al. 2013). As NH_3 volatilisation occurs predominately at pH > 7.5, relatively little is expected from restoration planting sites compared with limed agricultural soils. Ammonia volatilisation is not reviewed in this thesis.

Denitrification of NO_3^- to gaseous N occurs in terrestrial and aquatic systems. Denitrification can be both detrimental and beneficial to the environment (Bolan et al. 2004). On one hand, N_2O is formed during denitrification, a greenhouse gas that contributes to global warming (van Zwieten et al. 2009). On the other hand, denitrification can be used as a means to remove N from soils or waterways, minimising NO_3^- contamination (Bolan et al. 2004). Conversion to gaseous N_2 may beneficially reduce both NO_3^- and N_2O losses from the soil. Due to the potent nature of N_2O, this review details factors regulating its production from soil.

The regulation of N_2O emissions is complex, and involves the rate of nitrification (Section 2.3.1) and denitrification, the ratio of the end products of denitrification and the diffusion of N_2O through the soil profile (de Klein et al. 2008). These are affected by soil aeration and moisture status, as well as factors that affect denitrification, such as mineral N (particularly NO_3^-), C availability, soil pH and temperature.

Soil aeration and moisture

Changes in soil aeration and moisture content influence the denitrification rate and the diffusion of N_2O through soil (de Klein et al. 2008). Low soil moisture (water-filled pore space (WFPS) < 40 %) limits soil biological activity (Saggar et al. 2009) and corresponding aerobic conditions decrease the activity of N_2O-reductase in denitrification (Bakken and Dorsch 2007), limiting N_2O emissions. Under increased moisture conditions (55 and 65 % WFPS) emissions increase as the soil environment becomes more favourable for bacterial activity, N_2O formation is through nitrification as the soil is still aerobic (Saggar et al. 2009). At higher moisture levels (WFPS between 60 and 95 %), emissions can rise further as the soil becomes anaerobic which favours denitrification (Saggar et al. 2009). Denitrification losses are greatest in late-autumn to early-spring in New Zealand (Cameron et al. 2013). Reduced soil aeration reduces the N_2O:N_2 ratio of denitrification (Bolan et al. 2004). This is likely to be because restricted N_2O diffusion through the soil profile increases the chance of N_2O reduction and its subsequent emission as N_2 (de Klein et al. 2008).

Nitrogen inputs

The addition of N as excreta or fertiliser increases concentrations of the NO_3^- for denitrification and subsequently N_2O emissions (de Klein et al. 2008). In grazed pastures, the majority of the N_2O originates from animal urine patches rather than from fertilisers (Cameron et al. 2013). The proportion of N input that is emitted as N_2O is referred to as the emission factor (IPCC 2007). Nitrification inhibitors, such as DCD can reduce N_2O emissions from urine patches by up to 70 % (reviewed by Luo et al. 2010). Animal excreta deposited during grazing can produce large N_2O emissions (0.1-4 % of applied N) (de Klein et al. 2001). Nitrate concentrations influence the N_2O:N_2 ratio of the denitrification products by inhibiting the reduction of N_2O to N_2. Dinitrogen is predominant at low NO_3^- concentrations and N_2O dominates at high NO_3^- concentrations (Bolan et al. 2004).

Carbon availability

The supply of readily available organic C in soil is critical in controlling the rate of denitrification (Bolan et al. 2004). Land management practices that increase the organic C content of soil that is available to soil denitrifiers (such as animal excreta deposited during grazing or land application of effluents) stimulates the denitrification process (de Klein et al. 2001, Luo et al. 2008, Bhandral et al. 2010). As C availability increases the N_2O:N_2 ratio decreases (Bolan et al. 2004).

Soil pH

Soil pH affects both the rate of nitrification and denitrification as well as the ratio of N_2O:N_2 produced by denitrification (Bolan et al. 2004). Neutral pH conditions (pH 6-8) are optimal for denitrifying bacteria, although denitrification can occur across a broad pH range. In acidic conditions,

denitrification tends to slow down, but can still occur at pH values as low as 3.5. The mechanism by which pH affects denitrification is unclear. The proportion of N_2O emitted increases as pH decreases and N_2O is often the dominant product of denitrification in acid soils. This is potentially due to increasing amounts of NO_2^- at lower pH levels (Bolan et al. 2004).

Soil temperature

The denitrification rate increases with increasing soil temperature, up to 30 ^oC (Sherlock 1992, Bolan et al. 2004). In New Zealand, a relatively high denitrification rate is often observed during winter despite low soil temperatures (<10 ^oC), this is associated with high soil moisture contents (Luo et al. 2000b). Under field conditions, diurnal patterns of N_2O release closely follow fluctuations in soil temperature (Sherlock 1992).

2.4 Adverse effects of soil nitrogen losses

2.4.1 Eutrophication

Increased nutrient inputs can have a profound effect on the quality freshwater rivers and lakes and coastal waters (Carpenter et al. 1998, Smith and Schindler 2009). An increase of N and P supplies to aquatic ecosystems commonly results in an increase in the abundance of algae and aquatic plants, leading to eutrophication (Smith 2003). Thus, N leaching from agricultural soils can initiate or exacerbate the eutrophication of water bodies. In addition to the nuisance growth of aquatic algae and plants, nutrient inputs to freshwater and coastal ecosystems cause a variety of undesirable impacts (Table 2.1). The breakdown of large amounts of plant and algal residue leads to low dissolved oxygen concentrations in water which can have adverse effects on fish and other aquatic life (Diaz 2001).

Nutrient inputs from agricultural land in New Zealand have been linked to surface water eutrophication (Vant 2001, Hamill and McBride 2003, Larned et al. 2004, Monaghan et al. 2007). Median inorganic N concentrations in New Zealand's lowland pastoral streams exceeded guideline levels over a four year sampling period (1998-2002) (Larned et al. 2004). Nitrate concentrations in monitored rivers have risen over the past two decades and it is estimated that a third of lakes in New Zealand are likely to have high nutrient levels and poor water quality (Ministry for the Environment 2007). The microbial contamination and nuisance (sometimes toxic) algae associated with agricultural eutrophication are of concern for the contact recreational use of waterways (Muirhead and Monaghan 2011).

Table 2.1 Potential effects of eutrophication, caused by excessive inputs of N and P to lakes, reservoirs, rivers and coastal oceans (adapted from Smith and Schindler 2009).

Effects of eutrophication
• Increased biomass of phytoplankton and macrophyte vegetation
• Increased biomass of consumer species
• Shifts to bloom-forming algal species that might be toxic
• Increased biomass of benthic and epiphytic algae
• Changes in species composition of macrophyte vegetation
• Declines in coral reef health and loss of coral reef communities
• Increased incidence of fish kills
• Reductions in species diversity
• Reductions in harvestable fish and shellfish biomass
• Decreases in water transparency
• Taste, odour and drinking water treatment problems
• Oxygen depletion
• Decreases in perceived aesthetic value of the water body

The N-input possible before the onset of eutrophication depends on the physical, chemical and biological characteristics of the receiving waters (Smith and Schindler 2009). Phosphorous enrichment has an important contributing effect. With few exceptions, reductions in P inputs have reduced eutrophication; however the role of N appears more complicated (Smith and Schindler 2009).

2.4.2 Drinking water quality

Nitrate leaching into ground or surface waters used as drinking water supplies is a threat to human health. Nitrate build up in drinking water creates a risk of methaemoglobinaemia in bottle-fed babies (known as "blue-baby syndrome") and has been linked to cancer and heart disease (Grizzetti et al. 2011). Blue-baby syndrome is a potentially fatal condition that occurs when haemoglobin in an infant's red blood cells is oxidised to methemoglobin, which is unable to transport oxygen (Knobeloch et al. 2000). Suspected cases of blue-baby syndrome caused by high NO_3^- concentrations in drinking waters were first reported in the 1940's, but reported cases have been rare in recent years (Golden and Leifert 1999). The 214 cases of blue-baby syndrome recorded in the USA were associated with infants fed formula made up from well water with high NO_3^- concentrations (Avery 1999). However, it is recognised that NO_3^- may be one of a number of co-factors that play a sometimes complex role in causing the disease (Fewtrell 2004). High drinking water NO_3^- concentrations can also cause methaemoglobinaemia in livestock and induce abortions in cattle (Di and Cameron 2002b).

To protect human health, the World Health Organisation (WHO) (2007) and the New Zealand Ministry of Health (2008) have set guidelines recommending that NO_3^--N concentrations in drinking

water should be below 11.3 mg L^{-1} to be suitable for human consumption. Despite this, Grizzetti at al. (2011) found that 20 % of the European population live in areas where drinking water NO_3^- concentrations exceed this value. In New Zealand, 39 % of ground water monitoring sites had NO_3^- concentrations above background levels, likely due to human activities such as the application of fertiliser and livestock effluent (Ministry for the Environment 2007). The national median NO_3^- concentration at groundwater monitoring wells between 1995 and 2008 was 1.7 mg NO_3^--N L^{-1}, well below the drinking water guidelines (Figure 2.3). However, 5 % of monitoring sites had median NO_3^- levels exceeding guidelines (Ministry for the Environment 2009).

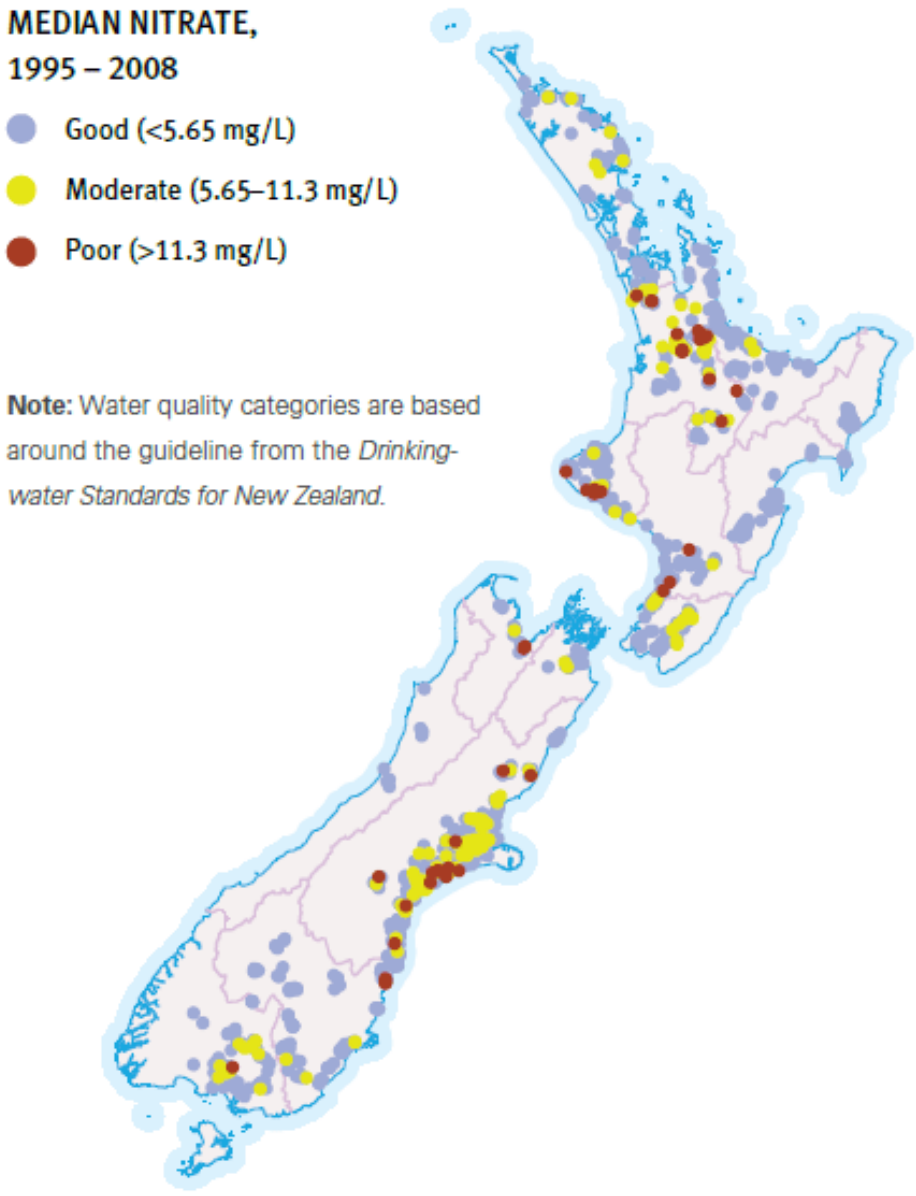


Figure 2.3 Median NO_3^--N levels for the period 1995-2008 for 914 groundwater-monitoring sites in New Zealand (Ministry for the Environment 2009).

2.4.3 Greenhouse gas emissions - nitrous oxide

Natural emissions of N_2O arise from nitrification and denitrification in soils (two thirds of total emissions) and in the ocean (one third of total emissions) (Nevison et al. 1995, Kroeze et al. 1999). Anthropogenic emissions of N_2O have increased since pre-industrial times and are environmentally important as N_2O is a potent greenhouse gas with a global warming potential 298 times that of carbon dioxide (van Zwieten et al. 2009). More than a third of N_2O emissions are anthropogenic and are primarily from to agriculture (IPCC 2007).

In New Zealand, agricultural greenhouse gas emissions play a major role in its national emission profile (contributing 47.1 % (Ministry for the Environment 2012). N_2O makes up approximately 7 % of New Zealand's greenhouse gas emissions (Ministry for the Environment 2012), contributed largely from ruminants grazing in pastoral ecosystems. There has been a 25 % increase in N_2O emissions from agricultural soils in New Zealand since 1990, following an increase in N fertiliser use and animal excreta (Ministry for the Environment 2012). N_2O emissions are the highest from dairy-grazed pastures (10–12 kg N_2O-N ha^{-1} yr^{-1}), intermediate from sheep-grazed pastures (4-6 kg N_2O-N ha^{-1} yr^{-1}), and lowest from forest, shrubland, and ungrazed pastoral soils (1-2 kg N_2O-N ha^{-1} yr^{-1}) (Saggar et al. 2008, Kirschbaum et al. 2012). In order to reduce future total greenhouse gas emissions in New Zealand there is a need to find ways to mitigate agricultural N_2O emissions (Clark et al. 2011).

2.4.4 Economic losses to farmers

In addition to adverse environmental effects, losses of N from agricultural soils reduce soil fertility and plant yields (Cameron et al. 2013). Agricultural systems rely on N inputs from fertiliser to sustain productivity; particularly in New Zealand where many of the introduced crops grown are adapted to soil that is more fertile. Fertiliser use represents a large cost to the farmer. It is estimated that 60-90 % of the N ingested by grazing cows is not metabolised efficiently and is returned to pastures as excreta (Haynes and Williams 1993). In New Zealand, livestock are grazed and require pasture year-round. Large N leaching losses occur in situations when N inputs exceed the growth requirements of the pasture, such as in winter or under urine patches (Di and Cameron 2002a). This N represents a financial loss to the farmer and the agricultural sector (Zaman et al. 2009). Loss of N in the form of N_2O also represents an important economic loss (van Zwieten et al. 2009). Considerable efforts have been made to develop farming practices and technologies to reduce N losses. Methods include application of nitrification inhibitors, optimising N and water application to meet plant needs, and renewing pastures less frequently (Cameron et al. 2013). Legislation to reduce NO_3^- leaching has become a major constraint on agricultural land use in many countries (Cameron et al. 2013). The protection of the

"100 % Pure New Zealand" brand also needs consideration, as its deterioration may result in decreased demand and reduced market value of the country's agricultural products.

2.5 The interaction of plants with soil nitrogen

2.5.1 The role of plants in the soil nitrogen cycle

Plants perform an important role in biogeochemical cycles (Jobbágy and Jackson 2004). It is well known that vegetation can strongly influence soil properties and individual plant species play an important role in shaping soil fertility in natural ecosystems (Hobbie 1992). Plant species can effect N cycling directly through N uptake, use and loss during litter fall and indirectly by the provision of organic C required for the functioning of soil microbes and root associated herbivores (Hobbie 1992). Recently, there is increasing recognition of the complex feedbacks between both above and below ground components of terrestrial ecosystems (Wardle et al. 2004). In addition to plants modifying soil properties, soil microbial decomposers break down dead plant material and determine the supply of soil N to the plant (Wardle et al. 2004).

Degradation of natural vegetation communities in terrestrial systems alters uptake by plants and changes the flux of nutrients in soils (Jobbágy and Jackson 2004), potentially amplifying solute transport to water bodies (Dosskey et al. 2010). This is evident in comparisons of nutrient losses between pristine forested and degraded catchments (Herlihy et al. 1998, Band et al. 2001, Larned et al. 2004) and nutrient losses following forestry harvest (Vitousek and Matson 1985, Hendrickson et al. 1989, Vitousek et al. 1997). Conversion of fertile pasture land to planted forests in New Zealand rapidly reduces soil N leaching losses (Parfitt et al. 2002, Parfitt and Ross 2011), indicating forests have a potential role to play in reducing N leaching losses to rivers, lakes and groundwater. The role of riparian zone vegetation in reducing nutrient transport to waterways is frequently cited (Hill 1996, Naiman and Décamps 1997, Correll 2005, Mayer et al. 2007), although there is a lack of consensus regarding the mechanisms involved. Riparian plantings have been described as a buffer, filtering out N from overland flows and groundwater interacting laterally with the stream channel (Correll 1997). The interaction of plants with soil nutrients has received some attention concerning phytomanagement, which encompasses a range of techniques used to manipulate the soil-plant environment to control the flux of contaminants (reviewed by Dickinson et al. 2009, Robinson et al. 2009, Vangronsveld et al. 2009). Typically, phytomanagement has involved trace-element contamination, however the principles involved can be applied to N. Improved management of N in land-applied wastes has been achieved through planting tree species, such *Eucalyptus*, *Populus* and

Salix, as short rotation forests (Kutera and Soroko 1994, Sims and Riddell-Black 1998, Roygard et al. 2001, Guo et al. 2002).

2.5.2 Vegetation, the rhizosphere and soil nitrogen

The presence of plants significantly modifies the soil N environment (Figure 2.4). Plants take up water and soluble nutrients from the soil. Above-ground vegetative parts of plants add leaf litter to the soil and stabilise the soil surface. Plants regulate soil processes through their rhizosphere, the area of soil influence by plant roots (Hartmann et al. 2008, Hinsinger et al. 2009). Rhizosphere soil is distinct from surrounding soil due to the root exudation of soluble compounds, water uptake nutrient mobilisation by roots and microorganisms; and rhizosphere-mediated organic matter decomposition. Through these mechanisms the rhizosphere mediates virtually all aspects of nutrient cycling. The rhizosphere effect on soil organic matter decomposition is often large in magnitude and significantly involved in plant-soil interactions (Pinton et al. 2007). These plant and rhizosphere processes influence the quantity, speciation and mobility of N in the soil environment and are reviewed in more detail here.

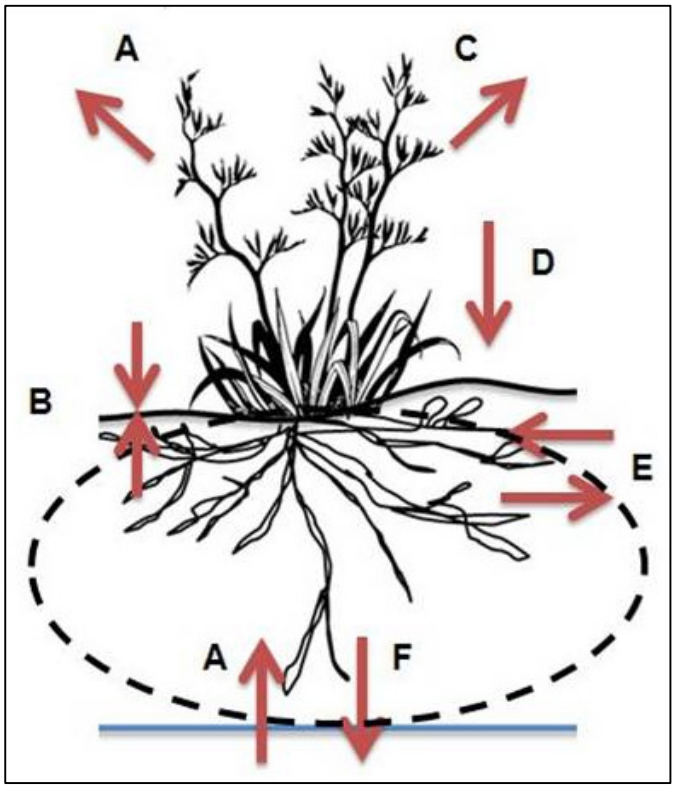


Figure 2.4 Key soil-rhizosphere-plant interactions and N pathways (adapted from Robinson et al. 2009). The area inside the dashed line represents the rhizosphere and the blue line is the water table. (A) Plant transpiration reduces water flux through soil and subsequent N leaching, rainfall directly to plant foliage evaporates to the same effect. (B) Roots act to stabilise the soil, reducing surface transport of N as eroded soil. (C) Nitrogen accumulated in the plant tissue may be removed from the system via browsing or harvesting. (D) Leaf fall returns N to the soil surface where it may be incorporated into soil organic matter or mineralised by microbial activity. (E) Roots change the soil pH, aeration, organic C content and can alter the microbial community; this may promote or inhibit functions of the soil N cycle. Specific root exudate chemicals may affect the functioning of N cycle bacteria. (F) Roots also create macropores that may increase N leaching.

Plant nitrogen uptake

Plant uptake of nutrients results in a transfer of soil N to plant biomass. Most plants preferentially absorb soluble mineral soil N. Plant species may be adapted to absorb the form most present in their native soil (due to the specificity of carrier proteins involved) (Atkin 1996). For example spruce, which grow on acid soils, preferentially absorb NH_4^+ over NO_3^- due to its prevalence (Kronzucker et al. 1997). The use of NO_3^- requires more energy as plants must absorb and then assimilate NO_3^- (converting it to NH_4^+) before use (Aerts and Chapin 2000). Evidence suggests that plants in marginal environments are also capable of absorbing soluble organic N (Kielland 2001). Of course, plant N may be subsequently returned to the soil through litter fall or removed from the plant through grazing or harvest.

Plant roots provide water quality services by filtering N that passes as lateral or vertical groundwater flow (Correll 1997). The filtering effect may be direct, whereby roots absorb nutrients into plant tissues, or indirect, as plant transpiration reduces water flux through soil. Mass flow of soil solution to the root caused by transpiration carries soluble N to the root surface, where it is available for uptake (Aerts and Chapin 2000). Mass flow supplies a large proportion of total N delivery to the roots of plants, due to the high mobility of N in soils (Lambers et al. 2008). Diffusion and the transport by mycorrhizal fungi are the other major mechanisms that move nutrients to the root's surface (Aerts and Chapin 2000). Additionally, some plant species (such as those in the Fabaceae family) are capable of acquiring N through symbiotic N fixation. Typically, when N is absorbed as an organic acid or NH_4^+, plants absorb an excess of cations over anions and secrete H^+ to maintain charge balance, acidifying the soil (Marschner 1995). Because the bulk of the nutrients required for plant growth usually enter the plant by means of root and/or mycorrhizal uptake, the size of the root system is an important determinant of nutrient acquisition capacity (Aerts and Chapin 2000).

Soil stability

Vegetation increases soil stability, which reduces N transport through soil erosion (Marden et al. 2005). Mechanisms involved are hydrological (interception of rainfall by leaves and transpiration of water from soil) and mechanical (roots system reinforces soil) (Phillips et al. 2000). Soil aggregation is increased by the enmeshment of soil particles in roots and the binding action of root exudates (Bronick and Lal 2005). Riparian vegetation reduces bank collapse and corresponding transport of sediment-bound N to streams (Marden et al. 2005). The presence of litter and roots also increases the roughness of the soil surface and the increased friction slows water velocity allowing for increased interception of overland flow (Correll 1997). In contrast, the formation of root channels provides pathways for the rapid transport of solutes to groundwater and may increase N leaching. This reduces the contact time

of the soil solution with the soil matrix and soil organisms, which could otherwise slow leaching through sorption and transformation processes (Jarvis 2007).

Root exudates

Plants can generally acquire C more efficiently from photosynthesis than they can nutrients from soil. Root exudation of C containing compounds stimulates microbial activity in the rhizosphere (Brzostek et al. 2013) and may enhance nutrient uptake. Organic acids form a major proportion of plant exudates and can acidify the rhizosphere (Salisbury and Ross 1992). Numerous studies have shown that root inputs of labile C increase soil organic matter decomposition (termed rhizosphere priming, Fontaine et al. 2003, Kuzyakov 2010, Dijkstra et al. 2013). The consequences of root exudation and rhizosphere priming for soil N cycling (and subsequent N availability for plant uptake or leaching) depend on the balance between plant uptake, microbial mineralisation and microbial immobilisation (Dijkstra et al. 2013). Root exudates may increase N supply to plants in some cases (Dijkstra et al. 2009, Drake et al. 2011, Phillips et al. 2011b), but not in others (Bengtson et al. 2012). Root exudates may enhance N supply by stimulating microbial grazers such as amoebae and nematodes, which feed on bacteria and excrete excess N (Clarholm 1985). Annual uptake of N by vegetation is often twice that produced through mineralisation in soil in the absence of roots. This discrepancy could involve the more rapid nutrient cycling in the rhizosphere compared to bulk soil, as stimulated by root exudates (Aerts and Chapin 2000).

Certain plant species release allelochemicals from their root systems, which are capable of inhibiting the nitrification process (Bremner and McCarty 1993, Paavolainen et al. 1998, Subbarao et al. 2006, Fillery 2007). Inhibition of nitrification is potentially an adaptation mechanism to conserve and use N efficiently in systems that are N limiting (Lata et al. 2004, Subbarao et al. 2012b). Monoterpenes have been shown to inhibit the growth of *Nitrosomonas europaea* and are implicated in the low nitrification rates observed in coastal California redwood (Ward et al. 1997) and Norway spruce (Paavolainen et al. 1998) forests. Low nitrifier populations and NO_3^- levels grasslands (Sylvester-Bradley et al. 1988, Lata et al. 1999) have been used as evidence of suppression by the dominant species. However, more direct measures of plants capacity to suppress nitrifier activity in field soil are required (Fillery 2007).

Litter fall - addition of soil organic matter

In addition to soluble organic C from root extracts, rhizosphere soil can receive inputs of organic matter via the fall of senescing leaves (Aerts and Chapin 2000). Chemically, the presence of organic matter increases the buffering and exchange capacity of soils, as humic substances provide multiple absorption sites (McLaren and Cameron 1996). Organic matter stabilises soil aggregates, and may

increase the aeration and temperature of the soil (Bronick and Lal 2005). Decomposition of plant litter is a key process in the terrestrial N cycles. Nutrients that are not reabsorbed during senescence are re-mineralised and made available to the ecosystem (Aerts and Chapin 2000). In general, litter decay rate is inversely related to the litter C:N ratio and lignin content, and positively related to N content (Hobbie 1992). Climate also has a strong direct influence on litter decay rates (Hobbie 1992). Annual net N mineralisation rates are well correlated with foliar litter decay rates (McClaugherty et al. 1985).

Soil microbes and macrofauna

In the rhizosphere, litter fall, root turnover, sloughed cells, exudates and secretions provide C as an energy source to heterotrophic microfauna and microbes (Hobbie 1992). The rhizosphere generally supports a higher microbial biomass and populations, with higher activity levels, than bulk soil (Van Veen et al. 1989). N cycling may be increased in the rhizopshere as a result (discussed previously). Organic matter attracts earthworms, which influence the levels and cycling of C and N in soils. Earthworms have been shown to selectively remove litter with a low C:N ratio, thereby increasing the overall C:N ratio of remaining litter (Bohlen et al. 1997). The redistribution of surface litter by earthworms has consequences for the spatial heterogeneity, microbial activity, and nutrient content of soil (Bohlen et al. 1997). Earthworms may also alter soil pH and soluble organic C (Sizmur and Hodson 2009).

2.5.3 Riparian vegetation interaction with nitrogen

Worldwide there has been much research on the role of plants in mitigating the flow of agricultural N across the riparian zone. Riparian zones, depending on aspect and adjacent land use, can receive high concentrations of N in subsurface flow. Early work using N budgets demonstrated that riparian forests in the United States were effective at trapping N and reducing levels entering streams (Lowrance et al. 1984, Peterjohn and Correll 1984, Jacobs and Gilliam 1985). Riparian buffers can be examined in three dimensions; longitudinal (regarding the length of planted riparian zone needed to remediate degraded conditions), transverse (relating to setting appropriate buffer widths) and vertical (effects of root zone and canopy structure) (Lee et al. 2004). Scant research has been conducted within vertical dimensions, including the rhizosphere of plants, where there is high potential for the interaction biological and chemical mechanisms with N (Polyakov et al. 2005, Sutton 2006, Van Appledorn 2009). The mechanisms discussed in the following section relate to riparian zones, but are transferable to a paddock edge or patch planting that receives hydrological flux due to slope or application of water or effluents.

Mitigation of nitrogen flux

There is consensus that riparian zones are important in the reduction of non-point source N entering waterways and that plant uptake of N and denitrification are the most important processes of N removal. Rhizosphere filtration is as implicit assumption in this consensus, although there appears to be little quantitative evidence. Furthermore, there is debate as to the relative importance of these mechanisms across different landscapes (Hill 1996, Correll 1997). Riparian zone hydrology regulates N transport. Soil properties and connection to the water table may determine residence time, which is important. Under slow flow conditions even a slow rate of denitrification or uptake may remove a lot of N (Gilliam et al. 1997). The lack of a shallow aquifer may reduce the N removal abilities of a riparian zone, as N may pass below plant roots (Gilliam et al. 1997).

The moist, organic and often anaerobic soil (with ground water close to the surface) of riparian zones, make them potential hotspots for N_2O production in agricultural landscapes (Groffman et al. 2000). Organic riparian soils also have a large role in catchment-wide NO_3^- removal (Cooper 1990). Compared with plant uptake (where N is eventually returned to the soil and then water in litter fall), denitrification has been described the more desirable means of NO_3^- removal from drainage waters, as the end product (N_2) is naturally abundant in the earth's atmosphere (Martin et al. 1999). However, if N_2O is produced, pollution swapping may occur, where the benefits of reduction in NO_3^- due to denitrification are offset by greenhouse gas emission (Dhondt et al. 2004). As described previously (2.3.2), the ratio of N_2O:N_2 production during denitrification in riparian soil is dependent on NO_3^- and oxygen concentrations, temperature, the availability of organic matter, soil pH and the microbial populations present (Hefting et al. 2003). However, Houghton (2005) suggests that overall, N_2O emissions from riparian zones are unlikely to contribute significantly to the overall emissions compared with those from agricultural land.

The literature shows some apparent contradictions. Some studies have considered vegetation assimilation to be the primary mechanism of N removal, but it is also assumed that uptake would only occur during the growth season. Significant N removal has been found in winter under dormant hardwood forests in the United States, making uptake as a mechanism appear unlikely (Gilliam et al. 1997). Alternatively, denitrification is often invoked as the primary mechanism for N removal. However, long term nutrient balance studies show that N is removed at all times of the year and in soil conditions in which it is unlikely that denitrification occurs extensively (Gilliam et al. 1997). Spatial and temporal variability in denitrification may explain these conflicting theories and results (Polyakov et al. 2005).

2.5.4 Species-specific plant-nitrogen interactions

Plant nitrogen uptake and foliar nitrogen

By immobilising N into tissues via uptake, plants can directly affect the amount of N available in soil for leaching or denitrification. Thus, species growth rate and biomass production can determine the amount of N that is removed from soil. In agricultural studies, biomass production and winter growth rate of pasture grass species correlate positively with plant N uptake and negatively with N leaching losses (Crush et al. 2005, Moir et al. 2013, Malcolm et al. 2014). High growing season biomass of a *Populus* sp. riparian buffer has been shown to facilitate the immobilisation of more N in plant material than grass buffer zones or adjacent crop fields (Tufekcioglu et al. 2003). Nitrogen uptake from effluent and wastewater has been shown to be highest in high biomass producing tree species used as short rotation energy crops (Guo et al. 2002, Tzanakakis et al. 2009, Pandey et al. 2011).

In addition to biomass production, foliar N concentration varies between species and may contribute to differences in species-specific N uptake rates. Nitrogen concentration in foliar tissues is higher than in stems and roots, therefore species with a high proportion of plant biomass allocated to leaves may take up more N. Generally speaking, leaf N concentration and leaf lifespan are negatively correlated (Reich et al. 1997); plants in nutrient-poor environments tend to grow slowly and use nutrients more efficiently. Nitrogen is conserved in long-lived low N foliage, which has the additional benefit of deterring herbivores (Hobbie 1992, Aerts and Chapin 2000). In contrast, plants growing in nutrient-rich environments generally produce large amounts of N-rich leaves. Such leaves are readily degradable, which releases large amounts of nutrients for potential re-use by the plant (Hobbie 1992, Aerts and Chapin 2000). This has been posed as an explanation for the dominance of evergreen species in low nutrient environments (Aerts 1995). Nitrogen-fixing species often also have high tissue N concentrations and low C:N ratios (Wright et al. 2004).

Some species are able to absorb N in excess of their immediate growth requirements (termed luxury consumption or uptake (Aerts and Chapin 2000). Luxury uptake of nutrients has been proposed a strategy that has associated with slow growing plants adapted to low fertility conditions. During a flush of nutrients inputs such species can absorb high qualities, used to sustain continued slow growth until nutrients are next available (Chapin 1980). In fertilisation experiments in the USA, several forest species (Tripler et al. 2002) and temperate grass species (Moir et al. 2013) have shown luxury uptake.

Litter fall and soil nitrogen availability

Litter fall and decomposition is an important link in the terrestrial N cycle. Because plant species differ in both the quantity and quality of resources that they return to soil, individual plant species may have important but different effects on soil chemistry, soil biota and the processes that they regulate (Wardle et al. 2004). Leaf litter that has low N content decomposes slowly resulting in soil low in N (Hobbie 1992, Aerts 1995). While N-rich leaf litter rapidly decomposes and releases N, developing soil that is high in available N (Hobbie 1992). This promotes rapid nutrient cycling in soil compared with low nutrient conditions, but is less efficient as the plant may not re-absorb all N released from fallen leaves. Species-specific differences in soil N cycling have been identified (Finzi et al. 1998, Chen and Stark 2000, Lovett et al. 2004). A range of studies have found increased mineralisation of N in soil dominated by species adapted to high nutrient conditions (Berendse 1998, van der Krift and Berendse 2001, Laughlin 2011). In New Zealand, N concentrations are higher in litter and associated soil of an introduced N-fixing species (*Ulex europaeus*) than in other shrubs and trees (Magesan et al. 2012).

Root structure

The role played by vegetation in improving slope stability and preventing soil erosion is well recognised (Greenway 1987). However, the international literature of below-ground root data for grasses and woody tree species is limited, probably due to the time-consuming nature of root system extraction (Phillips et al. 2000). Root biomass allocation varies between plant species. In particular, monocotyledonous herbaceous species generally allocate relatively more biomass to the roots and less to above ground parts, compared with dicotyledonous herbaceous species with the same growth rate (Garnier 1991). Root depth, spread and density vary between species and influence the plants' ability to capture and take up soil N and the extent of soil receiving rhizosphere effects (e.g. root exudates and mass-flow of solutes etc). For example, the deep rooting system of alfalfa allows it to capture N from up to 3 m depth, while shallow rooted crops, such as potatoes leave large N residues in soil which are prone to leaching (Webb et al. 1997). The use of appropriate deep-rooted perennial species may reduce N losses and increase N cycling efficiency (Ledgard et al. 2001). Root architectural simulations by Dunbabin et al. (2003) suggest that high rooting dentistry in the top soil is important to reduce rates of NO_3^- leaching in high-rainfall environments. Higher nutrient sequestration by poplar and switchgrass compared with corn and soybean has been attributed to the abundant fine and deep roots of these species (Tufekcioglu et al. 1998). A strong relationship between fine root biomass and N uptake was found for species planted in a constructed wetland receiving effluent waste water (Tanner 1996). However, Malcolm et al. (2014) found that species specific plant growth and root metabolic activity was more important than root architecture and depth in reducing NO_3^--N leaching losses.

Root exudates and the soil microbial community

Root exudation rates differ several-fold among species, but the relationship between exudation rates and plant growth forms is poorly understood (Aerts and Chapin 2000). Brzostek et al. (2013) measured variation in rhizosphere effects between temperate forest species in terms of rates of root exudation, extracelluar enzyme activity and soil N availability. The composition of soil microbial communities differs among grassland (Bardgett et al. 1999) and forest species (Saetre and Bååth 2000), potentially due to species-specific rhizosphere effects. In a monoculture experiment involving nine grassland species, root traits were found to be strongly correlated with the biomass of soil bacteria relative to fungi and rate of C cycling (Orwin et al. 2010).

Root zone denitrification

Plant biomass and vegetation cover have been found to show positive correlations with denitrifying enzyme activity, suggesting that vegetation characteristics should also be considered in assessing soil denitrification capacity and potential N_2O emissions (Liu et al. 2011). The positive relationships between root biomass and soil organic matter, and between soil organic matter and denitrification potential, imply that establishing deep-rooted vegetation may increase the depth of the active denitrification zone (Gift et al. 2010). Some forest studies demonstrate species-specific effects on N_2O production from denitrification (Menyailo and Huwe 1999, Butterbach-Bahl et al. 2002, van Haren et al. 2010), while others show no effect of vegetation type (Borken and Beese 2006, Christiansen and Gundersen 2011). Similarly, in the riparian zone, in some instances no difference has been found between vegetation types in terms of denitrification and N_2O production (Clément et al. 2002, Liu et al. 2011, Jacinthe et al. 2012). Several studies have detected differences between grass and forest buffers (Groffman et al. 1991, Hefting et al. 2003), but there are no consistent findings (Polyakov et al. 2005).

Additional confounding factors

Other factors may also influence N mobility in the plant-soil environment, albeit indirectly. The rates of plant growth and N uptake, litter decay and various microbial activities, are mediated by abiotic factors, in addition to any species-specific effects. For example, rates of denitrification in forest and riparian zone soils vary seasonally (Pinay et al. 1993). Forests in young and old growth phases also differ in their effects on soil N cycling. Young riparian forests have shown high N removal due to plant N uptake during active growth (Mander et al. 1997), while denitrification rates were higher in older oak and spruce forests (Christiansen and Gundersen 2011) and *Kunzea ericoides* shrubland in New Zealand (Price et al. 2010).

2.6 Ecosystem services provided by New Zealand native vegetation

In addition to mitigating the adverse effects of soil N losses New Zealand native plants offer a range of ecosystem services that may benefit agricultural landscapes. Ecosystem services are those provided by nature that support life through a wide range of processes and functions (Myers 1996, Daily et al. 1997). The ecosystem services provided by indigenous species in New Zealand were estimated in 1999 to be worth over NZD 30 billion per year (Patterson and Cole 1999). Research has demonstrated that conventional arable farming in the Canterbury region has reduced the functioning of indigenous ecosystem services which are of significant financial value (Sandhu et al. 2008). The reintroduction of native vegetation into New Zealand's agricultural landscapes may enhance terrestrial and aquatic biodiversity, play a role in greenhouse gas sequestration and erosion control, and has potential to provide plant products of commercial and cultural value.

Terrestrial native biodiversity

The establishment of native plant vegetation on farms not only increases native plant diversity in agricultural landscapes but also the diversity of native fauna utilising the habitat and resources provided (Meurk and Swaffield 2000). Native riparian, shelterbelt, hedgerow and paddock edge plantings may act as corridors for fauna such as birds and lizards to move through the agricultural landscape. These corridors may connect larger patches of suitable habitat such as paddock corner plantings and designated restoration areas (Bennett 1998). Such planting may facilitate the re-introduction of birds such as Tūi to the Canterbury Plains and ensure isolated populations do not become genetically isolated. A recent study has found that the use of native plant species as shelterbelts on New Zealand dairy farms yielded higher species richness of native spiders and beetles compared with exotic plants (Fukuda et al. 2011). The incorporation of a range of native plants into cropping systems is likely to increase the diversity of invertebrates found in such systems, which may provide both biological control (via predators and parasitoids of pest insects) and enhanced pollination services for cropping systems (Tsitsilas et al. 2006, Kremen and Chaplin-Kramer 2007).

Aquatic biodiversity

A variety of ecological benefits are provided by vegetated riparian buffers. Riparian vegetation has been found to reduce sediment transport to streams (Hubble et al. 2010, Pollen-Bankhead and Simon 2010), provide inputs of leaf litter and woody debris and reduce water temperature and light penetration via shading (Sweeney et al. 2004, Mander et al. 2005, Finlay et al. 2011). These features enhance the habitat quality for aquatic invertebrates and fish and provide resources to sustain aquatic food webs (Gregory et al. 1991). Studies in New Zealand have found physical in-stream conditions are remediated faster than water quality through the presence of riparian vegetation (Storey and Cowley 1997, Scarsbrook and Halliday 1999, Parkyn et al. 2003).

Greenhouse gas sequestration

As part of the Emissions Trading Scheme in New Zealand, increased tree planting has been suggested as a way in which greenhouse gas emissions can be reduced (Ministry for the Environment 2011). It has been estimated that New Zealand native scrubland (*K. ericoides-L. scoparium*) could accumulate as much C in biomass as exotic forestry plantations, which are currently used to offset a the country's greenhouse gas emissions (Scott et al. 2000). Several studies suggest the reversion of the estimated 1.45 M ha of low fertility grasslands in New Zealand to native shrubland or forest, as a means of C sequestration (Scott et al. 2000, Whitehead et al. 2004, Trotter et al. 2005). Methane may sequestered by the soil associated with native plants. Enhanced oxidation of methane has been reported in soil under *K. ericoides* shrublands compared with pastures (Price et al. 2004, Price et al. 2010). Lower N_2O emissions are also likely form soil planted with native vegetation compared with productive pastures due to reduced input and availability of N (Saggar et al. 2008, Liu and Greaver 2009).

Erosion control

Erosion rates in New Zealand are high by world standards and the benefits provided by vegetation in terms of its control are a key ecosystem service to New Zealand (Basher 2013). A range of exotic vegetation has been used for erosion control in New Zealand, with more limited use of indigenous species (Basher 2013). The establishment of native vegetation on slopes, marginal land and river banks, has the potential to reduce soil erosion in New Zealand (Marden et al. 2005, Phillips et al. 2011a, Basher 2013).

Commercial value of native plant products

New Zealand native plant products have a range of traditional and modern uses that may offer increased value to land set aside for environmental protection (Cooper et al. 1991). Rongoā (traditional Māori medicine) makes use of many New Zealand native plants. Studies of native plant phytoextracts has led to interest in their use in modern medicine (Lis-Balchin et al. 1995, Old 2013).

The honey and essential oils of *Kunzea* spp. and *L. scoparium* are sold worldwide as natural remedies for minor infections and ailments (Stephens et al. 2010). Methylglyoxal has been identified as the principle component responsible the non-peroxide antibacterial activity in the honey (Stephens et al. 2010). While, leptospermone, which occurs in the leaves of *L. scoparium*, is an antibacterial β-triketone found in the essential oils, but not honey, potentially due to its insolubility in water (Weston et al. 2000). The value of New Zealand's mānuka honey industry was estimated at NZD 75 million in 2010, and is predicted to grow towards NZD 1.2 billion per annum (Landcorp 2014). Production of these high value plant products may make the incorporation of native plants into farm systems more economically viable.

Potentially, the growth of native plants as short rotation shrubs and trees may provide fibre (McGruddy 2006) or wood products (Bergin et al. 1995). The integration of *P. tenax*, into land management systems has received considerable attention (McGruddy 2006). Māori used the strong *P. tenax* leaves in weaving and European settlers developed a fibre export industry which was productive until the 1920's (Wardle 2002). In addition to the removal of excess N from the farm system, the harvest of *P. tenax* leaves and flowers may be used to supply fibre, gel, seed oil and other extractives, to growing industries which make use of modern technologies and traditional knowledge (McGruddy 2006).

Cultural and aesthetic values

Native vegetation has the ability to provide both cultural and aesthetic values to New Zealand's rural landscapes (Meurk and Swaffield 2000). In Māori culture there are clear links between healthy ecosystems and people's cultural and spiritual wellbeing (Harmsworth and Awatere 2013). An important cultural concept is kaitiakitanga or stewardship of the land; this is reinforced and demonstrated through the presence of native species (Morad and Jay 2000). Māori cultural protocols are increasing being incorporated into land management decisions (Meurk and Swaffield 2000). A range of individuals, community groups and authorities in New Zealand are actively promoting the establishment of native species, not only within conservation initiatives but also within urban design and amenity plantings (Meurk and Swaffield 2000, Meurk and Hall 2006, Sullivan et al. 2009).

Chapter 3

Species selection, description and comparative investigation of native plant rhizosphere profiles

This chapter firstly provides a description of the choice of species for this research, including details of each species' morphology (above- and below-ground) and ecology (Section A). Secondly, this chapter outlines literature knowledge of root system variation of native species commonly used in restoration, and describes an observational study of native rhizosphere soil profiles at the principal field research site at the Lincoln University Dairy Farm (Section B).

Section A: Species selection and description

3.1 Native species for restoration planting in agricultural landscapes

In the Canterbury Plains region, widespread vegetation loss has left few native remnants, which remain only as non-regenerating ageing populations (Meurk 2008). Targeted restoration planting in Canterbury will help prevent further native vegetation loss and will encourage natural regeneration. Local authority restrictions of catchment nutrient losses have encouraged the fencing of stream margins and protection with native plants. Over the past decade, conservation organisations have made advances in re-establishing native vegetation on the Canterbury Plains.

Limited anecdotal evidence and early studies indicated New Zealand native species have shallow root systems that are slow to develop compared with exotic tree species (Phillips 2005). In contrast, the work of Marden at al. (2005) and evidence from restoration planting over the last few decades, shows the majority of native lowland riparian species have good establishment and biomass growth within 3 years of planting, although growth and rooting depth may not be as extensive as exotic trees (Phillips et al. 2011a). Nevertheless, native species are increasingly planted in agricultural landscapes. Marden at al. (2005) conclude that native species provide effective bank protection for small streams, with many riparian species achieving appreciable growth and canopy cover 7-10 years after establishment. Further description of native plant root systems is provided in Section 3.4.

3.2 Species and site selection

Plant species were selected for this research based on suitability for and current use in restoration planting throughout New Zealand and their availability at local restoration sites for field research. The plants are native New Zealand species of early successional status, frequently found growing naturally in unstable environments, such as riparian banks. They are suitably hardy and fast growing for restoration purposes and most have been previously studied in riparian bank stabilising trials (Marden et al. 2005, Marden and Phillips 2009).

Two native restoration sites on the Canterbury Plains in the Selwyn District were chosen for field study. The use of planted restoration sites provided plants of the same age, planted on the same soil type, typically in an evenly spaced, randomised arrangement. The sites have a history of agricultural use prior to establishment of native vegetation, making the findings directly applicable to management decisions regarding on-farm planting. Species present at these sites are considered to have formed part of the kahikatea-mataī forest and wetland communities on low-lying swampy land on the Canterbury Plains (Williams 2005, Meurk 2008). Some species present (*Phormium tenax, Austroderia richardii* and *Carex* spp.) are more typical of swamp-wetland plant communities (although are found in both community types). These wetland species are used commonly in riparian planting due to their tolerance of high soil moisture (Williams 2005, Meurk 2008). Dominant trees in the original vegetation tōtara (*Podocarpus totara),* kahikatea (*Dacrycarpus dacrydioides*) and mataī (*Prumnopitys taxifolia*) were not included in this research as they are slow to establish and not used as ubiquitously in restoration planting.

The field sites chosen were the Lincoln University Research Dairy Farm (LUDF), near Lincoln, and Selwyn Huts (SH), near Te Waihora (Lake Ellesmere) (Figure 4.1, Chapter 4). These sites were chosen for their differences in soil type and availability of soil N, as well as the significant overlap in the native species present (nine suitable native species were present at both sites). Further description of the field sites is given in Chapter 4. The sites are well established, with plants having developed significant root systems and accumulated leaf litter on the soil surface. In addition, these sites contain species of a similar age (4-5 years old at the time of the research). *Leptospermum scoparium* was not sampled at the field sites, as it was only present in limited numbers at one site, and is not commonly planted in Canterbury due to high incidence of mānuka blight disease.

Native seedlings used for the glasshouse-based pot trial experiments (Chapter 5) were grown from locally sourced seed, propagated by the Department of Conservation, Motukarara Conservation Nursery. *L. scoparium* was included in the pot trials in order to provide comparison to closely related

species *Kunzea robusta* and identify any points of difference. Both species are the topic of much current research, due to their high value honey and the potential application of their antimicrobial properties to bio-waste disposal (Prosser et al. 2014). The exotic species *Lolium perenne* was used as a comparison to the native plants in the field survey and glasshouse experiments. This is the predominant grass species used in New Zealand grazed-pasture systems (Charlton and Stewart 1999), thus would typically be present in adjacent paddocks.

3.3 Description of plant species

Native monocotyledons and dicotyledons are grouped accordingly, followed by a description of *Lolium perenne*. For each native species, the most common Māori and English names follow the species name (family name in brackets). Naming, description of phylogenetic relationships and above-ground morphology were compiled from the work of Dawson and Lucas (2011), Wardle (2002) and Metcalf (2009). Information on below-ground morphology was obtained from Marden et al. (2005) and Marden and Phillips (2009). A photo of each species growing at either the Lincoln University Dairy Farm or Selwyn Huts site is provided.

3.3.1 Native monocotyledons

Cordyline australis, tī kōuka, cabbage tree (Asparagaceae)

Cordyline australis (G. Forst) Endl. (1883) (Plate 3.1), a distinctive monocotyledon tree, is one of five *Cordyline* species endemic to New Zealand (Wardle 2002). Species of this genus differ from normal monocotyledonous growth form in their above-ground arborescent habit, secondary cambium thickening and their conspicuous rhizome and root system (Tomlinson and Fisher 1971). *C. australis* is widely distributed throughout New Zealand, to 1000 m above sea level (a.s.l.), in a range of environments from swamps, rivers, and lake margins to rocky places and forests (Simpson 2000). *C. australis* can grow 8-12 m tall with a trunk up to 1.0 m in diameter and often has a many branched crown (Dawson and Lucas 2011). Thick, tough leaves are tufted on the end of branches (0.3-1.0 m long and 30-60 mm wide) and may persist as skirts around the trunk of young trees. Large branched inflorescences are largely insect pollinated and produce fruits eaten by native and exotic birds (Dawson and Lucas 2011).

Plate 3.1 ***Cordyline australis*, tī kōuka, cabbage tree**

Generally, monocotyledons do not develop an anchoring taproot, but the stems of juvenile *C. australis* branch at the base and send a shoot downwards, which develops a taproot-like rhizome (Dawson and Lucas 2011). These large starch-storing rhizomes were traditionally eaten by Māori (Simpson 2000). *C. australis* also forms many long, "spaghetti-like", true roots, which grow horizontally or obliquely away from the rhizome (Simpson 2000). In younger trees (1-5 years), roots are concentrated in the top 0.5 m of soil and do not spread more than 1.25 m from the trunk (Czernin and Phillips 2005, Marden et al. 2005). While in older trees (25 years), multiple rhizomes are common and roots have been found to reach depths of 2.0 m and spread 3.0 m in diameter (Czernin and Phillips 2005). *C. australis* was amongst the species providing fastest soil reinforcement of natives through root site occupancy modelling (Phillips et al. 2011a) due to its large root biomass (>50 kg, 38 % of total biomass for 25 year old trees, Czernin and Phillips 2005).

Phormium tenax, harakeke, New Zealand flax (Xanthorrhoeaceae)

Phormium tenax, J. R. Forst. et G. Forst. (1976) (Plate 3.2), one of two herbaceous monocotyledons in the *Phormium* genus, is indigenous to New Zealand (Wardle 2002). This bush-forming species (up to 3 m in height) is widespread in lowland, coastal and montane vegetation communities, often associated with wetlands and rivers throughout New Zealand (up to 1370 m a.s.l.) (Metcalf 2009). The leaves of *P. tenax* (1-3 m by 50-120 m,) are arranged in fans or sheaths of up to 10 leaves, each taking 18-22 months to grow to maturity, before stating to decay (Metcalf 2009). The red/brown flowers are

arranged on tall stalks (up to 5m) and are often pollinated by native birds feeding on the nectar (Metcalf 2009). The tough fibrous leaves of *P. tenax* provide one of the world's strongest natural fibres. Māori used *P. tenax* in weaving and European settlers developed a fibre export industry which was productive until the 1920s (Wardle 2002). Stock are known to browse *P. tenax* leaves (McGruddy 2006).

Plate 3.2 *Phormium tenax*, harakeke, New Zealand flax

P. tenax has an extensive fibrous root system, known to extend as wide and as deep as the above-ground bush (McGruddy 2006). The bulk of the roots are in the top 0.5 m of soil. Stout orange roots extend parallel, diagonal and vertical to the surface, with the upper layers branching into networks of fine, white roots (McGruddy 2006). Some roots also descend more deeply, these absorb water through the epidermis, which is very thin towards the apex of the root (Atkinson 1922). Marden and Phillips (2009) report roots of 3 year old *P. tenax* extending 4 m in diameter (but with 80 % of roots within 1.0 m diameter) and comprising 17 % of total plant biomass (265 m total length of roots >1 mm). The roots of flax contain a range of phytochemicals, some with antimicrobial activity; hence, root extracts were used in traditional medicine (Wehi and Clarkson 2007).

Austroderia richardii, toetoe (Poaceae)

Austroderia richardii (Endl.) N. P. Barker et H. P. Linder (2010) (Plate 3.3), is one of five grassy monocotyledons in the native genus *Austroderia*. These species were recently revised from the *Cortaderia* genus (Linder et al. 2010), which includes South American species *Cortaderia selloana* (pampas grass) which has been introduced to New Zealand.

Plate 3.3 ***Austroderia richardii*, toetoe**

A. richardii (formerly *Cortaderia richardii*) is the smallest of the New Zealand *Austroderia* species, occurring naturally throughout the South and Stewart Islands (up to 600 m a.s.l.) (Metcalf 2009). Common along stream banks, riverbeds, lake margins and in disturbed areas, this species is very hardy, fast growing and can withstand harsh conditions (Metcalf 2009). *A. richardii* forms large tussocks (1.5 to 2 m height), with coarse, narrow, sharp-edged leaves (1-3 m by 20−50 mm), and tall upright flowering stems (up to 4 m) with wind dispersed seeds (Metcalf 2009). Māori used the leaves in house thatching and in weaving (Metcalf 2009). The leaves have low palatability to stock (Lambert et al. 1989b).

The fibrous root system of *A. richardii* is likely to be extensive. The roots of related species, *Austroderia toetoe*, are known to spread up to 2 m from the plant and represent 10 % of biomass for 3 year old plants (Marden and Phillips 2009). Marden and Phillips (2009) found more than 70 % of roots were distributed within 0.5 m of stump (394 m total length of roots >1 mm).

Carex virgata, pukio, swamp sedge (Cyperaceae)

Carex virgata Sol. ex Boott (1853) (Plate 3.4), is part of a large worldwide genus of perennial sedges. *C. virgata* is one of 61 species from the *Carex* genus that are endemic to New Zealand (Wardle 2002). This species thrives in a variety of habitats, including swamps, drain margins, seepages and wet pastures (up to 1000 m a.s.l.) throughout New Zealand (Wardle 2002). As fast growing colonisers, *Carex* species are widely used in swale, wetland and riparian restoration planting, but will also grow on dry sites (Hudson and Harding 2004, Haines and Margetts 2006, Peters and Clarkson 2010). *C. virgata* has sharp-edged long, narrow leaves (0.5-2 m by 3-4 mm) which are densely clumped to form a tussock (1 m height). Flower panicles are erect and up to 1 m in length (Moore and Edgar 1970). *Carex secta*, known as makurau, is also common in riparian plantings throughout New Zealand. *C. secta* is slightly larger than *C. virgata* (1.5 m height), and may be raised on pillars of matted roots and decaying leaves (Salmon 1991).

Plate 3.4 ***Carex virgata*** **pukio, swamp sedge**

C. virgata is known to have an extensive root system effective in binding soil (Haines and Margetts 2006). Marden and Phillips (2009) report the total root length for 3 year old plants of related species *C. secta* (959 m total length of roots >1 mm) as ten times that of the dicotyledon species studied and the most extensive of the monocotyledons (17 % of plant biomass) (Marden and Phillips 2009). The Cyperaceae have generally been considered non-mycorrhizal, although recent evidence suggests that mycotrophy may be widespread among sedges (Miller et al. 1999).

3.3.2 Native dicotyledons

Coprosma robusta, karamu (Rubiaceae)

Coprosma robusta Raoul (1844) (Plate 3.5), a shrub or small tree (up to 6 m), is one of a morphologically diverse array of *Coprosma* species (c. 50) native to New Zealand (Dawson and Lucas 2011). *Coprosma* species have inconspicuous male and female flowers occurring on different plants, are wind pollinated and leaves generally have domatia. *C. robusta* is prevalent in coastal regions and lowland forest (up to 1200 m a.s.l.) throughout New Zealand (Dawson and Lucas 2011). *C. robusta* has thick glossy leaves (70-120 by 30-50 mm) with spreading branches. Fruits are orange/red and may be eaten by native birds and lizards (Dawson and Lucas 2011). Extracts of *C. robusta* leaves and bark were used in Māori medicine (Wardle 2011). The leaves are palatable to stock, but only marginally palatable to possums (Marden et al. 2008a). The fast growth rate and ability to tolerate moderate winds and frosts make *C. robusta* an excellent restoration species (Wardle 2011).

C. robusta develops a heart root system with many fibrous roots. Heart-rooted systems are those which are compact with many obliquely or vertically descending roots arising from or near the base of the trunk (Marden et al. 2005). *C. robusta* was classed as one of the better performers in terms of developing a soil stabilising root system after 3-5 years with a maximum root depth of 0.4 m (Marden et al. 2005). Five year old *C. robusta* plants had 1.3 kg below-ground biomass (21 % of plant biomass, Marden et al. 2005). In young plants, roots were described as fleshy and with low tensile strength (Watson and Marden 2004).

Plate 3.5 ***Coprosma robusta*, karamu**

Kunzea robusta, kānuka, white tea-tree (Myrtaceae)

Kunzea species were originally placed in the *Leptospermum* genus, but were revised as *Kunzea*, a genus mostly restricted to Australia (Wardle 2011). Previously, all New Zealand *Kunzea* species were described as *Kunzea ericoides* (A. Rich) Joy Thomps. (1983), although it was recognised that populations were extremely variable (de Lange 2014). Following recent research, using cytological and molecular variation, ten species of *Kunzea* endemic to New Zealand are now recognised (de Lange 2014). *Kunzea robusta* de Lange et Toelken (2014) (Plate 3.6) is studied in this thesis and is the only *Kunzea* species present on Banks Peninsula (de Lange 2014). Although another species, *Kunzea serotina*, forms isolated remnant stands on the Canterbury Plains (de Lange 2014), both seedlings used in the greenhouse experiments and those planted at the two field sites (in 2008 and 2009), were from Banks Peninsula seed sources. As most literature was published prior to the revision of the *Kunzea* genus, *K. ericoides* is also referred to in this thesis when discussing previous research.

Plate 3.6 ***Kunzea ericoides*, kānuka, white tea-tree**

K. robusta is the species that has most usually been described in ecological studies of "*K. ericoides*" as it is the most widespread member of the genus in New Zealand (de Lange 2014). *K. robusta* is a broadly distributed in the North and South Islands (up to 1000 m a.s.l.), although absent from most of parts of the Nelson region and the Canterbury Plains (de Lange 2014). *K. robusta* occurs scarcely in Otago, the southern limit for the species (de Lange 2014). *Kunzea* are light demanding species and have a role as colonisers of disturbed sites (Wardle 2002). *K. robusta* is known to frequently colonise marginal and slip-prone hill country, particularly the clay soils of the

drought-prone eastern parts of both islands (de Lange 2014). It is sometimes regarded as a weed in these habitats because of its ability to rapidly reclaim rough pasture land (de Lange 2014). *Kunzea* species often occur with *L. scoparium* in seral shrubland, where *L. scoparium* tends to decline overtime and the taller *Kunzea* species become dominant (Allen et al. 1992, Wardle 2002).

Although often as a shrub, *K. robusta* can reach heights of 30 m with a trunk up to 1 m diameter (de Lange 2014). In open habitats, *K. robusta* branch profusely, producing a bushy shrub or small rounded tree, whereas in dense stands the branches and foliage are confined to the canopy (de Lange 2014). Leaves (8 by 1 mm) are in branchlets and are unpalatable to mammals. Small (5 mm), insect pollinated flowers occur in clusters (Dawson and Lucas 2011). *K. robusta* flowers abundantly when young producing large amounts of wind-blown seeds (Wardle 2002).

Below-ground descriptions are only available for specimens described previously as *K. ericoides*. Although *K. ericoides* was not studied by Marden et al. (2005), it is likely to have a heart root system, as for the other woody species. Roots of *K. ericoides* have been found to reach a maximum depth of 1.5-2.2 m for 6-32 years old plants (Watson et al. 1995). In that study, rooting depth was not correlated with age, but rather to the slope and stoniness of soil.

Leptospermum scoparium, mānuka, red tea tree (Myrtaceae)

Leptospermum scoparium J. R. Forst. et G. Forst (1776) (Plate 3.7) is part of the largely Australian *Leptospermum* genus, and is native to Australia and New Zealand (Dawson and Lucas 2011). The morphology and chemistry of *L. scoparium* is highly variable suggesting this species probably comprises several forms, not yet described (Dawson and Lucas 2011). *L. scoparium* has been described as the most widespread and important indigenous shrub species in New Zealand, which has undergone the most development as an economic plant (Stephens et al. 2005). *L. scoparium* is found throughout New Zealand and the Chatham Islands, mostly on infertile sites (Dawson and Lucas 2011). *L. scoparium* is an early successional (seral) species on disturbed sites and may invade ungrazed pasture, like *K. robusta* (Wardle 2002). In the 1930s *L. scoparium* was infected with a disease termed "mānuka blight" (caused by an Australian scale insect), which inflicted serious damage, particularly in the Canterbury lowlands where the species is now uncommon (van Epenhuijsen et al. 2000).

Plate 3.7 ***Leptospermum scoparium*, mānuka, red tea tree**

L. scoparium is mostly a shrub, but can form a tree up to 10 m in height, with a trunk around 150 mm diameter. Leaves are thick and stiff (4-12 by 1-4 mm) and flowers mostly white (12 mm diameter). Flowers form capsules that do not open until a year after formation or following a fire (Dawson and Lucas 2011). Māori used *L. scoparium* for food, medicine and timber, and early colonists used the leaves as a tea substitute (Stephens et al. 2005). The essential oils (consisting of triketones, sesquiterpenes and monoterpenes) derived from *L. scoparium* leaves and the honey (containing methylglyoxal) of this species have received considerable attention due to their anti-bacterial properties (Stephens et al. 2005).

L. scoparium develops a heart root system (Marden et al. 2005), which consists of a few main structural roots which give rise to a dense network of fine roots (Watson and O'Loughlin 1985). Marden et al. (2005) found 5 year old *L. scoparium* had roots to a depth of 0.25 m, which spread 1.5 m in diameter (roots form 19 % plant biomass). Studies of mature *L. scoparium* (13-50 year old) have found roots that penetrated to a depth of 0.5 m on stony soils and 0.8 m on sandy soils, however sinker roots were recorded to depths of 1.2 m (Watson and O'Loughlin 1985). The root system of *L. scoparium* seedlings is capable of developing in waterlogged conditions (Cook et al. 1980). Many types of ectomycorrhizae and endomycorrhizae are associated with *L. scoparium* and are thought to improve phosphorus uptake, allowing rapid growth on unfavourable sites (Stephens et al. 2005).

Pittosporum tenuifolium, kōhūhū, black matipo (Pittosporaceae)

Pittosporum tenuifolium Sol. ex Gaertn. (1788) (Plate 3.8) is one of 26 species in the widely distributed *Pittosporum* genus which are native to New Zealand (Wardle 2011). The distribution of *P. tenuifolium* is throughout the North Island and in the east of the South Island (up to 900 m a.s.l.) (Dawson and Lucas 2011). *P. tenuifolium* is present on the margins of coastal and hill country forests, forms secondary shrublands and is a common species on reverting farmland. *P. tenuifolium* is hardy and tolerant of most soils and weather conditions, thus is widely used in landscaping (Dawson and Lucas 2011). The fast growth and tall dense foliage make this species useful as a shelter and nurse crop species (Marden et al. 2008b). *P. tenuifolium* forms a small, rounded tree up to 8 m in height (trunk 0.3-0.4 m diameter). The thin leaves are undulate (20-40 by 10-20 mm) and flowers (10 mm diameter) are dark purple to black. Flowers are pollinated by moths and other night flying insects (Wardle 2011). A pungent odour is released when the branches are broken (Dawson and Lucas 2011).

P. tenuifolium develops a heart root system, with lateral roots (spreading up to 2 m when mature) with branched extremities. At 5 years of age *P. tenuifolium* has a maximum rooting depth of 0.3 m and mean root spread of 2.3 m diameter (roots form 24 % plant biomass, Marden et al. 2005). Roots have moderate tensile strength (Watson and Marden 2004) and trees are susceptible to topple on poorly drained soils(Marden et al. 2008b).

Plate 3.8 ***Pittosporum tenuifolium*, kohuhu, black matipo**

Sophora microphylla, kōwhai, small-leaved kōwhai (Fabaceae)

Sophora microphylla Aiton (1789) (Plate 3.9), an iconic New Zealand species with bright yellow flowers, is one of 8 species in the widespread *Sophora* genus which are present in New Zealand (Dawson and Lucas 2011). *S. microphylla* has the widest distribution of the New Zealand *Sophora* species, found throughout the country except for Stewart Island. It grows on lowland flood plains, lake edges and stony hillsides, especially on fertile soils (Dawson and Lucas 2011). *S. microphylla* colonises new surfaces well, but is light demanding and long-lived, so not often present in forest (Wardle 2011). *S. microphylla* is semi- or brevi-deciduous, losing leaves gradually during winter (more pronounced in cooler regions) and can be completely leafless during flowering (McGlone et al. 2004).

Plate 3.9 ***Sophora microphylla,* kōwhai, small-leaved kōwhai**

S. microphylla has a distinct juvenile divaricating growth phase with intensely tangled branches and a spreading growth form (Wardle 2011). The leaves are sparse and considerably smaller than the adult phase. The length of time spent in the juvenile phase varies considerably and can be up to 20 years (Wardle 2011). Once the tree reaches 2-3 m in height, a main leader branch develops and branches develop into an adult tree with a weeping shape (maximum height 10-12 m). The leaves of adult *S. microphylla* are pinnately compound (up to 150 mm long, well-spaced leaflets, 4-12 by 2-5 mm) and flowers are golden yellow (20-50 mm long). Fruit are dry pods (up to 200 mm long) which are constricted into one–seeded compartments. Seeds (10 mm long) have hard, waxy coats, which can take years to open. There is evidence for seeds being transported long distances in streams and

in the sea and subsequently germinating (Dawson and Lucas 2011). Many native bird species feed on the nectar and flowers (Wardle 2011).

Root system information is available for related species *Sophora tetraptera*, (Marden et al. 2005). *S. tetraptera* develops a heart root system, with a mean root spread of 2 m diameter, maximum depth of 0.3 m and 0.9 kg below-ground biomass (31 % of total) after 5 years (Marden et al. 2005). Additionally, *S. tetraptera* develops sinker roots (Marden et al. 2005). The spindly, long reaching, shallow roots of *S. tetraptera*, have high tensile strength (Watson and Marden 2004), making it suitable to stabilising steep rocky sites (Marden et al. 2008c). *S. tetraptera* was slow growing, but by 5 years of age had invested the most biomass in roots of the species studied by Marden et al. (2005). *Sophora* species are legumes and their roots have nodules harbouring the N-fixing bacteria rhizobia, which form a symbiotic relationship with the plant (Wardle 2011).

Olearia paniculata, akiraho, golden akeake (Asteraceae)

Olearia paniculata (J.R.Forst. & G.Forst.) Druce (1917) (Plate 3.10), is one of about 38 species in the tree daisy genus, *Olearia*, which is largely confined to Australia and New Zealand (Wardle 2011). *O. paniculata* has had its taxonomy changed several times (Wardle 2011). The distribution of *O. paniculata* is from the latitude of Tauranga in the North Island, southwards to Greymouth in the west, and Oamaru in the east of the South Island (Wardle 2011). It occurs on the margins of lowland and hill country shrubland and forests and frequently in exposed coastal sites (Wardle 2011). The relatively fast growth rate and ability of this species to withstand harsh conditions have led to its common use for hedging, shelter and restoration planting (Reay and Norton 1999). *O. paniculata* can tolerate dry infertile soils, but not those that are poorly drained (Wardle 2011).

O. paniculata forms a dense shrub to small tree, up to 6 m tall, with a trunk up to 0.4 m in diameter, and is often multi-trunked (Dawson and Lucas 2011). The leaves are undulate (30-100 by 20-40 mm) and inflorescences are many-branched, with small flower heads enclosed in overlapping scales. Extracts of *O. paniculata* bark and leaves are known to contain sesquiterpenes (Corbett et al. 1964). Many native bird species feed on the fruit. Two forms of *O. paniculata* are observed; the South Island form has slightly larger, more dark-green leaves than that of the North Island (Dawson and Lucas 2011) form. The root system of *O. paniculata* was not described by Marden et al. (2005) or other available literature, although it could be assumed to have a heart root system as for the other dicotyledon species.

Plate 3.10 ***Olearia paniculata*, akiraho, golden akeake**

Plagianthus regius subsp. regius, manatu, lowland ribbonwood (Malvaceae)

Plagianthus is a small endemic New Zealand genus, with only two species. *Plagianthus regius* is a tree with two subspecies (Dawson and Lucas 2011). *Plagianthus regius* subsp*. regius* (Poit.) Hochr. (1907) (Plate 3.11) is found throughout the country, although is uncommon north of Auckland (*P. regius* subsp. *chathamicus* is endemic to the Chatham Islands). This thesis will refer to *P. regius* subsp*. regius* as *P. regius* for simplicity.

P. regius occurs in the lowlands and lower mountains, often on fertile sites, such as newly formed river deltas (Wardle 2002). *P. regius* is one of New Zealand's few fully deciduous trees (McGlone et al. 2004). The species has a distinct juvenile form, as a shrub up to 2 m tall, with dense, interlaced branches (Dawson and Lucas 2011). The juvenile toothed leaves are small (5-15 by 3-10 mm) with various shapes. Mature trees are many-branched with a widely spreading crown. Leaves are larger (30-75 by 5-50 mm) and more uniform in adult trees and have prominent teeth. *P. regius* flowers profusely in branched, laterally hanging inflorescences (flowers 3-4 mm diameter). Male and female flowers are on different trees, with the male flowers forming considerable litter fall after releasing their pollen (Dawson and Lucas 2011). The rapid early growth rate and erect, tall habit make it suitable for mid-tier shelter (Marden et al. 2008d).

Plate 3.11 ***Plagianthus regius subsp. regius*, manatu**

P. regius develops a heart root system, with a mean root spread of 2.6 m diameter, maximum depth of 0.4 m and 1.8 kg of root biomass (26 % of total) after 5 years (Marden et al. 2005). Notably, long laterally-spreading roots have been found in 5 year old plants (Marden et al. 2005). Roots have low to medium tensile strength (Watson and Marden 2004). *P. regius* was identified as one of the better performers in terms of developing a soil stabilising root system, due to its development of a deep, wide-spreading root system, which quickly occupied the site (Marden et al. 2005, Phillips et al. 2011a).

3.3.3 Exotic pasture grass species

Lolium perenne, perennial ryegrass (Poaceae)

Lolium perenne Linnaeus (1753) (Plate 3.12), is widely used in pastures across the temperate world (Casler and Kallenbach 2007) and is the dominant grass used in New Zealand grazed-pasture systems (Charlton and Stewart 1999). *L. perenne* cv. Ceres *ONE*^{50} was used in greenhouse experiments in this research and *L. perenne* (unknown variety) was present at the field sites.

L. perenne grows well in a range of moist fertile situations and forms a compatible mix with white clover (Charlton and Stewart 1999). However, it performs poorly during hot dry conditions, when many deeper-rooted grasses may maintain production (Charlton and Stewart 1999). *L. perenne* is a loosely to densely tufted perennial grass, growing 0.1-0.9 m high (Hubbard 1954). The culms are erect or spreading, slender, with 2-4 nodes. The leaves are green and hairless with smooth sheaths.

The basal area is usually pinkish when young (Hubbard 1954). *L. perenne* has fine roots, 0.2-3 mm in diameter (Malcolm 2013). The root system is moderately shallow (Kemp et al. 2004), with approximately 80 % of its root mass in the top 0.15 m of the soil (Haynes and Williams 1993, Bolinder et al. 2002).

Plate 3.12 *Lolium perenne*, perennial ryegrass

L. perenne cv. Ceres *ONE*^{50} is a "High performance ryegrass" developed using a cross of New Zealand and Spanish perennial ryegrass genetics, which has high dry matter production, particularly through the winter months (Agricom 2011). *L. perenne* cv. Ceres *ONE*^{50} is a late-heading diploid perennial ryegrass, of medium leaf and tiller size, with exceptional production, available with AR1 novel endophyte and LE (Agricom 2011). The recommended sowing rate is 20 kg ha^{-1} (Agricom 2011).

3.4 Overview of literature on native plant root systems

New Zealand native species which are typically planted at restoration sites vary considerably in terms of root system type, rooting depth (Figure 3.1) and spread (Figure 3.2), root length and root tensile strength (Marden et al. 2005, Marden and Phillips 2009, Phillips et al. 2011a). This has implications for their interaction with soil. Most information on the root systems of native species comes from research which compared their potential to improve slope stability and prevent soil erosion. This has largely involved 12 native woody species (summarised in Marden et al. 2005, Phillips et al. 2011a), which were established at a trial site in Gisborne, New Zealand (Plate 3.13). Several other

monocotyledonous species were included in a smaller trial (Marden and Phillips 2009). An air gun was used to remove the soil from roots during destructive harvesting, allowing quantitative analysis of rooting morphology, after 1 to 5 years of growth (Marden et al. 2005).

Plate 3.13 Field trial site for research conducted on the stabilising characteristics of New Zealand native riparian plants. The site is adjacent to the Taraheru River, in Gisborne, North Island, New Zealand. Two year old plants of 12 native species were planted in 3 blocks and extracted 1, 2 (1 m spacing) and 3 (1.5-2 m spacing) years after planting for analysis of biomass and root morphology. Biomass for 1 and 2 years is for container-grown plants. Image from Phillips et al.(2008) and methodology as described in Marden et al. (2005).

The growth rate and below-ground morphology of New Zealand native species was variable. Of those studied, monocotyledons were faster growing and had higher root biomass than woody-dicotyledons of the same age (Marden and Phillips 2009). *A. toetoe, P. tenax* and *C. secta* had a dense mass of fine-fibrous roots, while *C. australis* formed a deep taproot (Marden et al. 2005, Marden and Phillips 2009). The native dicotyledons had less extensive but more substantially sized woody roots, which formed compact heart-rooted systems (Marden et al. 2005).

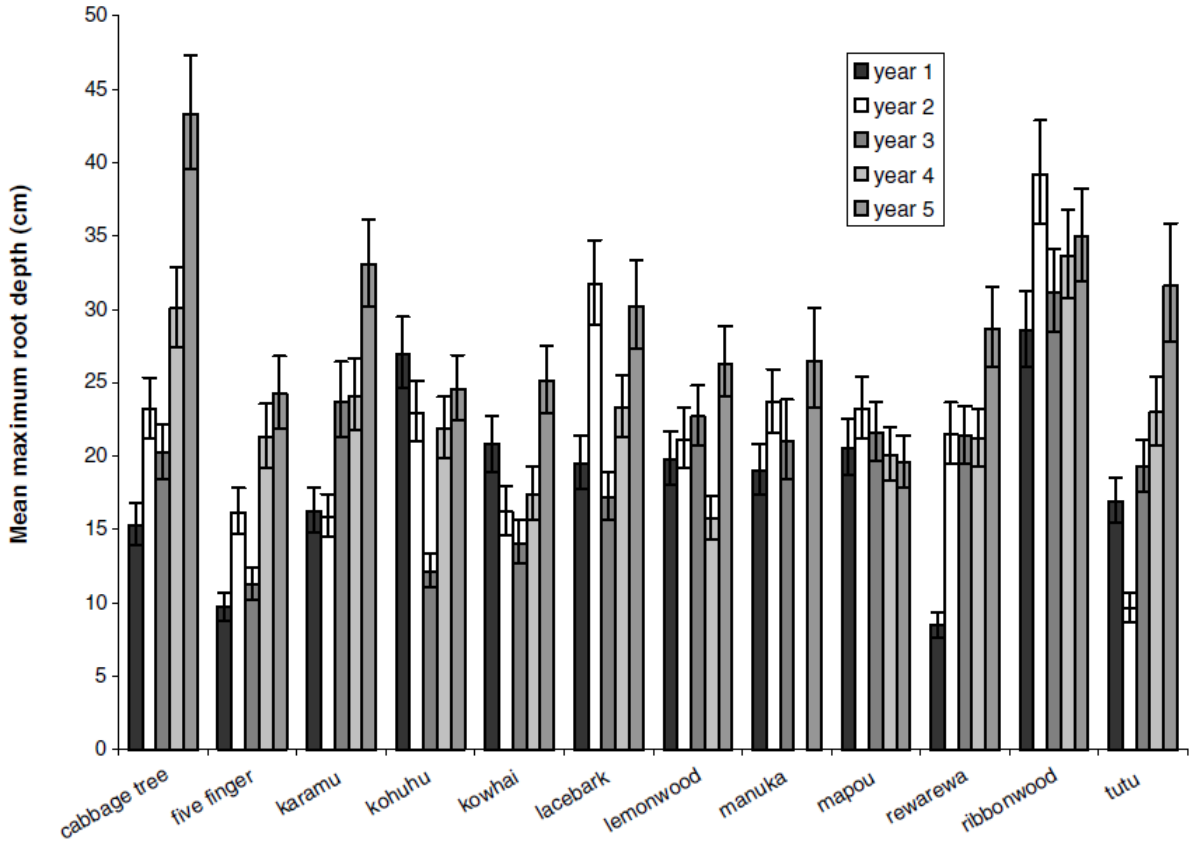


Figure 3.1 Changes in mean maximum root depth over a 5 year period for 12 New Zealand native plant species (from Marden et al. 2005 (Figure 2), with permission).

Rooting depth and spread varied considerably between native species. *C. australis* (cabbage tree) and *P. regius* (ribbonwood) were the deepest rooting species (Figure 3.1), while *Pittosporum eugenioides* (lemonwood) had the widest spreading roots (Figure 3.2). *P. regius*, *P. eugenioides*, *P. tenuifolium* (kōhūhū), *C. australis* and *C. robusta* (karamu) were identified as the best performers in terms of quickly developing a root system with soil stabilising properties (Marden et al. 2005). These species were recommended for riparian planting where the bank height does not exceed 2 m. The toxicity of *Coraderia arborea* (tutu) to grazing stock limits its application to agricultural plantings (Marden et al. 2005). The roots of *Myrsine australis* (mapou), *Knightia excelsa* (rewarewa), *Pseudopanax arboreus* (fivefinger), *L. scoparium* (mānuka) and *S. tetraptera* (kōwhai) were less extensive than other species (Figure 3.1, Figure 3.2). In other studies, differences between closely related species have been noted, for example mature *L. scoparium* can root to depths of 0.8 m (Watson and O'Loughlin 1985), while the rooting of *K. ericoides* is recorded to depths of 2.2 m (Watson et al. 1995).

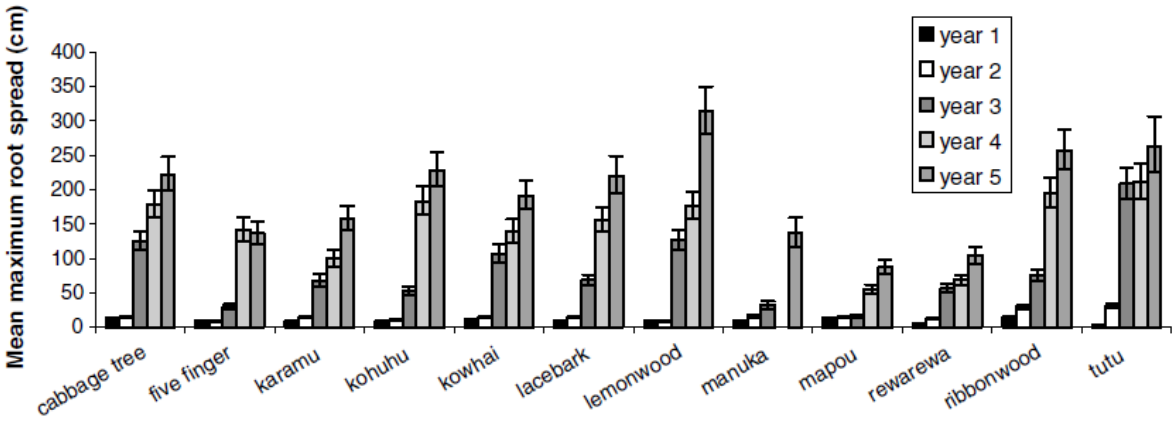


Figure 3.2 Changes in mean maximum root spread over a 5 year period for 12 New Zealand native plant species (from Marden et al. 2005 (Figure 4), with permission).

Other significant differences between root systems were also identified. The monocotyledons studied had 3-10 times longer structural root length than the native dicotyledons (Marden and Phillips 2009). Of the woody species, *P. eugenioides*, *C. arborea* and *P. regius* had the longest root length after 5 years, and *L. scoparium* and *M. australis* the least (Phillips et al. 2011a). In terms of root tensile strength *S. tetraptera* was the strongest and the fleshy roots of *C. robusta* the weakest (Phillips et al. 2011a). Root site occupancy modelling predicts *P. regius* and *C. australis* would stabilise soil the most effectively (Phillips et al. 2011a).

Section B: Comparative investigation of native rhizosphere profiles

The below-ground morphology of New Zealand native plants are known to vary considerably. This section provides an overview of the literature involving comparative studies of native species root systems. In addition, the findings of a preliminary field investigation are presented. The rhizosphere soil profiles of six native species were described (i) to identify the extent of their root systems at the LUDF restoration site and compare this with the literature (Marden et al. 2005, Marden and Phillips 2009) and (ii) to identify variation in soil chemistry parameters that warrant further investigation.

3.5 Native rhizosphere soil profiles at the Lincoln University Dairy Farm field study site

Soil pedology, soil physico-chemistry and root structure were compared in soil profiles beneath one of each of six native plant species (*C. robusta, P. tenuifolium*, *K. ericoides, P. tenax, A. richardii* and

C. australis) at the LUDF site. Plants had been established for 5 years at the time of this research. The LUDF site is on a Templeton silt loam soil (Hewitt 1998) (Typic halustept soil, Soil Survey Staff 2014). A further description of the soil properties and history of this site can be found in Chapter 4 and Chapter 6. Plants were selected in relatively open areas, in the centre of the plot, to minimise the influence of both the roots of other plants and variation of local soil conditions.

3.5.1 Soil investigation

Soil pits were excavated by hand in October 2013 to a depth of 0.9-1.0 m, directly adjacent to the centre of each individual plant (to reach the vertical extent of roots). Branches and leaves of the plants were cut away on one side to position the exposed soil profile below the stem or centre of the plant. Pits were 1.0-1.5 m wide across the principal face, extended in order to reveal the lateral extent of roots (in the case of *C. australis* roots extended further than the pit, 1.5 m wide). The sides of the pits were of approximately 0.5 m breadth to allow access. Soil was carefully removed from around the roots leaving them intact protruding from the pit wall to facilitate accurate description of root size and distribution. Horizon depths, root size and density and other morphological features were described using standard methodology (Milne et al. 1995) on the principal wall (rhizosphere wall) and an adjoining, side wall (chosen for least influence of surrounding plants). The adjoining wall was described for comparison to the rhizosphere profile.

Soil samples were collected from directly below the centre of the principal face, at 0.15, 0.3, 0.45, 0.6 and 0.9 m depth, for physico-chemical analysis. Soil samples were immediately sieved (4 mm) and then stored in a fridge until analysis the following day. A subsample of soil was dried at 105 °C to determine gravimetric soil moisture content. A 4 g subsample of moist soil was shaken with 40 mL of 2 M potassium chloride (KCl) for 1 hour, centrifuged at 2000 rpm for 10 minutes and then filtered (Whatman No. 41) (Blakemore et al. 1987). The KCl extracts were frozen until they could be analysed by Flow Injection Analyser (FIA) (FOSS FIAstar 5000 triple channel with SoFIA software version 1.30; Foss Tecator, Hoganas, Sweden) for ammonium-N (NH_4^+-N) and nitrate-N (NO^{3-}-N). The remaining soil was air dried (35 °C for 48 hours), ground and sieved (2 mm) for subsequent analysis. Soil pH was measured in suspension with water (10 g of air-dried soil to 25 mL of water) (S20 SevenEasy™ pH; Mettler-Toledo, Switzerland) (Blakemore et al. 1987). Total organic carbon (C) was measured using the loss on ignition method (Blakemore et al. 1987), 10 g of air-dried soil was weighed (after desiccation at 100 °C for 2 hours), ignited in a muffle oven (500 °C for 4 hours), then re-weighed.

3.5.2 Rhizosphere profile descriptions

A comparative description of findings is presented here, as replication with statistical analysis was not possible. A full pedological description of the rhizosphere profiles (and adjacent walls for comparison) can be found in Table A.1. (see Plate A.1 for associated photographs). Figure 3.3 provides a comparison of profile coverage by roots of different size classes. The rooting profiles show a large amount of variation between native species (Plate 3.14, Plate 3.15, Plate A.1), consistent with the findings of Marden et al. (2005) and Marden and Phillips (2009) for plants of similar age.

Description and comparison of root structure

C. australis had a substantial (70-90 mm diameter) taproot-like structure to a depth of 0.8 m (Plate 3.15a, Figure 3.3). Lateral fine roots extended horizontally from the taproot in the upper 0.4 m of soil and diagonally from 0.4-0.6 m to a depth of 1.0 m. *C. australis* was the only species to develop a substantial amount of roots below the Ah horizon. The vertical and lateral extent of roots was greater than previously recorded for *C. australis* of this age (Czernin and Phillips 2005, Marden et al. 2005). Soil under *C. australis* was noticeably softer than under the other species.

The fibrous root systems of *P. tenax* and *A. richardii* were extensive (Plate 3.15b and c, Figure 3.3) and consistent in extent with minimal previous description for young plants (McGruddy 2006, Marden and Phillips 2009). The soil profile was more extensively covered by roots (<6 mm) under *A. richardii* than *P. tenax*, however the roots of *A. richardii* were largely confined to the top 0.3 m of soil (*P. tenax* to 0.4 m). Both species had a few very fine roots extending vertically to almost 1.0 m. Notably, two types of roots were present in *P. tenax*, a many branched network of orange roots in the upper soil profile and additional straight, transparent, water-filled roots descending vertically (Atkinson (1922) gave a similar description).

Plate 3.14 **Above- and below-ground morphology of *C. australis* (a), *P. tenax* (b), *A. richardii* (c), *C. robusta* (d), *K. robusta* (e) and *P. tenuifolium* (f) at the Lincoln University Dairy Farm site.**

Plate 3.15 **Excavated rhizosphere soil profiles of *C. australis* (a), *P. tenax* (b), *A. richardii* (c), *C. robusta* (d), *K. robusta* (e) and *P. tenuifolium* (f) at the Lincoln University Dairy Farm site.**

Root structure and extent varied between the woody, dicotyledonous species examined (Plate 3.15d-f, Figure 3.3). *C. robusta* and *P. tenuifolium* had shallow, laterally-spreading roots (up to 0.5 m from the trunk). Marden et al. (2005) report similar laterally-spreading roots for *P. tenuifolium*. This species additionally had several very coarse roots descending vertically to the base of the Ah horizon, and many coarse, medium and fine roots (Plate 3.15f, Figure 3.3). The root ball of *P. tenuifolium* was concentrated within 0.45 m of the trunk. While *C. robusta* was rooted to a similar extent, this species had less large roots. Two gross roots extended diagonally from the base of the *C. robusta* trunk, in addition to many medium and very fine roots (Plate 3.15d, Figure 3.3). The maximum rooting depth of *C. robusta* was similar to previous records (Marden et al. 2005). *P. tenuifolium* was deeper rooted at the LUDF site than when examined by Marden et al. (2005).

In contrast to *C. robusta* and *P. tenuifolium*, the root ball of *K. robusta* was narrower but deeper, with medium, fine and extremely fine roots extending vertically to 0.8 m (Plate 3.15e, Figure 3.3). Watson et al. (1995) found roots to a similar depth for 6 year old *K. ericoides*. The roots of *K. robusta* were the woodiest of the dicotyledonous species, with a bark-like covering of larger roots.

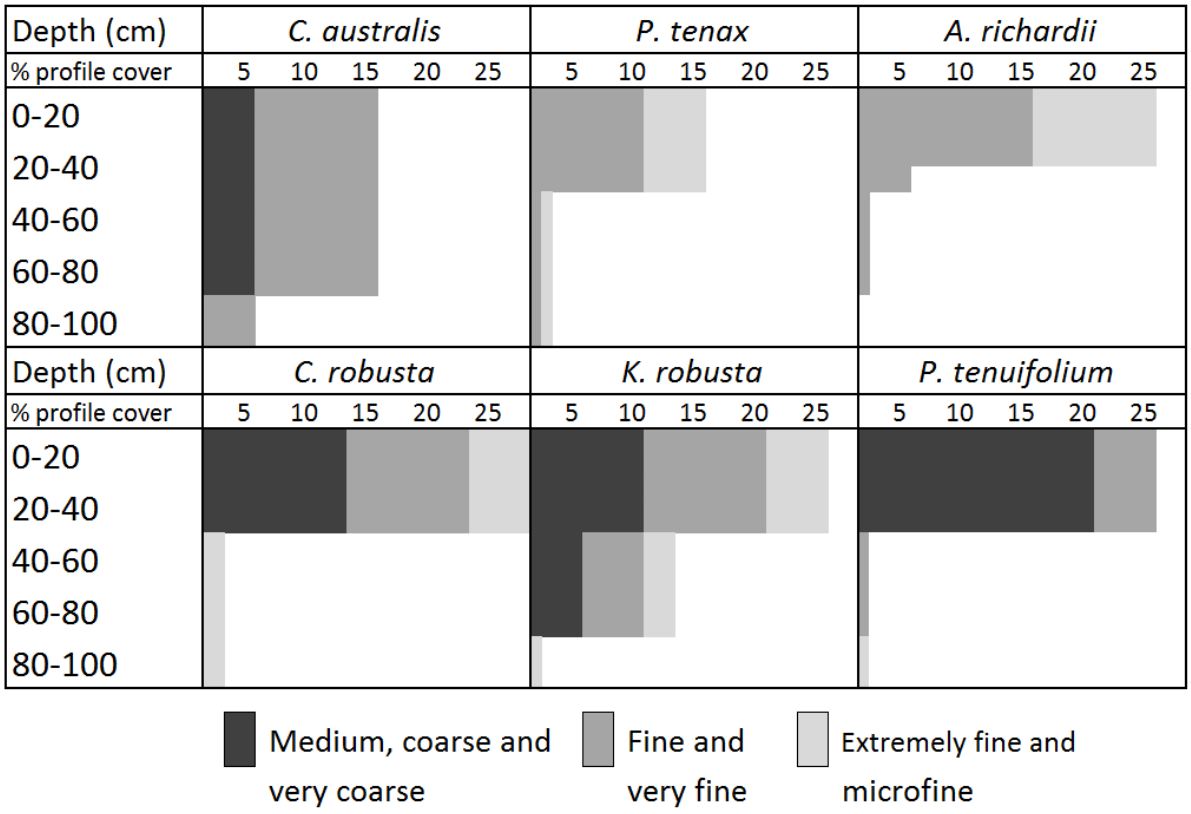


Figure 3.3 Distribution of roots in different size classes as described in rhizosphere soil profiles of six native species studied at the Lincoln University Dairy Farm site. Root size classes are those defined by Milne et al. (1995), in mm; very coarse, 60-100; coarse, 20-60; medium, 10-20; fine, 6-10; very fine, 2-6; extremely fine, 1-2; microfine, <1.

Comparison of soil pedology and physico-chemistry

The roots of *C. australis*, *P. tenax*, *C. robusta* and *P. tenuifolium* affected the boundary between the Ah and lower horizon. The Ah boundary is wavy, curving down where roots are present for these species, compared to a distinct or abrupt boundary on the adjoining wall (highlighted cells in Table A.1, and visible in Plate A.1a.1, b.1, d.1 and f.1). For *C. australis*, the boundary between the Bw and Bw(g) horizon was also affected. Roots were more abundant and of larger size classes in the Ah and Bw horizons of all species when compared to the adjacent side wall. Increased organic matter (root exudates and dead roots) surrounding roots in the Ah horizon has potentially contributed to the depression of this boundary. There were few other differences in pedological characteristics (colour, texture, consistence, structure or macrofabric) when comparing the rhizosphere profile and adjoining wall. Mottles were more abundant in the upper profile of the *K. robusta* rhizosphere and worm castings were spread deeper in the rhizosphere profile of the *C. australis*.

Between-species differences in soil physico-chemistry were more pronounced in the upper soil profile. Potentially the upper profile contains more roots, exerting variable influence over the soil. For soil moisture, total organic C and NH_4^+-N differences between species are reduced in the samples from 0.6 m depth and below (Figure 3.4). Soil pH increased with depth (Figure 3.4), typical of most soil profiles, due to increased leaching in the upper horizons and the displacement of metal cations by the hydrogen ions of organic acids (McLaren and Cameron 1996). Additionally exudates from the abundant roots in the upper profile may have acidified the soil. Total organic C decreased with increasing depth under most species (Figure 3.4), probably due to root exudates and other plant derived organic matter inputs in the upper profile. Nitrate-N and NH_4^+-N decreased slightly with depth (Figure 3.4), although NO_3^--N under *K. robusta* differed from the other species in that it increased in concentration with depth (Figure 3.4). Further sampling is needed to ascertain if this trend is related to *K. robusta* or some other feature of this particular soil profile.

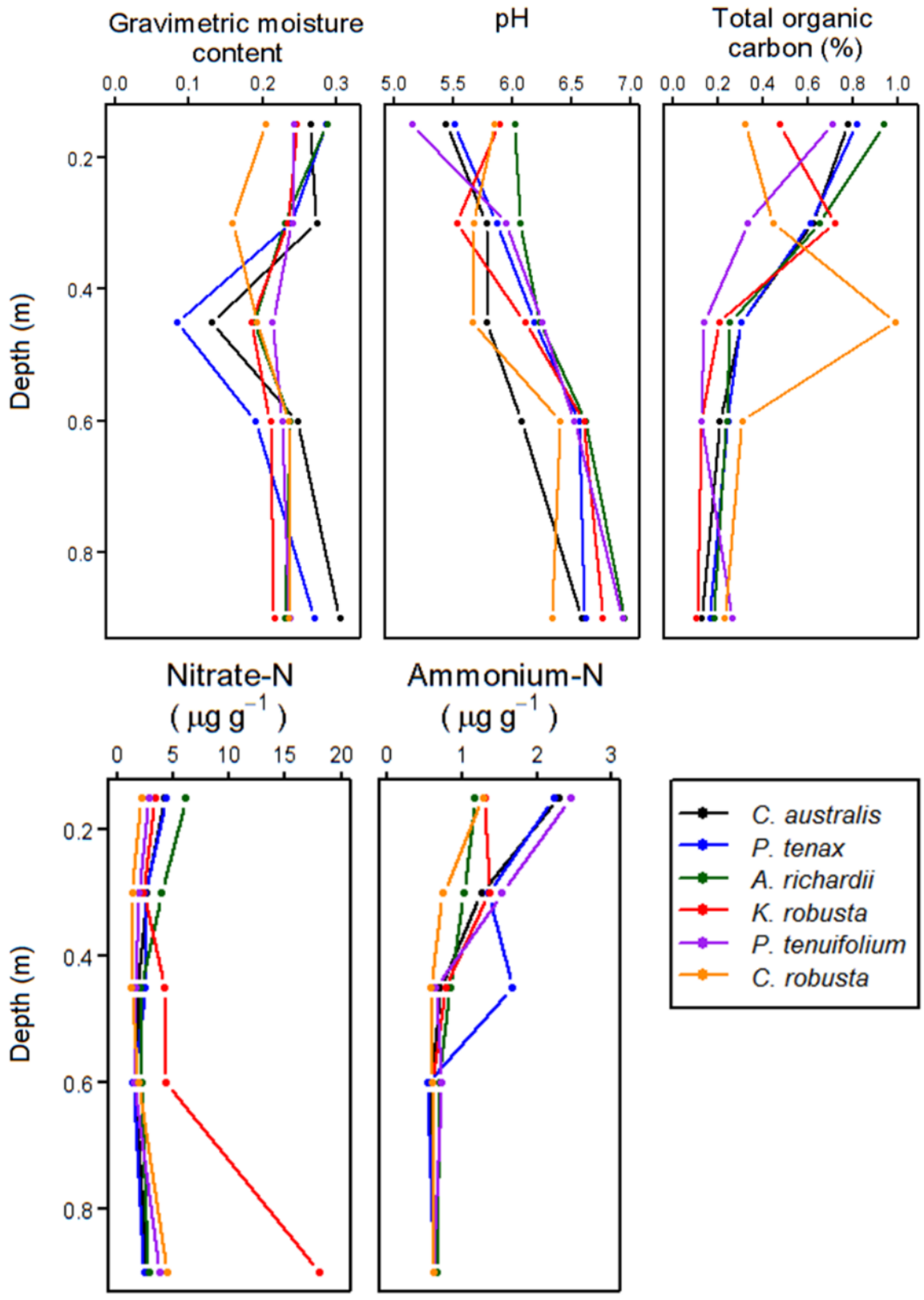


Figure 3.4 **Soil physico-chemical properties at increasing depth below six New Zealand native plant species. Samples were collected from directly below the plant centre/trunk of excavated rhizosphere profiles at the Lincoln University Dairy Farm site.**

3.6 Summary

The excavation of native plant rhizospheres at the Lincoln University Dairy Farm has largely confirmed root system morphology previously reported. It is clear that native species are capable of quickly (within 5 years) developing root systems with extensive coverage of the upper 0.3-0.5 m of the soil profile. Root systems varied between the large woody roots of the dicotyledons and the fine fibrous roots of the monocotyledons. *C. australis* has a unique taproot root system and many fine roots extending to greater depths than other native species. The depression of the Ah horizon around some species and variable soil physico-chemistry in the upper profiles may represent species-specific effects on the soil, with the potential for plants to influence soil N dynamics differentially.

Chapter 4

Rhizosphere and foliar nitrogen status of New Zealand native plants in agricultural landscapes

4.1 Introduction

Since human arrival, around 800 years ago, some 55% of New Zealand's native forest cover has been removed (Atkinson and Cameron 1993, Brockerhoff et al. 2008). In the productive Canterbury Plains region of the South Island, the landscape is highly modified and depauperate in native plants (Meurk 2008). Introduced species are used for all production agriculture in New Zealand and are planted in many shelterbelts and hedgerows (Meurk 2008). One third of New Zealand's land area is in grazed pastures (*Lolium perenne*, perennial ryegrass, most commonly), with dairy products being the country's biggest export commodity (Ministry for the Environment 2007). Recent interest in increasing native biodiversity in agricultural landscapes has seen New Zealand native species replace exotic trees and shrubs along paddock and riparian margins (Meurk and Swaffield 2000, Meurk and Hall 2006). Native plantings provide refugia and habitat corridors for native fauna to pass through agricultural landscapes (Meurk and Swaffield 2000). Native species also offer a range of valuable ecosystem services to agriculture, for example, increased crop pollination, pest and disease control (Sandhu et al. 2008).

Naturally fertile soils are of limited extent in New Zealand (Molloy 1998, Leathwick et al. 2003). The primary rocks are low in essential plant nutrients (McLaren and Cameron 1996) and many have been strongly leached or weathered (Molloy 1998, Wardle 2002). Extensive use of nitrogen (N), in the form of fertilisers and livestock effluents, has been necessary to maintain high productivity of introduced agricultural plant species, adapted to soils that are more fertile. Despite advances in the management of soil N on farms, N losses from agricultural land are widespread (Di and Cameron 2002b, Larned et al. 2004). Excess leaching of nitrate (NO_3^-), the most mobile form of soil N, can lead to eutrophication of waterways and endanger aquatic and human health (Carpenter et al. 1998). Additional N losses from soil can occur as nitrous oxide, a potent greenhouse gas, produced largely by biological denitrification (de Klein et al. 2001).

Plants perform important roles in below-ground nutrient cycles (Bardgett and Wardle 2010). Loss of native vegetation in terrestrial systems, particularly adjacent to waterways, alters nutrient uptake by plants and changes the flux of N in soils (Jobbágy and Jackson 2004). The role of vegetation

in removing N form vertical and lateral fluxes of water is well documented in riparian zone research (reviewed by Hill 1996, Mayer et al. 2007). Mechanisms proposed include: assimilation and retention by vegetation; denitrification; and transformation and retention of N in soils, but conclusive evidence is lacking (Correll 1997). The rhizosphere of plants is a potential "hotspot" for the action of biological and chemical mechanisms interacting with soil N (McClain et al. 2003), yet there has been scant species-specific research.

Like many temperate forest systems, natural forest in New Zealand is thought to be N limited (Vitousek and Howarth 1991). Many native species are adapted to soils that are low in available nutrients (Wardle 1985). Examples are *Leptospermum scoparium*, which forms early successional shrubland on disturbed infertile sites (Porteous 1993, Wardle 2002) and many native grass species, which have traits associated with low N availability (Craine and Lee 2003). Little is known about the below-ground soil chemistry of New Zealand native species or their ability to assimilate N. A preliminary study found species-specific variation in foliar macronutrients and rhizosphere soil physico-chemistry of native plants (Hahner et al. 2014). Building on these findings, this research investigates the pools of N in the rhizosphere soil of selected native species and their foliar N concentrations.

Differences in root morphology and physiology (such as root biomass, mycorrhizal associations and root exudates), as well as plant nutrient requirements, mean that rhizosphere effects on soil nutrient cycling are likely to vary between species (Richardson et al. 2009). The variable rooting profiles of New Zealand native plants are more extensive than those of exotic pasture grass species (Marden et al. 2005). The spread, density and arrangement of roots is important in determining the amount of solute with which the plant can potentially interact. Additionally, specific chemicals with bio-active properties, known to be associated with native species such as *Phormium tenax* (McGruddy 2006) and *L. scoparium* (Prosser et al. 2014), may influence the functioning of microbes involved in the soil N cycle.

Foliar N concentrations tend to be consistent within species and even across broad taxonomic groups (Wardle 2002). Plants that are adapted to low fertility soil usually have long lived leaves with lower N concentrations than those adapted to fertile sites, as less is potentially extracted from soil (Aerts and Chapin 2000). Therefore, native species may have lower foliar N concentrations than exotics plants which are adapted to more fertile soil (Craine and Lee 2003). It has been suggested that plant species from low fertility habitats have low rates of N mineralisation in the rhizosphere soil, as the conservative nutrient use of such species tends to produce leaf litter with a high carbon (C) to nitrogen ratio (C:N>30), which decomposes and releases nutrients slowly (Hobbie 1992, van der Krift

and Berendse 2001). Agriculture has greatly increased external contributions to the soil N cycle, but the response of native plants to elevated soil N is unknown. Knowledge of the interaction of New Zealand native species with soil N is important in developing their use in agricultural plantings designed to mitigate N losses.

This work presented in this chapter aims to determine variation in the foliar and rhizopshere N status of New Zealand native species at two planted restoration sites established on contrasting soils, and determine whether native plants are distinct from the exotic pasture species *L. perenne*.

4.2 Methods

4.2.1 Site descriptions

Two planted restoration sites in South Island, New Zealand, were selected in rural areas, with soil of contrasting type and N availability. The sites were situated on the Canterbury Plains, which are the largest alluvial plains in New Zealand, consisting of a series of gently sloping fans built up by four major rivers (Molloy 1998). The pre-colonisation vegetation of this lowland region (mild sub-humid climate, rainfall c. 800 mm yr^{-1}) consisted of podocarp-broadleaf forest on deeper soils, *Kunzea* shrubland on stony, free-draining soils, and some grassland species primarily on disturbed sites (McGlone 1989, Wardle 2002). The two restoration sites were planted with a range of native species (4-5 years prior to this study) which are commonly used in restoration and agricultural planting in Canterbury. The species included in this study were native species of early succession, frequently found growing naturally in riparian or marginal lowland environments, in Canterbury and throughout New Zealand. The sites were Lincoln University Dairy Farm (LUDF) and Selwyn Huts (SH) (Figure 4.1a).

The soil at the LUDF site is a Templeton silt loam (Immature Pallic, Hewitt 1998, Udic Haplustept, Soil Survey Staff 2014) developed from alluvium. This is one of the most fertile, agriculturally important soils in the Canterbury region, covering 10 % of the intermediate terraces on the plains (Molloy 1998). The LUDF site is a fenced triangular area (c. 1800 m^2), in the southwest corner a dairy farm (43°38'38.07" S, 172°26'1.96" E, Figure 4.1a and 4.1b). Drainage ditches on two sides and a dairy paddock on the third, border the site, which is beyond the reach of the centre-pivot irrigator. The farm was converted from former dry sheep pasture in 2001. The site was retired from dairy grazing in early 2008 (sprayed, ripped and rolled in preparation) and planted with one year old native seedlings in spring 2008.

The SH site is on an anthropic soil (Fill Anthrohpic, Hewitt 1998, Anthropic, Soil Survey Staff 2014) with a sandy loam texture and a thin organic matter layer, derived from river silt. The site is situated on the margins of Te Waihora/Lake Ellesmere, on top of a raised stopbank, adjacent to the Selwyn River mouth (43°44'31.73" S, 172°26'47.67" E, Figure 4.1a). The stopbank consists of silt dredged from the river in the late 1940's, designed to offer flood protection to the Selwyn Huts settlements (Singleton 2007). A section of the stopbank (c. 500 m long, 15-20 m wide), which runs from the settlement (Lower Selwyn Huts) to the lake edge, is fenced and planted. The surrounding land is periodically dry grazed with sheep, and it is likely that grazing also occurred within the site prior to fencing. High lake levels occasionally inundate the surrounding paddocks, but the bank is never flooded. The SH site was cleared of gorse and other weeds and planted with one year old native seedlings in 2009, either side of an access track.

At both sites, glyphosate weed control in first two seasons after planting has maintained a largely bare ground surface between plants. At the time of the present research, exotic weeds and the pasture species *L. perenne* had established in isolated patches between the native plants. Native plants were 6 and 5 years old at the LUDF and SH sites respectively during this research (established at the sites 5 and 4 years prior). Canopy closure had occurred in some areas at the LUDF site, with the fast growing native tree species (e.g. *Plagianthus regius* and *Cordyline australis*) >2 m in height.

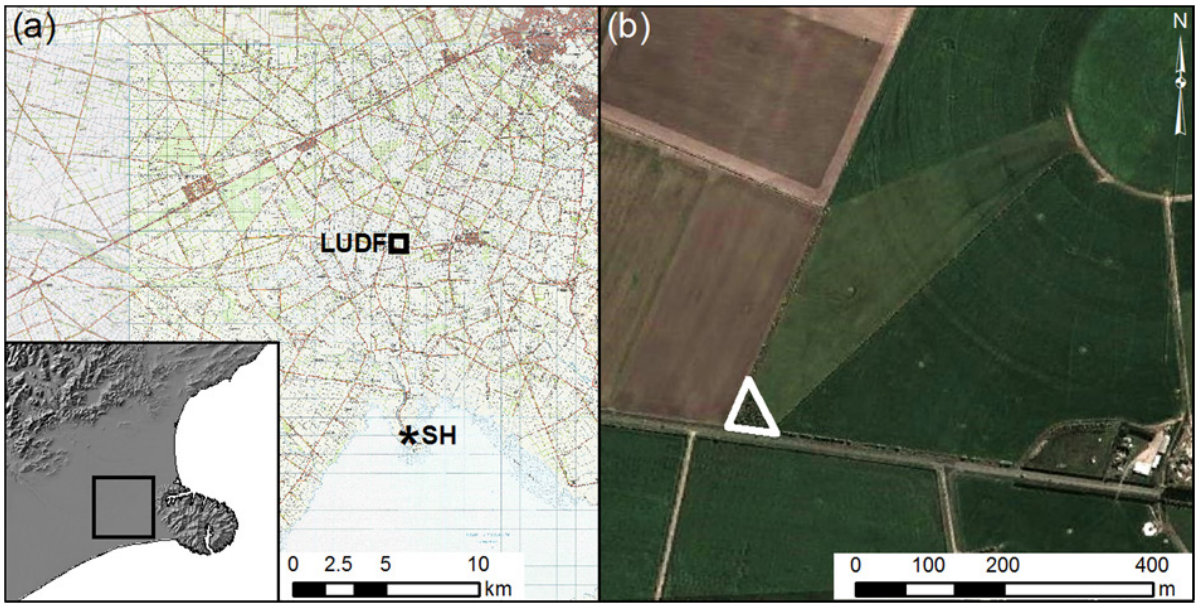


Figure 4.1 (a) The location of the Lincoln University Dairy Farm (LUDF) and Selywn Huts (SH) sites, southwest of Christchurch, New Zealand (Image sourced from Topo50 Map BX23 Lincoln Crown Copyright Reserved) and (b) the LUDF site in the corner of the Lincoln University Dairy Farm (Image from Google Earth, Image © 2014 DigitalGlobe, 17/02/2014, 43°38'25.95" S, 172°26'34.37" E, elevation 17m).

4.2.2 Field survey sampling strategy – soil and foliage

In October 2013, foliar and rhizosphere soil samples were collected from five replicate individuals of nine native species, selected at separate locations randomly distributed within each site (Table 4.1). Bulk foliar samples were collected from multiple parts of the canopy of each individual plant. Rhizosphere soil samples were collected using a hand trowel beneath each plant. Soil was taken from a depth of 0.15-0.30 m vertically below the trunk or centre of the plant, within a sampling zone no more than 0.1 m radially from the central point, ensuring the exclusion of the leaf litter layer (Figure 4.2). Bulked foliar samples of the exotic pasture species *L*. *perenne* were collected from five randomly selected patches growing within each site, in areas without native plants within a 2 m diameter. Rhizosphere soil (0.15-0.30 m depth) was taken from below each patch of *L*. *perenne*, from a similar soil extent as for the native species. At the LUDF site, native plants and patches of *L*. *perenne* established from seed, from which soil pore water was sampled (see 4.2.3), were excluded from random selection.

Table 4.1 The nine New Zealand native species sampled at each site. The scientific names and authority, family, allocation into monocotyledons or dicotyledons and the common Māori and English names, are given for each species.

Species	Family		Māori and English names
Cordyline australis [1] (G. Forst.) Endl. (1883)	Asparagaceae	monocotyledons	tī kōuka, cabbage tree
Phormium tenax [1] J.R.Forst. et G.Forst. (1976)	Xanthorrhoeaceae	monocotyledons	harakeke, New Zealand flax
Austroderia richardii [1] (Endl.) N.P.Barker et H.P.Linder (2012)	Poaceae	monocotyledons	toetoe
Coprosma robusta [1] Raoul (1844)	Rubiaceae	dicotyledons	karamu
Pittosporum tenuifolium [1] Sol. ex Gaertn. (1788)	Pittosporaceae	dicotyledons	kohuhu, black matipo
Kunzea robusta [1] de Lange et Toelken (2014)	Myrtaceaea	dicotyledons	kānuka, white tea tree
Sophora microphylla Aiton (1789)	Fabaceae	dicotyledons	kōwhai, small-leaved kōwhai
Olearia paniculata (J.R.Forst. & G.Forst.) Druce (1917)	Asteraceae	dicotyledons	akiraho, golden akeake
Plagianthus regius (Poit.) Hochr. (1907)	Malvaceae	dicotyledons	manatu, lowland ribbonwood

[1] Species at the LUDF site from which soil pore water was sampled.

4.2.3 Soil pore water sampling

Five replicate even-sized plants were selected, of a subset of six of the native species, at separate locations, randomly distributed within the LUDF site (Table 4.1, Figure 4.1b). Reference plots were also selected at locations without native plants within at least 2 m. Sampling pits were dug immediately beside each plant in 2011, as described in (Hahner et al. 2014). Briefly, a 0.3 m × 0.3 m pit (0.5 m depth) was dug, 0.40 m from the stem or centre of the plant, in a randomly chosen direction (Figure 4.2). Roots had grown out of the pit wall into the pit since the time of construction. Rhizon soil moisture samplers, (Eijkelkamp Agrisolutions Equipment, The Netherlands) 100 mm × 25 mm (0.1 µm pore size), were inserted into the rhizosphere soil at 0.15 and 0.30 m depth (Figure 4.2), in early September 2013. Samplers were inserted so that the filter was at least 20 mm behind the pit wall to avoid any effects of the exposed face on the soil chemistry (Figure 4.2). Seeds of the pasture species *L. perenne* (perennial ryegrass, cultivar ev one 50 AR1) were sown adjacent to sampling pits in reference plots, at 20 kg ha^{-1} (recommended rate, Agricom 2011) in winter 2013. Following germination, *L. perenne* was maintained by manually clipping the sward to a height of 0.10 m approximately fortnightly (clippings removed).

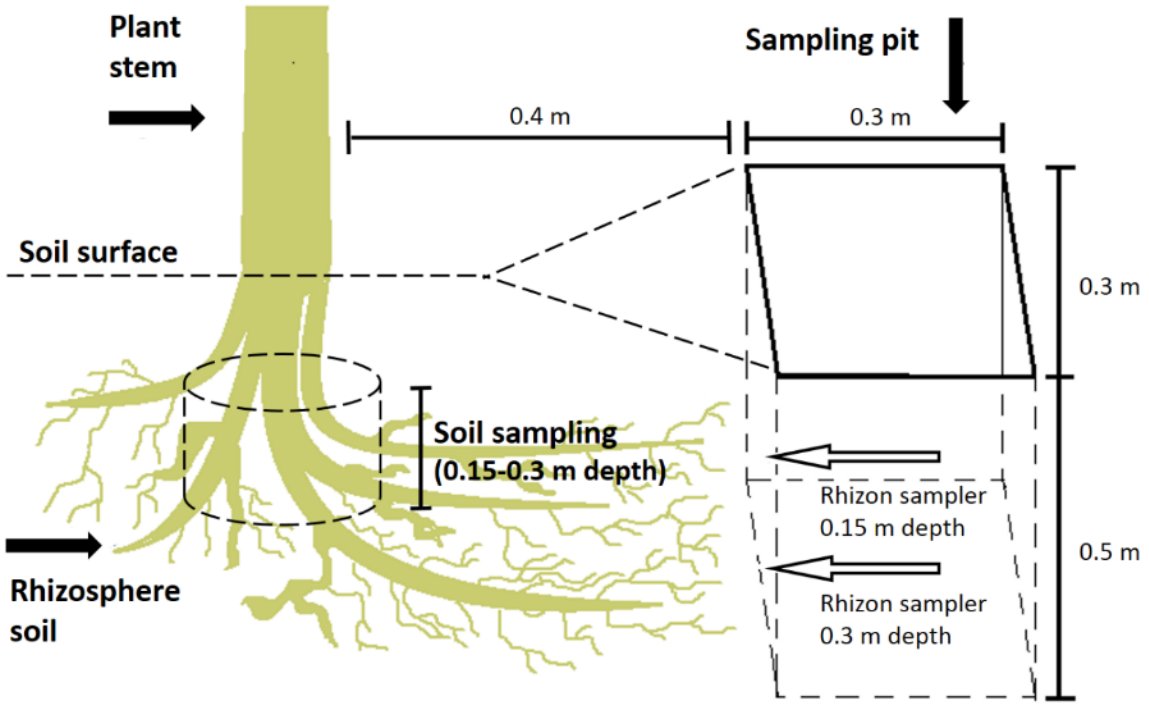


Figure 4.2 Schematic showing the location of rhizosphere soil and soil pore water collection (adapted from Hahner et al. 2014). Rhizosphere soil was collected from the soil sampling zone (0.1 m radially in line with plant stem/centre) beneath nine native species and *L. perenne*, at the Lincoln University Dairy Farm (LUDF) and Selwyn Huts sites. Soil pore water was collected from Rhizon samplers inserted into the sampling pits wall adjacent to six native species and *L. perenne* at the LUDF site (note that different individual plants were used for soil and pore water sampling).

After an equilibration period of 14 days following the installation of Rhizon samplers, pore water was sampled fortnightly, on four occasions during September and October 2013. A 30 mL syringe was attached to each Rhizon sampler, drawn out to 22 mL and held in place by a wooden stopper to create a vacuum. Water was transferred to a 30 mL plastic vial during sampling (frozen immediately for later analysis) and the syringe re-attached. During the summer period, pore water collection was not possible due to low soil moisture. In a previous study (2011) at this site dairy shed effluent application (50 kg N ha^{-1} applied inside wooden frames placed between the sampling pit and plant) did not lead to significantly increased flux N through the soil profile (Hahner et al. 2014). The effects of this previous application on soil N levels are considered negligible after two winters of rain passing through the soil and confirmed by comparison of N levels in spring samples from 2011 (Hahner et al. 2014) and those from 2013 in the present study.

4.2.4 Chemical analyses

Rhizosphere soil samples were immediately sieved (4 mm), transported to the laboratory and stored in a fridge for analysis the following day. A subsample of soil was dried at 105°C to determine gravimetric soil moisture content. A 4 g subsample of moist soil was shaken with 40 mL of 2 M potassium chloride (KCl) for 1 hour, centrifuged at 2000 rpm for 10 minutes and then filtered (Whatman No. 41) (Blakemore et al. 1987). The KCl extracts were frozen until they could be analysed by Flow Injection Analyser (FIA) (FOSS FIAstar 5000 triple channel with SoFIA software version 1.30; Foss Tecator, Hoganas, Sweden) for ammonium-N (NH_4^+-N) and nitrate-N (NO^{3-}-N). The remaining soil was air dried (35°C for 48 hours), ground and sieved (2 mm) for subsequent chemical analysis. Soil pH was measured in suspension with water (10 g of soil to 25 mL of water) (S20 SevenEasy™ pH; Mettler-Toledo, Switzerland) (Blakemore et al. 1987). Total organic C was measured using the loss on ignition method (Blakemore et al. 1987), 10 g of air-dried soil was weighed (after desiccation at 100 °C for 2 hours), ignited in a muffle oven (500 °C for 4 hours), then re-weighed. Foliar samples were dried (60 °C for 48 hrs) and ground to <200 µm. Total C and N in dried ground foliar material and soil samples were determined using Dumas combustion method on CNS Elemental Analyser (LECO Elemental Analyser, NSW, Australia). Soil pore water samples were thawed and analysed for NH_4^+-N and NO_3^-- N concentrations by FIA.

4.2.5 Statistical analyses

Mean and standard errors for pH measurements were calculated by conversion to the equivalent hydrogen ion concentrations and back calculation to pH. The overall effects of site, species and their interaction on foliar and rhizosphere soil parameters were assessed using two-way analysis of variance (ANOVA). Data were transformed as required to meet the assumption of homogeneity of variances.

Following the identification of a significant species x site interaction, one-way ANOVA with post-hoc tests (Fisher's least significant difference (LSD) test) was used to examine the effect of species, and differences between species, on foliar and soil at each site. Repeated measures ANOVA, was used to examine the effect of depth, species and sampling date on soil pore water NO_3^--N and NH_4^+-N. Type III sums of squares were used as the data were non-orthogonal. Following this, one-way ANOVA with post-hoc tests (Fisher LSD test) was used to examine the effect of plant species on pore-water NO_3^--N and NH_4^+-N at each sampling depth, on each date.

A principle components analysis (PCA) was conducted on the rhizosphere soil data from each site (soil moisture, pH, total organic C, NO_3^--N and NH_4^+-N, total N and total C), after transforming the data to meet the assumptions of normality, using the Euclidian distance function on standardised data. Pearson's correlation coefficient (r) was used to test for linear relationships between variables. All analyses were conducted using R version 3.0.1 (R Development Core Team, 2010, R Foundation for Statistical Computing, Vienna, Austria, http://www.r-project.org/) using base software, the "vegan" package to perform the PCA (Oksanen et al. 2009) and the "agricolae" package to perform post-hoc testing (de Mendiburu 2014).

4.3 Results

4.3.1 Foliar nitrogen status

Foliar N concentrations varied significantly between species (p<0.001) and there was a significant site by species interaction (p<0.001). There was no difference in mean foliar N concentrations between the two sites. *S. microphylla* and *P. regius* had significantly higher mean foliar N concentrations than other native species and *L. perenne* at both sites (p<0.001, Figure 4.3). *L. perenne* had significantly higher foliar N than the remaining native species at the LUDF site, and than *C. australis* and *O. paniculata* at the SH site (Figure 4.3). *C. australis* and *O. paniculata* had the lowest mean foliar N concentrations, significantly less than *L. perenne*, at both sites (Figure 4.3). Foliar N concentrations were not significantly correlated with rhizosphere soil total or available N at either site.

Plant species (p<0.001), site (p<0.01) and their interaction (p<0.001) had significant effects on the foliar C:N ratio. The mean C:N ratio at the SH (25.9) site was higher than at LUDF (23.8). *C. australis* had significantly higher mean foliar C:N ratios than all other species at both sites , while *S. microphylla* and *P. regius* had significantly lower C:N ratios than others (p<0.001, Figure 4.3). *C. australis, P. tenax* and *O. paniculata* had significantly higher mean foliar C:N ratios than *L. perenne* at both sites.

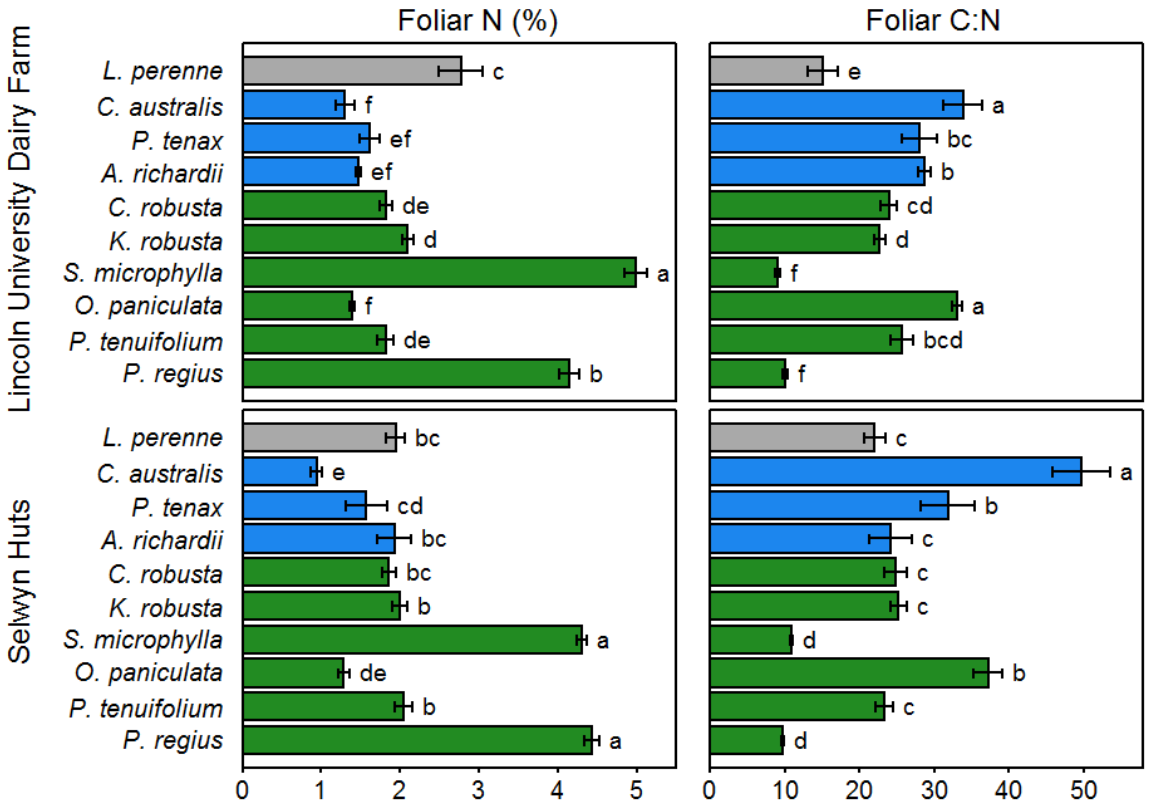


Figure 4.3 Mean (±SE) N concentration and C:N ratio of foliage. Samples collected from nine native species (green bars, dicotyledons; blue bars, monocotyledons) and *L. perenne* (grey bars) at the Lincoln University Dairy Farm and Selwyn Huts sites. Means for each site were compared using ANOVA (n=5). For each site, means which share a letter are not significantly different.

4.3.2 Rhizosphere nitrogen status

Plant species (p<0.001), site (p<0.001) and their interaction (p<0.01) had significant effects on the concentration of NO_3^--N in rhizosphere soil extracts. Mean NO_3^--N was higher at the LUDF site (7.04 µg g^{-1}) than at SH (1.55 µg g^{-1}). *A. richardii* and *P. regius* had significantly higher mean NO_3^--N concentrations in the rhizosphere soil at the both sites (LUDF, p<0.001 and SH, p<0.01), when compared to *L. perenne* (Figure 4.4). *A. richardii* had significantly more NO_3^--N than all other species at the LUDF site (Figure 4.4).

Rhizosphere NH_4^+-N concentration was significantly affected by plant species (p<0.05) and the interaction of species and site (p<0.001), but did not vary between sites. Ammonium-N was significantly more concentrated in rhizosphere soil of *P. tenax* than *L. perenne* at the LUDF site (p<0.05, Figure 4.4). Additionally, at LUDF, *P. tenax* and *K. robusta* had significantly higher mean NH_4^+-N concentrations than *A. richardii*, *C. robusta*, and *S. microphylla* (Figure 4.4). At the SH site, all native species, except *K. robusta*, had significantly higher mean NH_4^+-N concentrations than *L. perenne*

(p<0.001, Figure 4.4). Mean NH_4^+-N was significantly higher in rhizosphere soil of the native monocotyledon species and *P. regius* at the SH site when compared to *K. robusta* (Figure 4.4).

The total soil N concentration of rhizosphere soil varied significantly according to plant species (p<0.001), site (p<0.001) and their interaction (p<0.01). Mean total N concentration was higher at the LUDF site (0.29 %) than SH (0.18 %). When sites were examined separately, mean total N concentration did not vary among species at the LUDF site, but did at SH (p<0.001). *A. richardii* had significantly higher rhizosphere soil total N than all other species at the SH site, while *C. australis* had significantly less total N than the other native monocotyledons and *L. perenne* (data not shown). Total N was significantly positively correlated with NH_4^+-N and NO_3^--N in rhizosphere soil at the SH site (r=0.32, p<0.05 and r=0.45, p<0.01 respectively) but not at the LUDF site.

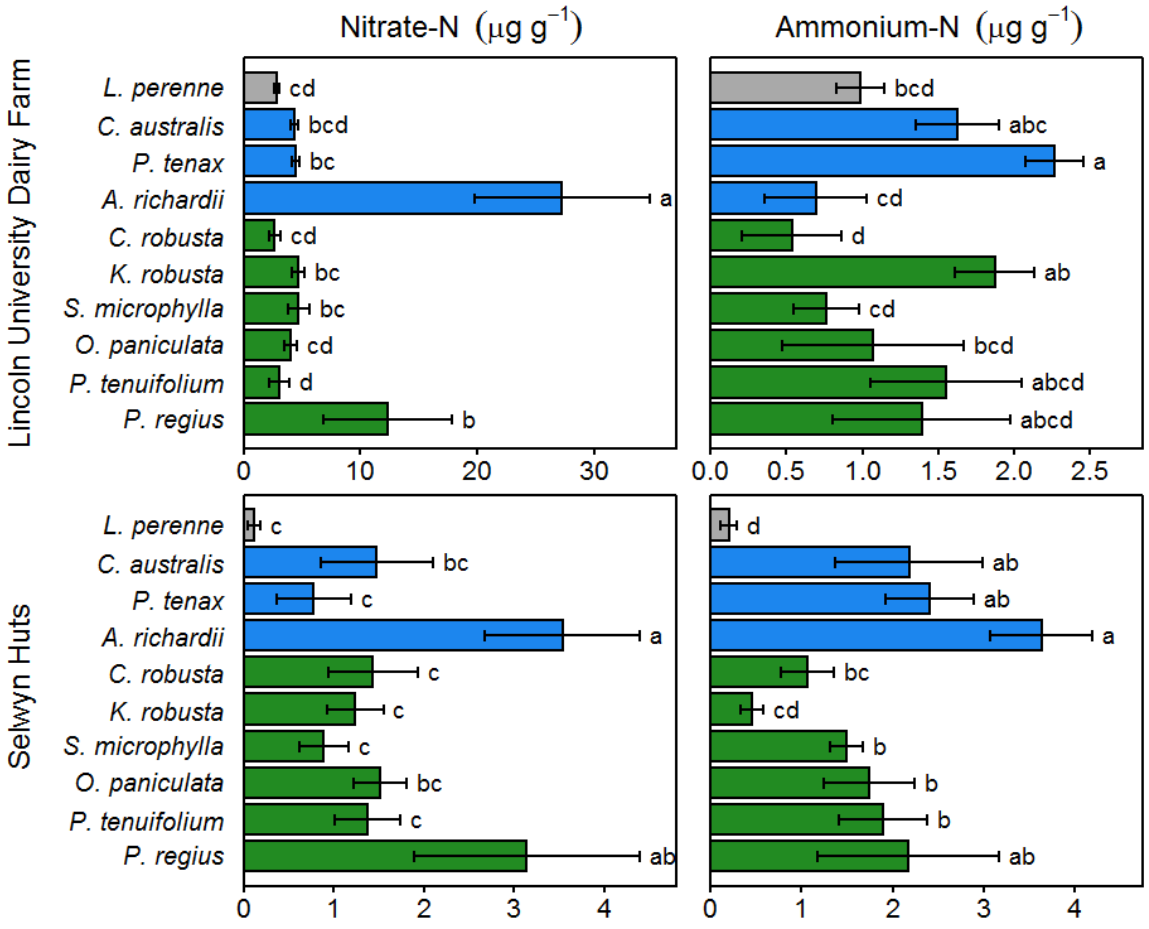


Figure 4.4 Mean (±SE) NO_3^--N and NH_4^+-N concentration in rhizosphere soil extracts. Samples collected from nine native species (green bars, dicotyledons; blue bars, monocotyledons) and *L. perenne* (grey bars) at the Lincoln University Dairy Farm and Selwyn Huts sites. Means for each site were compared using ANOVA (n=5). For each site, means which share a letter are not significantly different.

Soil pore water NO_3^--N and NH_4^+-N concentrations did not vary significantly according to the day sampled during the 2 month sampling period. Missing values occurred in the data for all species, as low soil moisture prevented sample collection in some instances. *C. robusta* and *P. tenuifolium* were not included in analysis as sample numbers were insufficient. Species (p<0.05) and sampler depth (p<0.05) had significant effects on soil pore water NO_3^--N concentrations but and had no effect on NH_4^+-N. *A. richardii* had significantly more NO_3^--N than *L. perenne* in pore water collected at both 0.15 and 0.30 m depth on each sampling occasion (p<0.05, Figure 4.5). In addition, *A. richardii* had significantly higher NO_3^--N concentrations than the other native species at 0.30 m depth (Figure 4.5). Inter-species differences in NO_3^--N concentrations were similar across sampling dates (data shown for 18 September 2013 only).

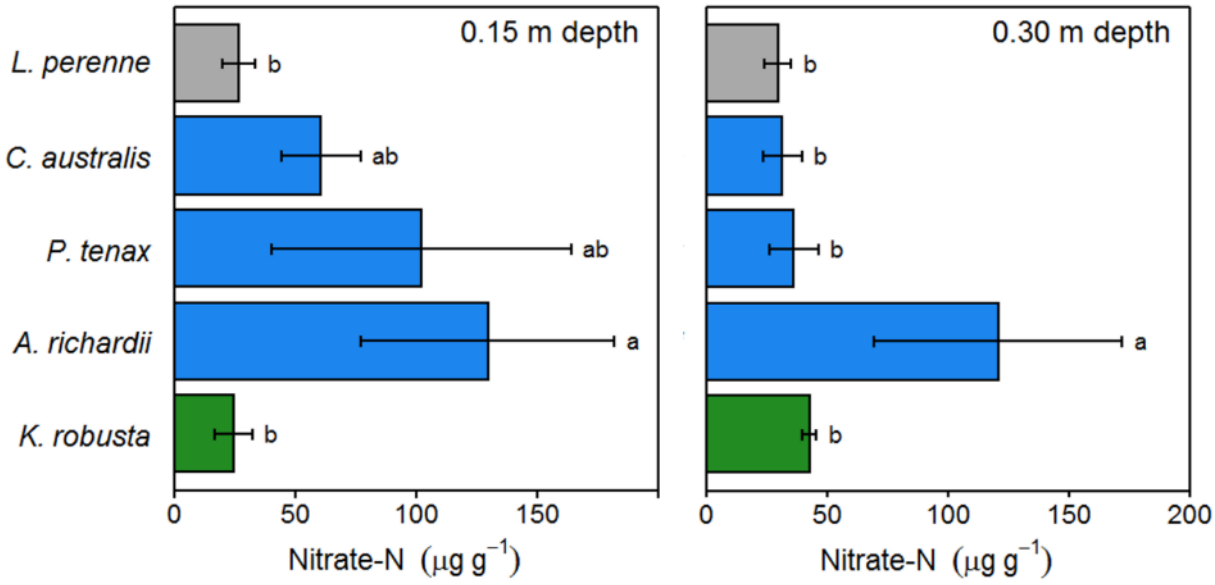


Figure 4.5 Mean (±SE) NO_3^--N in soil pore water samples collected 18 September 2013 at the Lincoln University Diary Farm site. Samples were collected from 0.15 and 0.30 m below 3 native monocotyledons (blue bars, n=4), a native dicotyledon (green, n=3) and an exotic pasture species (grey, *L. perenne*, n=5). Means for each depth were compared using ANOVA, those that share a letter are not significantly different.

4.3.3 Overall rhizosphere soil chemistry and relationship to soil nitrogen

The gravimetric moisture content, total C and total organic C concentrations of rhizosphere soil varied significantly between species (p<0.001) and sites (p<0.001) (and there was a significant species x site interaction, p<0.001). Mean soil moisture and total organic C were significantly higher at the LUDF site (31 % and 0.86 % respectively) compared to the SH site (23 % and 0.54 % respectively). At the SH site,

A. richardii and *P. tenax* had significantly higher mean total organic C and soil moisture than other native species and *L. perenne* (p<0.001, Figure 4.6). There were few between species differences in soil moisture at the LUDF site (*P. regius* had significantly lower soil moisture than *L. perenne, p*<0.01, Figure 4.6). Additionally, at the LUDF site, total organic C concentration was significantly lower in the rhizosphere of *L. perenne* than native species except *P. tenufolium* (p<0.001, Figure 4.6). Patterns in mean total C concentration were similar to those of total organic C (data not shown). Mean soil pH was significantly higher (p<0.001) at the LUDF site (5.4) than at SH (4.7), but did not vary between species.

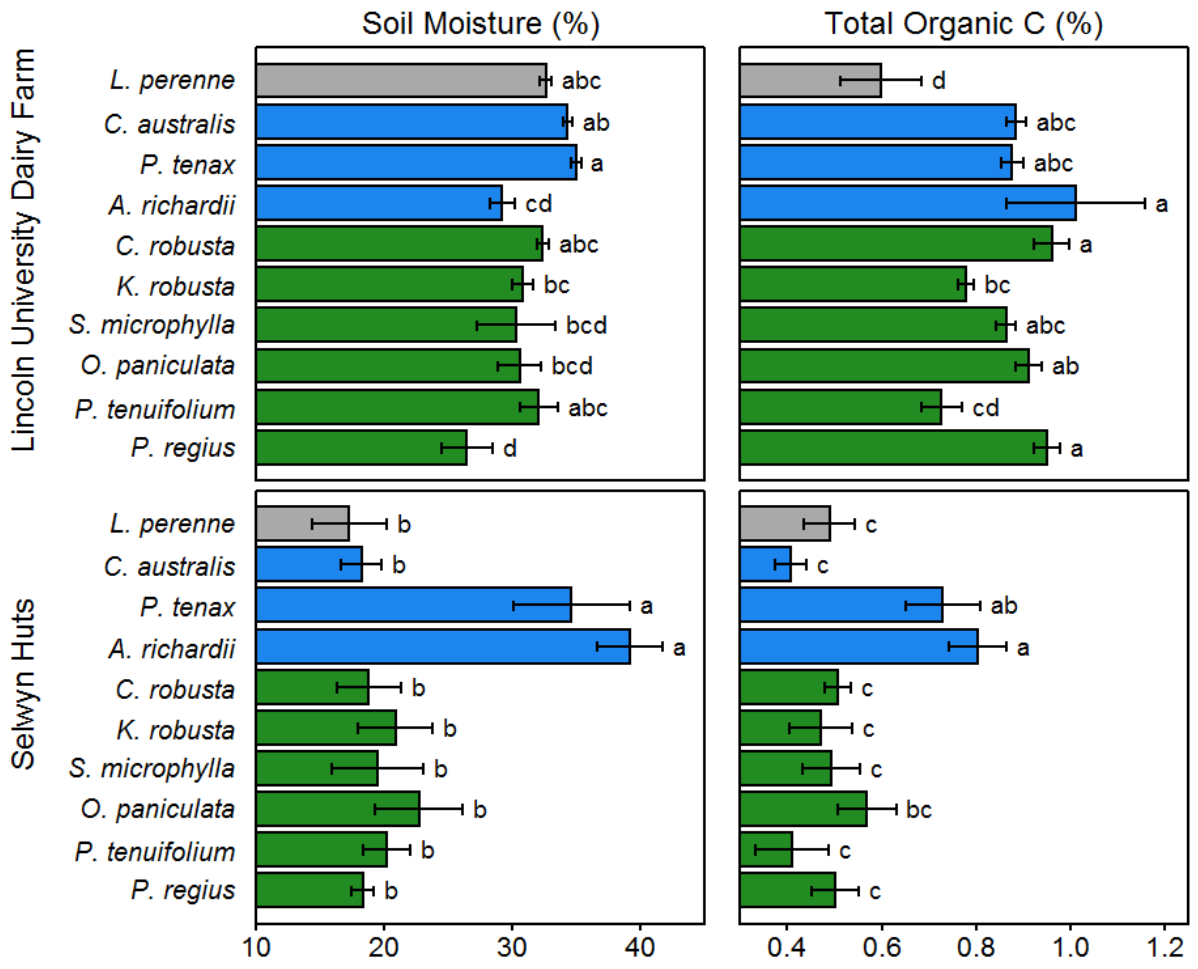
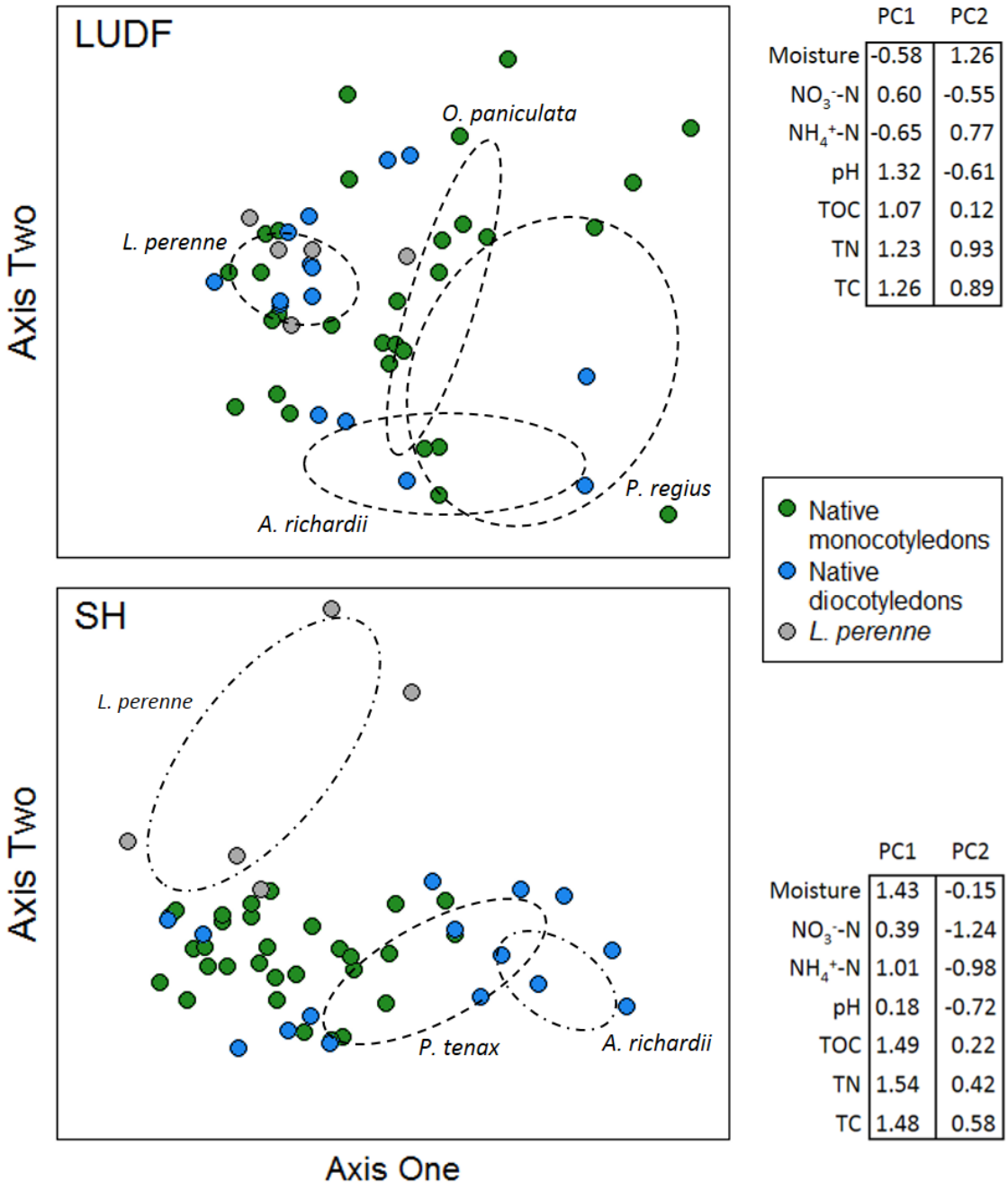


Figure 4.6 Mean (±SE) gravimetric moisture content and total organic C in rhizosphere soil. Samples collected from nine native species (green bars, dicotyledons; blue bars, monocotyledons) and *L. perenne* (grey bars) at the Lincoln University Dairy Farm and Selwyn Huts sites. Means for each site were compared using ANOVA (n=5). For each site, means which share a letter are not significantly different.

Multivariate analyses of the rhizosphere soil data revealed differences between species, at each site, in terms of overall patterns in rhizosphere soil physico-chemistry (Figure 4.7). At the LUDF site there was a distinction in the ordination space occupied by *L. perenne* and select native species; *A. richardii*, *P. regius* and *O. paniculata* (which were comparatively weighted towards the positive end of axis one) (Figure 4.7). Axis one explained 38 % of variation and was positively weighted by total organic C, NO_3^--N and pH. Axis two explained 24 % of variation and was positively weighted by NH_4^+-N and moisture. At the SH site, *L. perenne* was distinct from all native species except *K. ericoides* and *C. robusta* (Figure 4.7). *A. richardii* was distinct from other native species, except *P. tenax*, distributed to the positive end of axis one. Axis one explained 54 % of variation and was positively weighted by total C, total organic C, total N and moisture. *L. perenne* is distributed towards the positive end of axis two compared to the native species. Axis two explains 19 % of the variation and was negatively loaded by pH and NH_4^+-N.

Total soil N was significantly positively correlated with total organic C in rhizosphere soil at the LUDF ($r=0.32$, $p<0.05$) and SH ($r=0.97$, $p<0.001$y) sites. Total soil N was also correlated with soil moisture at the SH site ($r=0.75$, $p<0.001$). At the SH site NO_3^--N and NH_4^+-N were also positively correlated with total organic C ($r=0.32$, $p<0.05$ and $r=0.45$, $p<0.01$ respectively). NH_4^+-N was significantly positively correlated with soil moisture at the LUDF ($r=0.39$, $p<0.01$) and SH ($r=0.49$, $p<0.001$) sites, but NO_3^--N was not.



	PC1	PC2
Moisture	-0.58	1.26
NO_3^--N	0.60	-0.55
NH_4^+-N	-0.65	0.77
pH	1.32	-0.61
TOC	1.07	0.12
TN	1.23	0.93
TC	1.26	0.89

	PC1	PC2
Moisture	1.43	-0.15
NO_3^--N	0.39	-1.24
NH_4^+-N	1.01	-0.98
pH	0.18	-0.72
TOC	1.49	0.22
TN	1.54	0.42
TC	1.48	0.58

Figure 4.7 PCA ordination of rhizosphere soil physico-chemical variables at the Lincoln University Dairy Farm (LUDF) and Selwyn Huts (SH) sites. Points represent individual plants (n=5) of three native monocotyledons (green), six native dicotyledons (blue) and *L.* perenne (grey) from which rhizosphere soil was taken. Ellipses highlight the dispersion of selected species in ordination space (using the standard error of the weighted average of scores), further description of inter-species variation provided in text. The relative loading of the variables onto the axes is tabulated for each site. The first two axes account for 62 % and 73 % of the data variation at the LUDF and SH sites respectively.

4.4 Discussion

4.4.1 Foliar nitrogen status reflects plant strategy

Differences in foliar N concentration between native deciduous and evergreen species in this study were consistent at both sites and appear to reflect differences in plant growth strategy. The foliar N concentrations of *P. regius* (fully deciduous) and *S. microphylla* (brevideciduous in Canterbury) were within the upper limits recorded for New Zealand native deciduous trees (c. 2.0 -5.0 % N, unpublished data of G Hall in McGlone et al. 2004), more than double that of the evergreen native species and *L. perenne* in this study. Native evergreen species at these sites have foliar N concentrations consistent with existing data (c. 0.2-3.0 %, Wardle 2002, unpublished data of G Hall in McGlone et al. 2004, Bellingham et al. 2013, Hahner et al. 2014). *P. regius* and *S. microphylla* form part of about 5 % of New Zealand's woody flora which have marked winter leaf fall (McGlone 1989, McGlone et al. 2004). Leaf longevity and plant nutrient status are closely linked (Aerts and Chapin 2000). Short-lived leaves are typical of fast-growing plants in high fertility habitats. Deciduous trees invest in the rapid production of high nutrient, thin leaves, which decompose quickly in soil for re-absorption by the plant (Aerts and Chapin 2000, Givnish 2002). As high leaf turnover results in greater nutrient losses, in low soil fertility, plant growth is best catered for by retention of multiannual, thick, long-lived, nutrient-conserving evergreen leaves (Aerts 1995). Previous studies have found that New Zealand native deciduous trees often have distributions associated with nutrient rich alluvial soils (Wardle 2002), and have higher foliar N and less lignin (a measure of resistance to decay) in leaves than evergreen species (unpublished data of G Hall in McGlone et al. 2004). The ability of *S. microphylla* to fix N may also contribute to this species' high foliar N (Cornelissen et al. 1997), although New Zealand native legumes typically have low N-fixation rates compared with exotic species such as gorse, *Ulex europaeus* (Wardle and Greenfield 1991).

The low foliar C:N ratios of *P. regius* and *S. microphylla* (<11) compared to the native evergreen species (20-50), are typical of deciduous and N-fixing species, as a result of high foliar N concentrations (Aerts and Chapin 2000). Litter decomposition is rapid at C:N<20 (Taylor et al. 1989). Thus, leaf litter turnover at the restoration sites would be faster in the soil below the native deciduous species and *L. perenne* (C:N 15-20) than the native evergreen species. Of the native evergreen species, leaves of *C. australis* and *O. paniculata* (C:N>30) are likely to decompose most slowly. This is well documented for the tough, fibrous leaves of *C. australis* (Simpson 2000, Dawson and Lucas 2011).

Exotic pasture species, *L. perenne*, had approximately 50 % higher foliar N than evergreen native species at the LUDF site, but was no different from the majority at the SH site. Foliar N

concentrations for *L. perenne* were similar to those at unfertilised sites in New Zealand, where, as for the LUDF site, native evergreen species have comparatively lower foliar N (Lambert et al. 1989c, Hahner et al. 2014). Prior to agricultural inputs, many soils in New Zealand have been nutrient limited (Molloy 1998, Leathwick et al. 2003). It has been hypothesised that native plants have adaptive traits suitable for low fertility soils (Wardle 1985), such as low rates of N uptake compared to exotics plants adapted to more fertile soil (Craine and Lee 2003). In testing this, Craine and Lee (2003) found New Zealand native grass species consistently had lower foliar N concentrations than introduced grass species growing in the same conditions. Additionally, native grasses had comparatively denser and longer lived tissues, traits often associated with lower resource availability (Craine and Lee 2003). Further research, including more sites and exotic comparison species, is needed to interpret the inconsistent patterns found in the present study.

Foliar N concentrations of native species were no greater at the LUDF than SH site, despite the soil at LUDF having 60 % higher total N and 350 % higher NO_3^--N concentrations. This is inconsistent with previous observations of plasticity in foliar N content within New Zealand native species (Wardle 2002, Craine and Lee 2003). Globally plant populations at more fertile sites have higher foliar N (Ordoñez et al. 2009). However, recent international (Wardle et al. 2009, Sundqvist et al. 2012) and local (Bellingham et al. 2013) research has demonstrated that intra-species variation in the foliar nutrients is related to a range of complex drivers. In contrast to the native species, foliar N was higher for *L. perenne* at the more fertile LUDF site (compared with SH), with even higher foliar N concentration reported under fertiliser conditions (Keating and O'Kiely 2000, Moir et al. 2013). The results of the present study suggest that lowland native species are poorly adapted to increase their foliar N uptake in response to increased soil fertility (at the LUDF site). Study of these species in a controlled environment under elevated soil N conditions (equivalent to agricultural pastures) will allow further investigation of native species response (Chapter 5).

4.4.2 Nitrogen status of native plant rhizospheres

The variation in soil fertility and properties between the two sites is representative of differences in the soil type and site history. The application of N fertilisers, effluents and lime to grazed pasture that covered the LUDF site, prior to fencing and planting, has elevated soil total N, NO_3^--N and pH compared with the SH site. Nitrate-N made up a relatively high portion (c. 2%) of total soil N, as found previously by Hahner et al. (2014) at the LUDF site. In contrast, previous infrequent grazing and low-intensity management the SH site has added less N to the soil. In addition, the Anthropic soil at the SH site is sandy textured and free-draining. Thus, high leaching rates may have reduced soil NO_3^--N and total N

concentrations over time. The comparatively higher moisture and organic content of soil at the LUDF site reflects the higher proportion of clays in the Templeton silt loam.

Nitrate was consistently more concentrated in the rhizosphere soil of native species, *A. richardii* and *P. regius*, compared with exotic pasture species *L. perenne*, despite differences in absolute concentrations between site. In addition, NO_3^--N concentrations in soil pore water extracted under *A. richardii* at the LUDF site were higher than *L. perenne*, consistent with previous findings at this site (Hahner et al. 2014). Few differences in total soil N occurred between plant species and soil N parameters were not correlated with foliar N, suggesting that plant N uptake alone is unlikely to explain between-species differences in rhizosphere soil NO_3^--N. The similarities in soil total N also suggest that elevated NO_3^--N under *A. richardii* and *P. regius* is not the result of increased total soil N, but that the rhizosphere conditions have facilitated greater mineralisation of this total (largely organic) N pool. Soil NO_3^--N and NH_4^+-N concentrations vary through time, seasonally and with rainfall events (Di et al. 1999, Di and Cameron 2002b). The consistent N status of soil pore water across the spring period confirms that taking a one-off soil sample for extract analysis was sufficient to represent this period. Seasonal differences were not examined in this study.

Hahner et al. (2014) proposed reduced water infiltration and the concentrating effect of low soil moisture, as an explanation for the increased NO_3^--N beneath *A. richardii*. This is unlikely in the current study, as NO_3^--N and soil moisture were not correlated at either site. Native plants at the LUDF site were considerably smaller during the study of Hahner et al. (2014) (2 years prior). At the time of the present research, other species (such as *K. robusta*, *P. tennufolium, P. regius* and *O. paniculata*) had formed dense, closed canopies, which are likely to reduce rain infiltration to a greater degree than the foliage of *A. richardii*. Reduced NO_3^--N under the canopies of such species may result in elevated soil NO_3^--N compared species with more open forms. However, no consistent patterns arose for these species at the LUDF and SH sites. *Kunzea* spp. are capable of intercepting large amounts (c. 40 %) of total rainfall (Aldridge and Jackson 1968, Rowe et al. 1999), but no comparative studies exist for New Zealand native species. In addition, variation in plant species evapotranspiration may influence rhizosphere soil moisture and NO_3^--N leaching rates. This was not measured in the present study. At the SH site the only available location for sampling of *A. richardii* and *P. tenax* was on the water-logged lower slopes of the raised bank. This is reflected in the higher soil moisture levels and organic matter content compared to the other species.

The nature and volume of roots influencing the soil in the top 0.15-0.30 m (soil sampling zone) may have influenced rhizosphere mineral N concentrations in this study. *L. perenne* has a shallow root system (c. 80 % of roots in upper 0.15 m soil) compared to the New Zealand native species (Haynes

and Williams 1993, Bolinder et al. 2002). Variable rooting profiles of the native species (previously recorded by Marden et al. 2005) may differentially influence the soil physcio-chemistry, through inputs of soluble C which stimulate soil microbial activity (Esperschütz et al. 2009, Bardgett and Wardle 2010). The comparatively larger volume of soil influenced by the extensive, fibrous *A. richardii* root system (Marden and Phillips 2009) offers a possible explanation for the increased NO_3^--N status of this species, compared to *L. perenne* and the other natives. Increased microbial activity in root influenced soil may mobilise soil organic N into mineral form (Craine 2009). *P. regius* develops an extensive root system more rapidly than other native dicotyledons (Marden et al. 2005), potentially influencing soil NO_3^--N. The other monocotyledons sampled, *P. tenax* and *C. australis,* have similarly extensive fibrous root systems (Marden et al. 2005, Marden and Phillips 2009). Consistently elevated *P. tenax* rhizosphere NH_4^+-N concentrations may reflect an increased rhizosphere effect, but are not unique to this species.

The high N content of the deciduous *P. regius* foliage may have influenced the NO_3^--N concentrations in the soil below this plant, as these soft leaves readily decompose, forming mineral N. In addition, *P. regius* is fast growing and litter is produced in much larger qualities than for the other species. A range of studies report increased mineralisation of N in soil dominated by deciduous species with high foliar N (Berendse 1998, van der Krift and Berendse 2001). Despite a high foliar N and N-fixation, the rhizosphere NO_3^--N status of leguminous species, *S. microphylla*, was not distinct from the other native species or *L. perenne.* Nitrogen build up through litter fall occurs in soil beneath exotic N-fixing species (Magesan et al. 2012). In this case, the slow growth of *S. microphylla* may have resulted in limited litter fall compared to *P. regius.* Continual deposition of old leaves below *A. richardii* forms a dense matt around the base, which may also contribute N via leaching or degradation to the soil NO_3^--N pool.

Several native species had unique soil physico-chemistry in terms of the parameters measured, compared with *L. perenne*, after 4-5 years of growth at the restoration sites. This potentially provides habitat for a different suite of soil micro and macro fauna. In this way, re-vegetated set-aside land may serve as reservoirs for native earthworms, largely displaced when native vegetation was cleared for agriculture (Lee 1959). Much of the soil N cycle is microbial driven. Variation in the soil microbial community may contribute to species-specific impacts on N availability, in addition to other factors such as plant uptake and nutrient returns (Ordoñez et al. 2009). Soil N availability is additionally regulated through complex plant factors and soil physico-chemistry properties (which may govern nitrification and leaching), consequently few studies have examined the relationship between plant species and the pools of N in soil (Laughlin 2011). Further research,

involving study sites across a range of soil fertility and types, may elucidate the causal mechanisms determining rhizosphere N availability for New Zealand native species.

4.5 Conclusions

This study has found substantial differences in the foliar and soil N status between New Zealand native species and when compared to *L. perenne*:

- The pattern of foliar N had a similar profile between species at the two sites. Deciduous native species, *P. regius* and *S. microphylla* (also N-fixing) had consistently higher foliar N than evergreen native species and *L. perenne*.
- At these restoration sites, N concentrations in *L. perenne* foliage were similar to those of native evergreen species at these restoration sites and were less than reported values for fertilised pastures.
- There was no overall difference in foliar N concentrations between the two sites, despite elevated soil total N and NO_s^--N at the LUDF site.
- *A. richardii* and *P. regius* had raised NO_s^--N in rhizosphere soil compared with *L. perenne* at both sites, consistent with higher soil pore water NO_s^--N concentrations beneath *A. richardii* at the LUDF site.
- There were few differences in total soil N between plant species, suggesting that variation in rhizosphere soil conditions are likely responsible for greater NO_s^--N production or retention.
- Variation in canopy density (rain through fall), plant evapotranspiration, the volume of soil influenced by root exudates or inputs of N-rich leaf litter potentially contribute to differences in rhizosphere soil N cycling and warrant further investigation.

Chapter 5

Response of New Zealand native plants to agriculturally elevated levels of soil nitrogen

5.1 Introduction

Natural vegetation has been degraded in about one third of New Zealand's land area, which has been converted to high-producing grazed pastures (Ministry for the Environment 2007). Introduced plant species are used for all production agriculture and *Lolium perenne* (perennial ryegrass) is the most widely used pasture species (Charlton and Stewart 1999). Naturally fertile soils are of limited extent in New Zealand (Molloy 1998). The primary rocks are low in essential plant nutrients (McLaren and Cameron 1996) and many soils have been strongly leached or weathered (Molloy 1998). Extensive use of nitrogen (N) and phosphorus (P) fertilisers has been necessary to maintain high productivity of introduced agricultural plants, which are adapted to soils that are more fertile. Nitrogen is also applied as recycled dairy effluent (Longhurst et al. 2000) as well as being fixed by N_2-fixing clover incorporated into pastures (Cooper 1990). Additionally, many native soils are acidic, due to organic matter decomposition and high leaching rates, thus lime addition to soil is common in New Zealand to raise the pH to suit agricultural species (McLaren and Cameron 1996). Despite advances in the management of soil N on farms, N leaching losses from agricultural land have been implicated as a major cause of poor water quality in New Zealand (Smith et al. 1993, Larned et al. 2004). Excess leaching of nitrate (NO_3^-), the most mobile form of soil N, can lead to eutrophication of waterways and endanger aquatic and human health (Carpenter et al. 1998). Additional gaseous N losses from pasture soils as nitrous oxide (N_2O) are a major contributor to greenhouse gas emissions (de Klein and Ledgard 2005).

Globally, N leaching losses are less under forest than other land use types (Di and Cameron 2002b). Vegetation degradation, particularly in the buffer zone between agriculture and waterways has exacerbated the impacts of agricultural nutrients by reducing uptake by plants and changing the flux of nutrients in soils (Jobbágy and Jackson 2004), potentially amplifying N transport to water bodies. Natural and re-established riparian buffers may mitigate the flux of N from agricultural land to surface waters, and enhance terrestrial and aquatic habitats (Schultz et al. 1995, Hill 1996, Mayer et al. 2007). In order to preserve freshwater quality in New Zealand, many local authorities increasingly require the removal and fencing of riparian zones from grazing. Following growing interest in enhancing biodiversity in agricultural landscapes, native species are now planted in riparian zones, shelterbelts, paddock margins and other set-aside areas on farms, which have traditionally consisted

of exotic species (Meurk and Swaffield 2000, Meurk and Hall 2006). Native plantings provide refugia and habitat corridors for native fauna in agricultural landscapes and offer a range of ecosystem services of value to agriculture (Meurk and Swaffield 2000).

Plant uptake of soil N potentially reduces soil N losses. A portion of plant accumulated N may be subsequently returned to the soil through litter fall, or removed from the plant via grazing or harvest (Hobbie 1992). Plants may also influence soil N cycles by altering water infiltration rates, root-mediated inputs of organic carbon and changes to the soil microbial community (Hobbie 1992). Foliar N concentrations, species growth rate and total biomass production determine the amount of N removed from soil during the growing season. High biomass producing tree species have been used as short rotation energy crops to remove N from applied wastewater or effluent (Guo et al. 2002, Tzanakakis et al. 2009, Pandey et al. 2011). While variable rates of N removal through plant uptake have been recorded in herbaceous and forested riparian zones in Europe (Hefting et al. 2005), the relative importance of plant immobilisation, soil microbial immobilisation and denitrification is difficult to establish (Hill 1996, Correll 1997). Plants may immobilise more N than microbes during the growing season and denitrification N loses may be greater in cool, wet months (Cameron et al. 2013). Variation in root depth, spread and density influence plant species' ability to take up soil N and the extent of soil receiving rhizosphere effects (such as root exudates and mass-flow of solutes). New Zealand native species commonly used in agricultural plantings are known to quickly develop deeper, more extensive root systems than *L. perenne* (Marden et al. 2005, Phillips et al. 2011a). Planting native species in locations receiving high N inputs has the potential to reduce soil N losses compared with cover by pasture species, particularly on riparian slopes, stock camps or areas where effluent would otherwise be disposed to pasture.

As high fertility soils are relatively recent in the evolutionary history of New Zealand, it has been suggested that native species may not respond with increased productivity at agriculturally elevated levels of soil N (Craine and Lee 2003). Many native plants in New Zealand have evolved in soils that are low in available nutrients (Wardle 1985). For example, the root-associated mycorrhizae of native species, *Leptospermum scoparium*, are thought to improve nutrient uptake, facilitating rapid growth on infertile soils (Stephens et al. 2005). While in New Zealand, exotic pasture grasses, adapted to soils that are more fertile, typically require fertilisation to maintain consistent high productivity. Many temperate pasture species respond with increased growth following N application at rates up to 700 kg N ha^{-1} (Moir et al. 2013).

Nitrogen is the most important nutrient influencing plant growth and yield (Marschner 1995). There has been substantial research into the uptake and growth response of exotic agricultural and

horticultural species to applied N in New Zealand, however, limited research regarding the nutrient requirements, limits and growth of native plants. Of the few published studies, some suggest that fertilising soil produces negligible increases in the growth and survival of native plant seedlings (Ogle 1996, Pratt 1999, Douglas et al. 2007). In other studies, the growth of native species has been improved through fertiliser use (Hawkins and Sweet 1989, Langer et al. 1999). No studies have investigated N uptake by native plants growing in agricultural soils or receiving external N inputs. Luxury uptake of nutrients has been proposed as a strategy of plants adapted to low soil fertility (Aerts and Chapin 2000). During a flush of nutrients inputs such species can absorb high qualities, used to sustain continued slow growth until nutrients are next available. Yet it is unknown if this applies to New Zealand native species. There is lack of consensus over the response of native plants to elevated N levels and scant information relating to species typically planted in agricultural landscapes. It is possible that the exotic pasture species *L. perenne* will thrive in high N soils and accumulate large amounts of N into plant biomass. Alternatively, native species may show little or a negative growth response to elevated N levels that are typical of agricultural soils.

The aim was to quantify the growth and N uptake response of selected native species and *L. perenne* to elevated levels of soil N in controlled conditions. Experimental pot trials investigated plant response to N loading ranging from a typical annual farm application rate, 200 kg ha^{-1} (Di and Cameron 2002b) to a rate in excess of a typical urine patch, 1600 kg ha^{-1} (Di and Cameron 2012) which may occur under trees used by stock for shelter. The higher N application rates were set to test the tolerance and limits of native plants. Conducting this research in pots allowed some control over the variability frequently encountered in field trials and allowed N addition to the plants without soil leaching, controlling the provision of N to the plants.

5.2 Methods

5.2.1 Experimental setup

Plants species and soil growth medium

Two trials (2012 and 2013) were conducted in a greenhouse (ampelite wonderglass fibreglass roofing with an automated ridge, side vents and hot air heating) in the Nursery Greenhouse Complex, at Lincoln University. New Zealand native plant species selected for these trials (Table 5.1) are part of early plant succession which are found naturally in lowland riparian environments. These species are common in riparian or restoration plantings in the Canterbury Plains region. *L. scoparium* is planted less commonly in this region, due to the prevalence of the mānuka blight disease (van Epenhuijsen et

al. 2000), but is included in these trials for comparison to closely related species *Kunzea robusta* and due to the economic interest in these species for honey production.

Native seedlings were raised from locally sourced seed at the Department of Conservation Motukarara Conservation Nursery (Table 5.1). Seeds were sown in August-September 2010 and pricked out into 0.5 L pots in November 2011. The potting mix used was a bark-based media amended with lime and various nutrients (Media; 90 % horticultural grade bark (0-12 mm), 10 % stone chip (5 mm). Amendments (kg m^{-3} media); dolomite (1.5), lime (1), hydraflo (1), iron sulphate (0.5), osmoform pre-mix (1), fertilisers (N:P:K - 15:4:7.5) (5), tricodry tricoprotection (1), Motukarara Conservation Nursery, personal communication, 2013).

Table 5.1 The New Zealand native species investigated in the two greenhouse pot trials. The scientific names and authority, family, allocation into monocotyledons or dicotyledons and the common Māori and English names, are given for each, as well as the seed source location.

Species	Family		English, Māori names	Collection Location
Carex virgata Sol. ex Boott (1853)	Cyperaceae	monocotyledons	pukio, swamp sedge	Christchurch urban
Phormium tenax J.R.Forst. et G.Forst. (1976)	Xanthorrhoeaceae	monocotyledons	harakeke, New Zealand flax	Port Hills
Austroderia richardii (Endl.) N.P.Barker et H.P.Linder (2012)	Poaceae	monocotyledons	toetoe	Port Hills
Cordyline australis [1] (G. Forst.) Endl. (1883)	Asparagaceae	monocotyledons	tī kōuka, cabbage tree	McCleans Island
Leptospermum scoparium J.R.Forst. et G.Forst (1776)	Myrtaceae	dicotyledons	mānuka, red tea tree	Spencer Lagoon
Kunzea robusta de Lange et Toelken (2014)	Myrtaceaea	dicotyledons	kānuka, white tea tree	Waipuna Saddle
Sophora microphylla [1] Aiton (1789)	Fabaceae	dicotyledons	kōwhai, small-leaved kōwhai	Port Hills

[1] These species were only included in Trial Two.

The soil used as growth medium in the greenhouse trials was a sieved (<5 mm) low fertility Templeton silt loam (Immature Pallic, Hewitt 1998, Udic Haplustept, Soil Survey Staff 2014) collected immediately prior to each trial (0-0.3 m, after removing the pasture), near Springston, Canterbury (43°38'40.09"S, 172°23'29.15"E). The Templeton silt loam soil series is developed from weakly weathered greywacke alluvium-silt and sand over gravel and stones. The existing vegetation at the

site was mainly ryegrass and the area had been farmed with low intensity (sheep grazing) for the past 50 years (Randhawa 2003, R. McLenaghen, Lincoln University, personal communication, 2013). This soil was selected for these nutrient trials as it is has comparable structure to the soil at the Lincoln University Dairy Farm site (Chapter 4 and Chapter 6) but has negligible history of fertilisation. Total and available N concentrations (Table 5.2) were at the low end of the normal range suggested for arable pasture (Hill and Sparling 2009), raising the likelihood that plants would respond to nutrient treatments. The soil was also low in inorganic P (Randhawa 2003), total P and Olsen P (Table 5.2), below the guideline range for pasture (Hill Laboratories 2014). Total sulphur and sulphate-sulphur were also below recommended levels (Hill Laboratories 2014).

Table 5.2 Physico-chemical properties of the Templeton silt loam soil collected from the Gammack Estate (43°38'40.09"S, 172°23'29.15"E) at 0-0.3 m depth. Soil was analysed either at Lincoln University[(1)] or by Hill Laboratories (Hamilton, New Zealand) using the stated methodology. Units are in μg g^{-1} unless otherwise stated. Values are mean and standard error in brackets for analysis conducted at Lincoln University (n=10).

Soil property	Gammack Estate soil		Methodology
pH [1]	5.4		1:2.5 (v/v) soil:water
NO_3^--N [1]	0.59	(0.05)	Blakemore et al. (1987)
NH_4^+-N [1]	9.71	(0.6)	Blakemore et al. (1987)
Total N (%) [1]	0.27	(0.02)	Dumas method (LECO CNS-2000)
Total C (%) [1]	3.21	(0.06)	
C:N ratio [1]	11.8	(0.48)	
Olsen P (mg L^{-1})	15.6	(1.3)	Wantanabe and Olsen (1965)
Available N (kg ha^{-1})	162		Keeney and Bremner (1966)
Mineralisable N	117		
Total P	341		Blakemore et al. (1987) (ICP-OES)
Sulphate S	4		Watkinson and Perrott (1990)
Total S	369		Blakemore et al. (1987) (ICP-OES)
Total Base Saturation (%)	47		Hesse (1971)
CEC (cmolc kg^{-1})	16		
Exchangeable K	0.87		Blakemore et al. (1987) (ICP-OES)
Exchangeable Ca	4.8		
Exchangeable Mg	1.9		
Exchangeable Na	0.13		

[1] Analysis conducted at Lincoln University.

Seedlings were potted in 2.5 L plastic pots in two separate trials. The roots and potting mix were not disturbed on removal from the 0.5 L pots and were placed on top of 20 mm layer of soil, in the centre of the pot. Soil was packed around and above the plant roots (total 1.7 L per pot), leaving

a 10 mm gap below the pot rim, for ease of watering. Additional pots were packed with 2.3 L of soil (equivalent volume of potting mix and soil for native seedlings) for seeding of *L. perenne* and use as unplanted soil controls. Plastic saucers beneath pots prevented leaching loss of N during treatment application and watering.

Trial One

Twenty-four one year old seedlings of five native species (Table 5.1) were individually potted 8-11 October 2012 in the Gammack Estate soil (Table 5.2). In 24 additional pots, seeds of *L. perenne* (*Lolium perenne*, cultivar Ceres *One*^{50}, entophyte AR1) were sown equivalent to the recommended rate of 20 kg ha^{-1} (Charlton and Stewart 1999, Agricom 2011). Twenty-four pots remained unplanted (control). The pots were maintained in a greenhouse with average day and night temperatures of 22 and 17 °C respectively for the 9 week duration of Trial One. Four N treatments, with 6 replicate plants of each species, were arranged in a complete randomised block design.

Application of N treatments began 2 weeks after potting. Based on the surface area of the pots (0.019 m^2), N was applied at three levels equivalent to 200, 800 and 1600 kg N ha^{-1} (0.38, 1.52 and 3.02 g N per pot respectively). A control treatment (0 kg N ha^{-1}) consisted of tap water applied in equal volumes. The total N applied to each pot was split into 10 applications spread over a 5 week period. Urea (CH_4N_2O) was dissolved in 0.2 L of water for application to the native plant seedlings and 0.1 L for the *L. perenne* and unplanted pots (which required less water). Pots were watered on a per species basis, as required, between treatment applications (and during 2 weeks following), to maintain visually moist soil, consistently across all pots.

Plants were maintained in the greenhouse for two additional weeks following the final N application and harvested 10-13 December 2012. Plant height (all species), the length of three tagged branches (*L. scoparium* and *K. robusta*), number of tillers (*C. virgata* and *A. richardii*) and number of leaves (*P. tenax*) were measured prior to the first N application then fortnightly throughout the trial (four measures total) to measure native plant growth rate. *L. perenne* germinated in 7-10 days and was harvested fortnightly from 3 weeks after sowing, cut to height of 20 mm. The leaves, stems (*L. scoparium* and *K. robusta* only) and roots were harvested over four sequential days (proceeding in order of potting). All plant material was weighed fresh, then dried for 48 hrs at 60 °C, then re-weighed. During the harvest, soil was carefully collected from the rhizosphere established in the Templeton silt loam soil (avoiding collection of the potting mix remaining in the centre of the pot). Roots were washed of soil and potting mix, then patted dry (using absorbent paper) before weighing (fresh and dried).

Trial Two

As the pH, total P, total inorganic P and total S of the Templeton silt loam soil collected from the Gammack Estate are below the range suggested for arable farming (Hill and Sparling 2009), an additional experiment was set up to examine the effect of N on plant growth, after correcting for these soil deficiencies. In Trial Two, 1.5 year old native seedlings were used (Table 5.1), from the same batch as those in Trial One (stored in a shade house October 2012-June 2013). One year old *L. scoparium* seedlings (urban Christchurch seed source) were used, as insufficient numbers were available from the 2012 batch due to mortality. Twenty-four seedlings of the five native species used in Trial One, as well as two additional species (*S. microphylla* and *C. australis*), were individually potted 24-26 June 2013, in the Gammack Estate soil (Table 5.2). Twelve plants of each species were potted in soil amended with lime (6 g L^{-1} soil, to raise the pH approximately one unit to 6.5) and 12 plants were potted in unamended soil (control soil, pH 5.4). In 24 additional pots (12 lime and 12 control) seeds of *L. perenne* were sown and the foliage harvested fortnightly as for Trial One. Plants were maintained in the greenhouse over winter. After a period of dormancy, spring growth occurred.

A factorial experiment was conducted to test the effects of increasing soil pH (control and limed soil) and nutrient treatment (no N, 0 kg N ha^{-1}; N, 200 kg N ha^{-1}; and N-P-S, 200 kg N ha^{-1} + 100 kg P ha^{-1} + 60 kg S ha^{-1}), on the growth of native plant species and *L. perenne*. Plants of each species were divided amongst the 6 soil treatment combinations resulting from the factorial design. Four replicate plants per combination were arranged in a complete randomised block design.

The N (as urea, NH_2CONH_2) and N-P-S (P as monopotassium phosphate, KH_2PO_4 and S as potassium sulphate, K_2SO_4) treatments were split into 4 applications, spread over 4 weeks in September 2013. Chemicals were dissolved accordingly in 0.2 L of water per application, sufficient to saturate the pots with minimal leaching. Tap water was applied in an equal volume to the plants receiving no N. Pots were watered on a per species basis, as for Trial One. Two weeks after the final treatment application plants were harvested, 29-30 October 2013. Trial Two ran for approximately 4 months. During September and October the average day and night temperatures in the greenhouse were 20 and 16 °C respectively. The leaves, stems (*L. scoparium*, *K. robusta* and *S. microphylla* only) and roots were harvested, and rhizosphere soil collected, in the same manner as Trial One. Fresh weight of plant material was not recorded for Trial Two, following similarities in patterns of fresh and dry weight found in Trial One.

5.2.2 Chemical analyses

Rhizosphere soil samples were immediately sieved (<4 mm), transported to the laboratory and stored in a fridge for analysis the day following harvest A subsample of soil was dried at 105 °C to determine gravimetric soil moisture content. A 4 g subsample of moist soil was shaken with 40 mL of 2 M potassium chloride (KCl) for 1 hour, centrifuged at 2000 rpm for 10 minutes and then filtered (Whatman No. 41) (Blakemore et al. 1987). The KCl extracts were frozen until they could be analysed by Flow Injection Analyser (FIA) (FOSS FIAstar 5000 triple channel with SoFIA software version 1.30; Foss Tecator, Hoganas, Sweden) for ammonium-N (NH_4^+-N) and nitrate-N (NO_3^--N). The remaining soil was air dried (35°C for 48 hrs), ground and sieved (<2 mm) for subsequent chemical analysis. Soil pH was measured in suspension with water (10 g of soil to 25 mL of water) (S20 SevenEasy^{TM} pH; Mettler-Toledo, Switzerland) (Blakemore et al. 1987). *L. perenne* from the final harvest and the leaves, stems and roots of harvested native plant material were mechanically ground and sieved (<200 µm). Total C and N in dried ground plant material and soil samples were determined using Dumas combustion method on CNS Elemental Analyser (LECO Elemental Analyser, NSW, Australia). Available P in soil samples from Trial Two was estimated using the Olsen P method (Watanabe and Olsen 1965). Briefly, 1 g samples of air-dry soil were extracted with 0.5 M $NaHCO_3$ using a 1:20 soil to extractant ratio. Extracts were shaken for 30 minutes, then centrifuged for 10 minutes at 2000 rpm and filtered (Whatman No. 42). Phosphate concentration in the extracts was determined colourimetrically using Muphey-Riley colour reagent, with absorbance read at 880 nm on a UV spectrophotometer (UVmini-1240, Shimadzu, Japan).

5.2.3 Statistical analysis

In Trial One the rhizosphere soil properties, plant biomass, root to shoot ratio, plant N concentrations and distribution of N into soil and plant were statistically analysed using analysis of variance (ANOVA). The model included plant species, N application rate and their interaction as fixed effects, and experimental block as a random additive effect. Following the identification of a significant species x N interaction, one-way ANOVA was used to investigate the effect of N treatment on species biomass individually. The distribution of applied N into plant and soil was analysed by one-way ANOVA for each N application rate to identify species differences in uptake efficiency.

For the analysis of foliar N concentration and N uptake, following the identification of significant interaction effects (species x N), regression analysis and curve fitting was undertaken for all species individually in order to better interpret the results (SigmaPlot Version, Systat Software, San Jose, CA). Repeated measures ANOVA was used to test the effect of time period and N application rate on species growth rate in Trial One. Linear regression tested the relationship between plant N

(concentrations and total uptake) and above-ground biomass for each species. The relationships between root biomass, plant N uptake and soil N were also explored using linear regression, at each N application rate.

Trial Two was statistically analysed by conducting ANOVA. The model included plant species, N treatment, lime and all two-way interactions as fixed effects, and experimental block as a random additive effect. Three-way interactions (non-significant in all analyses) were removed to simplify the models and as the interpretation of such effects is complex. This model was used to examine the effect of species and soil treatment on rhizosphere soil properties, as well as above- and below-ground plant biomass.

Mean pH values were calculated by conversion to the equivalent hydrogen ion concentrations and back calculation to pH. Statistics were performed on the hydrogen ion concentrations. Plant N uptake was calculated by combining the total N concentration in plant materials with the dried biomass values. Data were transformed as required to meet the assumption of homogeneity of variances. Post-hoc tests (Fisher's least significant difference (LSD) test) were used to establish significant differences between treatment group means (p<0.05). All analyses were conducted using R version 3.0.1 (R Development Core Team, 2010, R Foundation for Statistical Computing, Vienna, Austria, http://www.r-project.org/) using base software and the "agricolae" package to perform the post-hoc testing (de Mendiburu 2014).

5.3 Results

5.3.1 Plant growth

Trial One

All native species remained healthy with no symptoms of N deficiency observed. The majority of species included in Trial One were unresponsive to N (Figure 5.1), though N addition did have significant overall effect on above- (p<0.01) and below-ground (p<0.05) dried biomass (Table B.1). The response patterns were similar for fresh plant biomass, data not shown. Nitrogen application affected the biomass of individual species differentially (p<0.05, species x N) (Table B.1). Of the native species, only *C. virgata* was responsive to N (p<0.05) and demonstrated a linear increase in above-ground biomass with increasing N application rates (Figure 5.1). Exotic pasture species, *L. perenne*, had a positive yield response N at the lowest application rate (200 kg N ha^{-1}) followed by a decrease at 1600 kg N ha^{-1} (p<0.01, Figure 5.1). Root biomass did not respond to N for most species, though N

addition had a significant negative overall effect (p<0.05, Table B.1). Root biomass of *A. richardii* was significantly reduced (p<0.05) at 1600 kg N ha^{-1} (Figure 5.1).

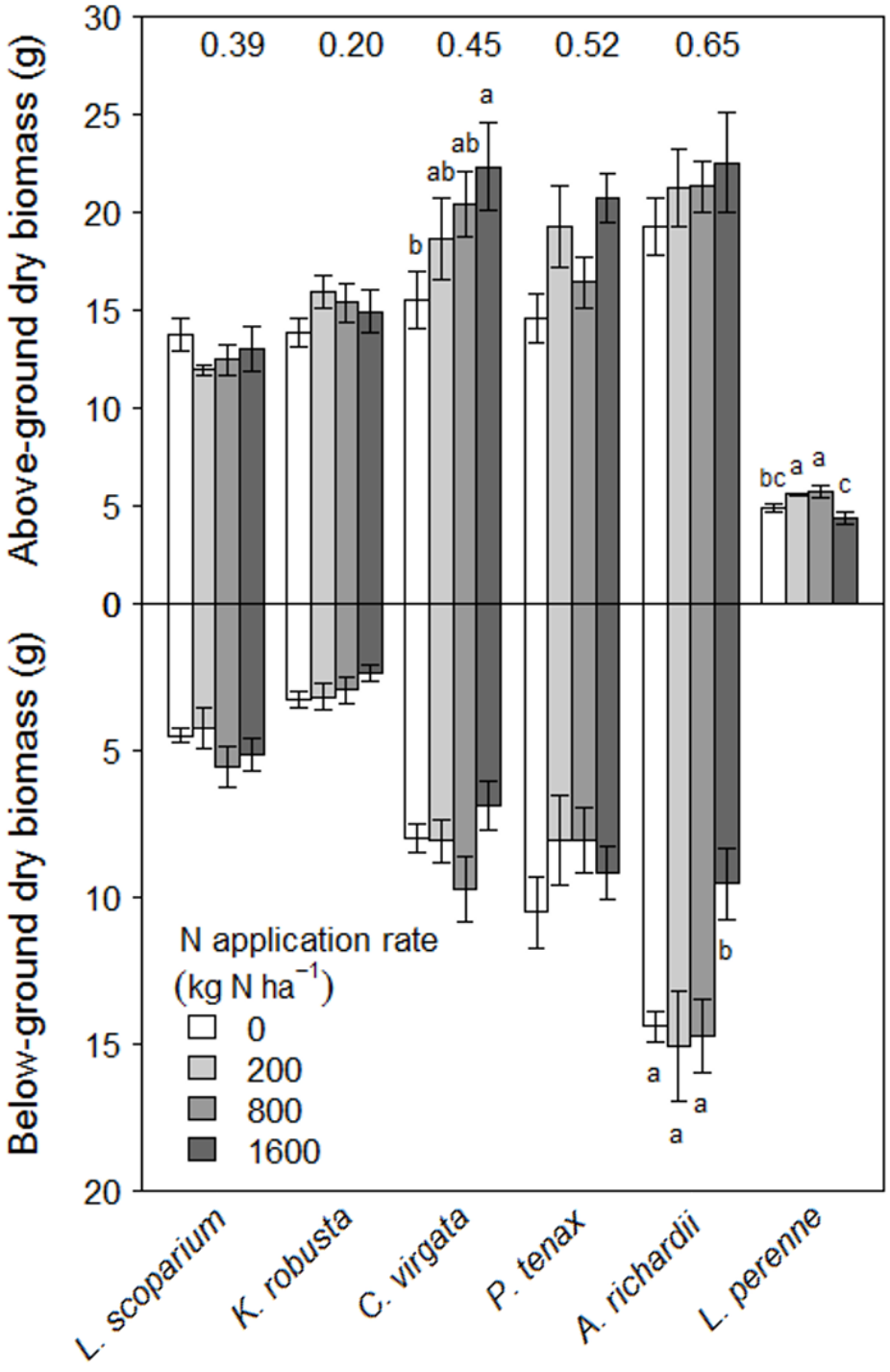


Figure 5.1 Trial One harvested plant biomass. Mean (± SE) dried plant above- (stems and leaves) and below-ground (roots) biomass of five native species and *L. perenne*, greenhouse grown in low fertility soil, supplied with increasing rates of N application (0-1600 kg N ha^{-1} equivalent). *L. perenne* roots were not measured. Values across the top of the plot are the mean root to shoot ratios for each species. The effect of N treatment was tested per species using ANOVA (n=6). For each species (group of bars) means which share letters are not significantly different, for groups without letters there was no significant effect of N treatment.

An early rapid growth phase was apparent following potting. The actual growth rate of measured plant attributes declined significantly throughout the trial for all native species, with the exception of the leaf length for the monocotyledons and leaf production in *P. tenax* (Table 5.3). The growth rate of *L. perenne* was greatest between weeks 3 and 5 of the trial (Figure B.1). Growth rate declined after 7 weeks in the 1600 kg N ha^{-1} treatment (Figure B.1). Parts of the sward of *L. perenne* in most 800 and 1600 kg ha^{-1} pots had died by the end of the trial. Few significant differences were found between N treatments in terms of the overall growth of measured plant attributes. *C. virgata* produced significantly less tillers in the control N treatment compared with plants which received N (data not shown).

Table 5.3 Change in growth rate of measured native plant attributes during Trial One. Significance values (*p*) for the effect of time in a repeated measures ANOVA (difference between fortnightly measures). The difference in growth rate between the first and last measurement period indicates the magnitude of changes. The effects of treatment and block were included in the model (non-significant, results not shown). *, ** and * indicate the treatment effect being significant at *p*<0.001, *p*<0.1 and *p*<0.05 respectively, n.s. indicates there was no significant effect. The tiller production was measured for *A. richardii* and *C. virgata* and leaf production for *P. tenax*.**

Species	*p*	Difference	*p*	Difference
	Height (mm $week^{-1}$)		**Branch length (mm $week^{-1}$)**	
L. scoparium	**	-50.2	***	-20.2
K. robusta	***	-50.3	**	-10.4
	Leaf length (mm $week^{-1}$)		**Tiller production (tiller $week^{-1}$)**	
P. tenax	n.s.		n.s.	
A. richardii	n.s.		***	-2.58
C. virgata	n.s.		***	-6.54

L. scoparium and *K. robusta* produced significantly less (*p*<0.001) dried biomass (above- and below-ground) than the native monocotyledons (Figure 5.1, Table B.1). The monocotyledons had fibrous root systems, while the roots of *L. scoparium* and *K. robusta* were woody. Although there were likely errors in the measurement of root biomass (due to root loss and inability to separate from soil), differences between species were large and represent inter-species variation well. The cumulative dried biomass harvested from *L. perenne* was significantly less (*p*<0.001) than native species biomass. The fine roots of *L. perenne* could not be separated from soil and were not harvested, but were visually less extensive than roots of the native species. *K. robusta* invested significantly less total biomass in roots than the other species, while *A. richardii* significantly more (*p*<0.001, root to shoot ratio, Figure

5.1, Table B.1). Stem material made up significantly more (p<0.05) of the total above-ground biomass of *K. robusta* (41 %) than *L. scoparium* (37 %) and was not affected by N application rate (data not shown). No effect of block was found in any analysis (Trial One or Trial Two) but it was retained in all models due to the experimental design.

Trial Two

In Trial Two, the native species *C. virgata*, *P. tenax* and *C. australis* and exotic *L. perenne* had significantly increased (p<0.01) growth in response to N addition, but had no further increase when N was added in combination with P and S (Figure 5.2). The other native species were unresponsive to N and N-P-S (Figure 5.2), although the nutrient treatment did have significant overall effect on above- ground biomass (p<0.05). Soil amendment with lime had no effect on plant biomass in Trial Two, and the biomass response to the nutrient treatments was similar for control soil and lime-amended plants.

Above- (p<0.001) and below-ground (p<0.001) biomass varied significantly between plant species (Figure 5.2). *C. virgata*, *K. robusta*, *S. microphylla* and *C. australis* produced significantly more above-ground biomass than the other native species and *L. perenne* (Figure 5.2). *L. scoparium* cannot be compared, as the plants were younger. Inter-species differences in native root system characteristics and biomass were similar to Trial One. *C. australis* had developed a 10-20 mm taproot structure and had many fibrous roots (as for the other monocotyledons). While *S. microphylla* had woody roots with root nodules. The monocotyledons *C. virgata*, *P. tenax* and *A. richardii* had significantly more root biomass than the other native species (Figure 5.2). As in Trial One, the roots of *L. perenne* were not harvested, but were visually less extensive than those of native plants.

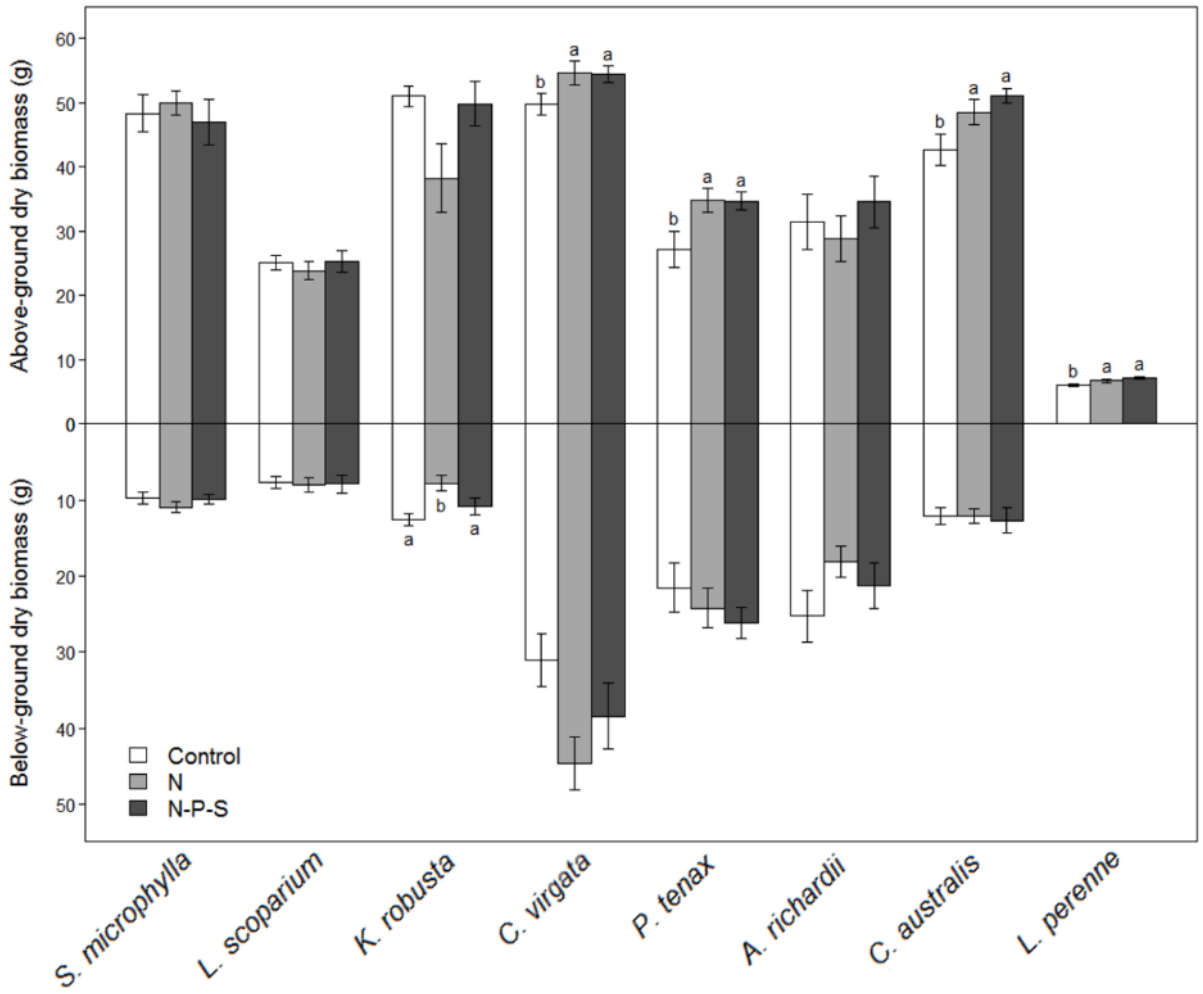


Figure 5.2 Trial Two harvested plant biomass. Mean (± SE) dried plant above- (stems and leaves) and below-ground (roots) biomass of seven native species supplied with additional nutrients (control, 0 kg N ha^{-1}; N, 200 kg N ha^{-1}; and N-P-S, 200 kg N ha^{-1} + 100 kg P ha^{-1} + 60 kg S ha^{-1}). *L. perenne* roots were not measured. Data are for the lime-control treatment (Lime data not shown). The effect of nutrient treatment was tested per species using ANOVA (n=4). For each species (group of bars) means which share letters are not significantly different, for groups without letters there was no significant effect.

5.3.2 Soil chemistry

Trial One

Total and plant available N in rhizosphere soil at the end of Trial One increased linearly with increasing N application rate and varied among plant species (Table 5.4). *L. scoparium* and *K. robusta* had significantly higher mean NO_3^--N concentrations than all other plant species and the soil control (Table 5.4). These native tree species, along with *L. perenne*, also had significantly more NH_4^+-N in soil. There were few significant differences between species in terms of the total N concentration in soil. Significant interaction effects indicated that individual plant species were differentially affecting soil

N (Table 5.4). Nitrogen addition significantly lowered the mean pH of soil (Table 5.4). Soil pH was almost 1 unit lower for pots that received 800 kg N ha^{-1}. *L. perenne* and *C. virgata* had significantly higher mean soil pH than the other species and control (Table 5.4). Soil gravimetric moisture content did not differ between plant species or N treatments (data not shown).

Trial Two

The increased soil pH following liming prior to Trial Two (from 5.4 to 6.5) was maintained in lime-treated pots over the course of the trial, despite an overall decrease in soil pH (Table 5.5). Treatment with N significantly lowered the rhizosphere soil pH (Table 5.5). Plant species identity did not affect soil pH in Trial Two. Plant species and soil treatment had no effect on soil gravimetric moisture content (data not shown).

Nitrogen addition in Trial Two raised mean NO_3^--N concentrations in soil tenfold (Table 5.5). The increase in NO_3^--N was lower when N was added in combination with P and S (Table 5.5). The native monocotyledons, with the exception of *A. richardii*, had significantly less NO_3^--N than other species, and *L. perenne* significantly more (Table 5.5). Ammonium-N was significantly higher for pots that did not receive N or lime (Table 5.5). *C. virgata* had significantly less NH_4^+-N in rhizosphere soil than the other species, while *P. tenax*, *A. richardii* and *L. perenne* had significantly more (Table 5.5). The effect of N treatment on the concentration of NO_3^--N and NH_4^+-N depended on the plant species identity (significant species x N interactions, Table 5.5).

Phosphorus addition (in N-P-S treatments) significantly raised the mean soil PO_4^{3-}-P concentration (Olsen P), approximately 10 units (Table 5.5). Lime addition was associated with a significant decrease soil PO_4^{3-}-P. *S. microphylla* and *C. australis* had significantly lower PO_4^{3-}-P concentrations than the other species and *L. scoparium* and *L. perenne* had significantly higher (Table 5.5).

Table 5.5 See over page. Chemical properties of rhizosphere soil in Trial Two. Mean soil NH_4^+-N, NO_3^--N, pH and PO_4^{3-}-P for seven native species and *L. perenne* (n=4). Plants were supplied with lime (control and lime) and N treatments with additional nutrients (control, 0 kg N ha^{-1}; N, 200 kg N ha^{-1}; and N-P-S, 200 kg N ha^{-1} + 100 kg P ha^{-1} + 60 kg S ha^{-1}). *, ** and * indicate the treatment effect being significant at $p<0.001$, $p<0.1$ and $p<0.05$ respectively. Means that share a letter are not significantly different following post-hoc tests. NH_4^+-N, NO_3^--N and PO_4^{3-}-P are in µg g^{-1}.**

Table 5.4 Chemical properties of rhizosphere soil in Trial One. Mean soil NH_4^+-N, NO_3^--N, total N and pH, for five native species, *L. perenne* (n=6, except total N, n=3) supplied with increasing rates of N application (0-1600 kg N ha^{-1} equivalent). *, ** and * indicate the treatment effect being significant at $p<0.001$, $p<0.1$ and $p<0.05$ respectively. Means that share a letter are not significantly different following post-hoc tests. NH_4^+-N, NO_3^--N are in µg g^{-1}.**

	NO_3^--N	NH_4^+-N	Total N (%)	pH
Species				
L. scoparium	246 [a]	230 [b]	0.30 [a]	4.68 [b]
K. robusta	271 [a]	248 [b]	0.31 [a]	4.63 [b]
C. virgata	154 [b]	186 [c]	0.29 [ab]	4.78 [c]
P. tenax	147 [b]	127 [d]	0.29 [bc]	4.56 [a]
A. richardii	146 [b]	161 [c]	0.28 [c]	4.62 [ab]
L. perenne	154 [b]	243 [b]	0.29 [ab]	4.87 [c]
Soil (control)	182 [b]	307 [a]	0.30 [ab]	4.60 [ab]
LSD (5%)	40.3	25.7	0.01	0.08
N rate (kg ha^{-1})				
0	1.00 [d]	2.24 [c]	0.25 [d]	5.31 [c]
200	60.1 [c]	16.6 [c]	0.27 [c]	4.72 [b]
800	227 [b]	211 [b]	0.30 [b]	4.39 [a]
1600	453 [a]	629 [a]	0.36 [a]	4.67 [b]
LSD (5%)	30.4	19.4	0.01	0.06
Significance of effects				
Species	***	***	**	***
N rate	***	***	***	***
Species x N rate	***	***	*	***
Block				

Table 5.5 Soil chemical properties Trial Two (full caption previous page).

	NO_3^--N	NH_4^+-N	pH	PO_4^{3-}-P
Species				
L. scoparium	28.8 [bc]	1.02 [b]	5.26	23.3 [a]
K. robusta	18.4 [cd]	0.92 [b]	5.23	20.8 [b]
C. virgata	0.77 [e]	0.49 [d]	5.49	20.4 [bc]
P. tenax	2.45 [e]	2.70 [a]	5.49	20.8 [b]
A. richardii	41.1 [b]	2.17 [a]	5.36	21.8 [b]
S. microphylla	10.0 [de]	1.07 [cd]	5.27	18.7 [d]
C. australis	1.87 [e]	1.54 [bc]	5.29	18.5 [d]
L. perenne	56.2 [a]	5.85 [a]	5.35	23.3 [a]
LSD (5%)	13.5	1.63		1.69
Lime treatment (pH)				
Control	17.7 [a]	2.49 [a]	5.08[a]	22.6 [a]
Lime	22.2 [a]	1.45 [b]	6.04[b]	19.3 [b]
LSD (5%)	6.76	0.82	0.1	0.85
Nitrogen treatment				
Control	2.63 [c]	2.65 [a]	5.59 [b]	17.3 [b]
N	36.3 [a]	1.44 [b]	5.21 [a]	17.2 [b]
N-P-S	20.9 [b]	1.82 [b]	5.29 [a]	28.3 [a]
LSD (5%)	8.27	1.00	0.12	1.04
Significance of effects				
Species	***	***		***
Lime	***	***	***	***
N	***	***	***	***
Species x Lime	*		***	***
Species x N	***	***	***	
Lime x N			***	
Block				

5.3.3 Plant nitrogen concentrations and uptake (Trial One)

Foliar, stem and root N

All species showed a trend of increasing foliar N concentration with increasing N application rate (Figure 5.3). Foliar N was affected by plant species, N application rate and their interaction (p<0.001) (Table B.1). Foliar N concentration in *L. scoparium*, *K. robusta* and *P. tenax* showed a linear increase in response to increasing rates of applied N (Figure 5.3). The increase in foliar N concentration across treatments was the greatest for *A. richardii* (c. 4 fold increase, Figure 5.3). *C. viragta*, *A. richardii* and *L. perenne* showed a more curvilinear increase in foliar N in response to applied N (gradual increase above 200 kg N ha^{-1}, Figure 5.3).

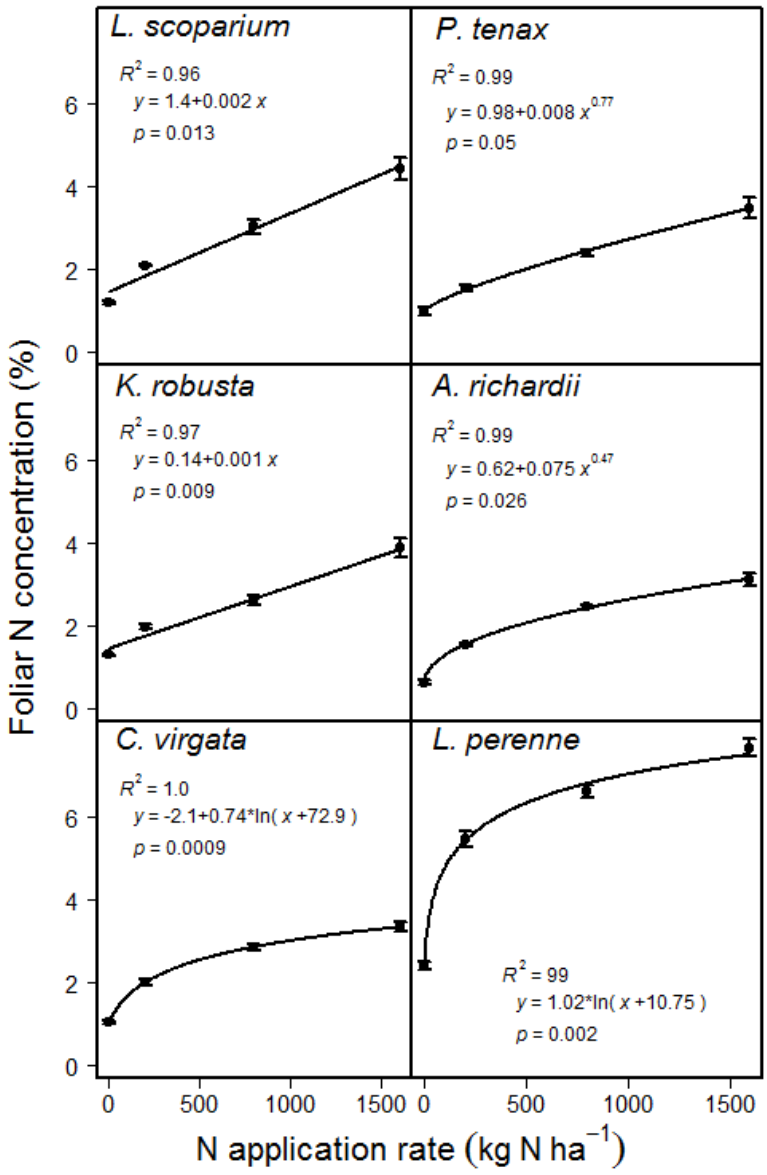


Figure 5.3 Comparison of foliar N concentration (%) of five native species and *L. perenne* to increasing levels of N application (ranging 0-1600 kg N ha^{-1} equivalent) in Trial One. Data are mean values ± SE (n=6), with p and R^2 values for the fitted curve showing the data trend.

The order of greatest mean foliar N concentration was *L. perenne* > *L. scoparium* >*K. robusta* >*C. virgata* >*P. tenax* >*A. richardii* (Table B.1). There was no discernible relationship between foliar N concentration and total above-ground biomass of individual species, except *C. virgata* (positive linear relationship, $p<0.05$, $F_{(1,22)}=5.15$, $R^2=0.19$).

The N concentration of the woody stems in *L. scoparium* and *K.* robusta was similar (means of 1.25 and 1.14 % respectively) and lower than foliar N concentrations. N application lead to significant ($p<0.001$) linear increase in N concentration in stem material (data not shown). *A. richardii* and *C. virgata* had significantly higher root N concentrations than the other native species ($p<0.001$) (Table B.1). Mean root N concentrations were lower than foliar N for all species, except *A. richardii*. There was a significant ($p<0.001$) linear increase in root N concentration in response to N addition, up to 800 kg N ha^{-1} (Table B.1).

Nitrogen uptake

Plant species, N application rate and their interaction significantly ($p<0.001$) affected N uptake into above-ground plant material in Trial One (Table B.1). *C. virgata* took up significantly more N than all other species (around double N uptake by *L. perenne*). The order of greatest mean N uptake into above-ground plant material was *C. virgata* >*A. richardii* > *P. tenax* >*K. robusta* > *L. scoparium* > *L. perenne* (Table B.1). The pattern of N uptake by the native species was similar in nature to that of the foliar N concentration. *L. scoparium*, *K. robusta* and *P. tenax* showed a linear increase in N uptake in response to increasing N application, while the response of *C. virgata* and *A. richardii* was more curvilinear (Figure 5.4). The N uptake response of *L. perenne* was curvilinear, with a maxima in uptake reached between 200 and 800 kg N ha^{-1} (Figure 5.4). The increase in N uptake across treatments was the greater for the native monocotyledons (4-6 fold increase) than the native dicotyledonous and *L. perenne* (c. 3 fold increase) (Figure 5.4).

A. richardii accumulated significantly more N in root biomass than the native other species (Table B.1). Root N uptake was affected by species ($p<0.001$), N application rate ($p<0.001$) and their interaction ($p<0.01$) (Table B.1). More N was taken up in above-ground (leaves and stems) biomass than roots. Root N accounted for between 12 % (*K. robusta*) and 40 % (*A. richardii*) of total plant N.

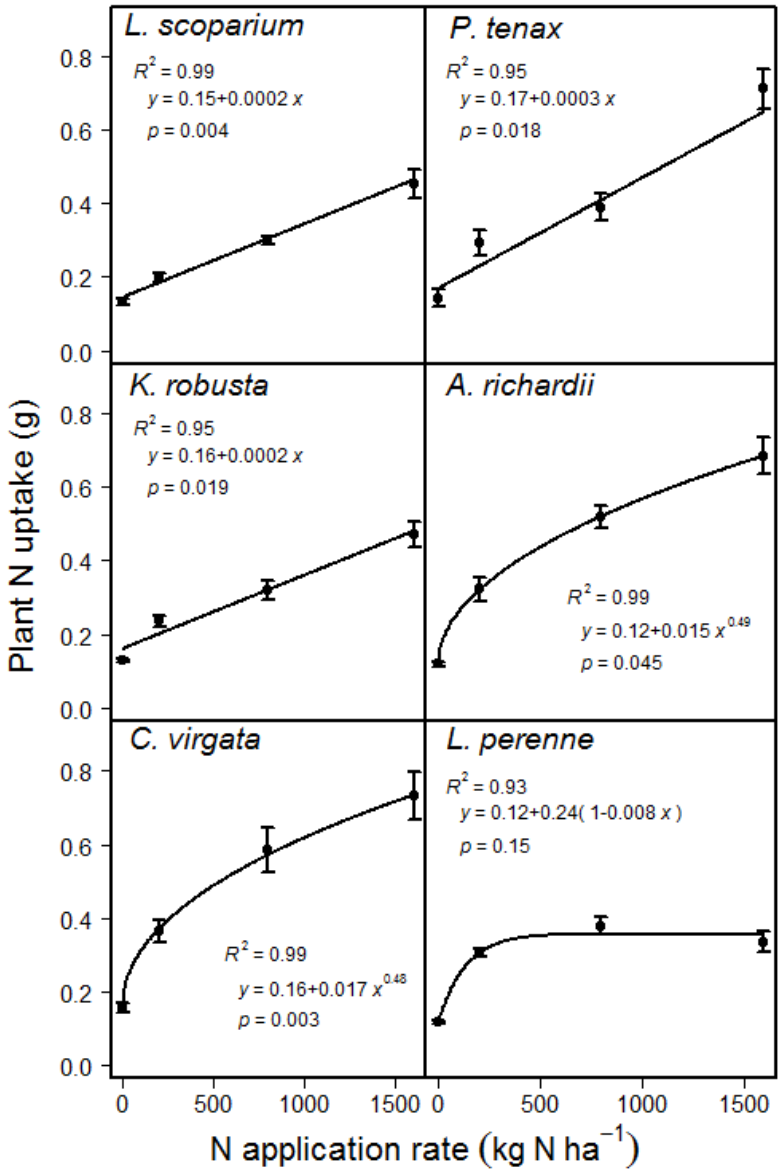


Figure 5.4 Comparison of above-ground N uptake of five native species and *L. perenne* to increasing levels of N application (ranging 0-1600 kg N ha^{-1} equivalent) in Trial One. Data are mean values ± SE (*n*=6), with *p* and R^2 values for the fitted curve showing the data trend.

The distribution of applied N in plant and soil

Plant N uptake as a percentage of N applied (after subtracting the mean of the 0 kg N ha^{-1} (control) to account for background plant N uptake) decreased significantly ($p<0.001$) with increasing N application rate. Uptake (combined roots, stems and leaves) of applied N was more efficient at 200 kg N ha^{-1} (63 %), than at 800 and 1600 kg N ha^{-1} N (27 and 17 % respectively). Plant species ($p<0.001$) and the interaction between species and N treatment ($p<0.05$) also significantly affected N uptake efficiency. There were few differences in N uptake efficiency between species at 200 kg N ha^{-1} (data not shown). Large error

and difficulties accounting for the distribution of the small amount of applied N prevented further interpretation of the plant-soil N budget at 200 kg N ha^{-1}. The native monocotyledons had significantly higher (p<0.001) N uptake efficiency than the dicotyledons and *L. perenne* at 800 and 1600 kg N ha^{-1} (Plant N, Figure 5.5).

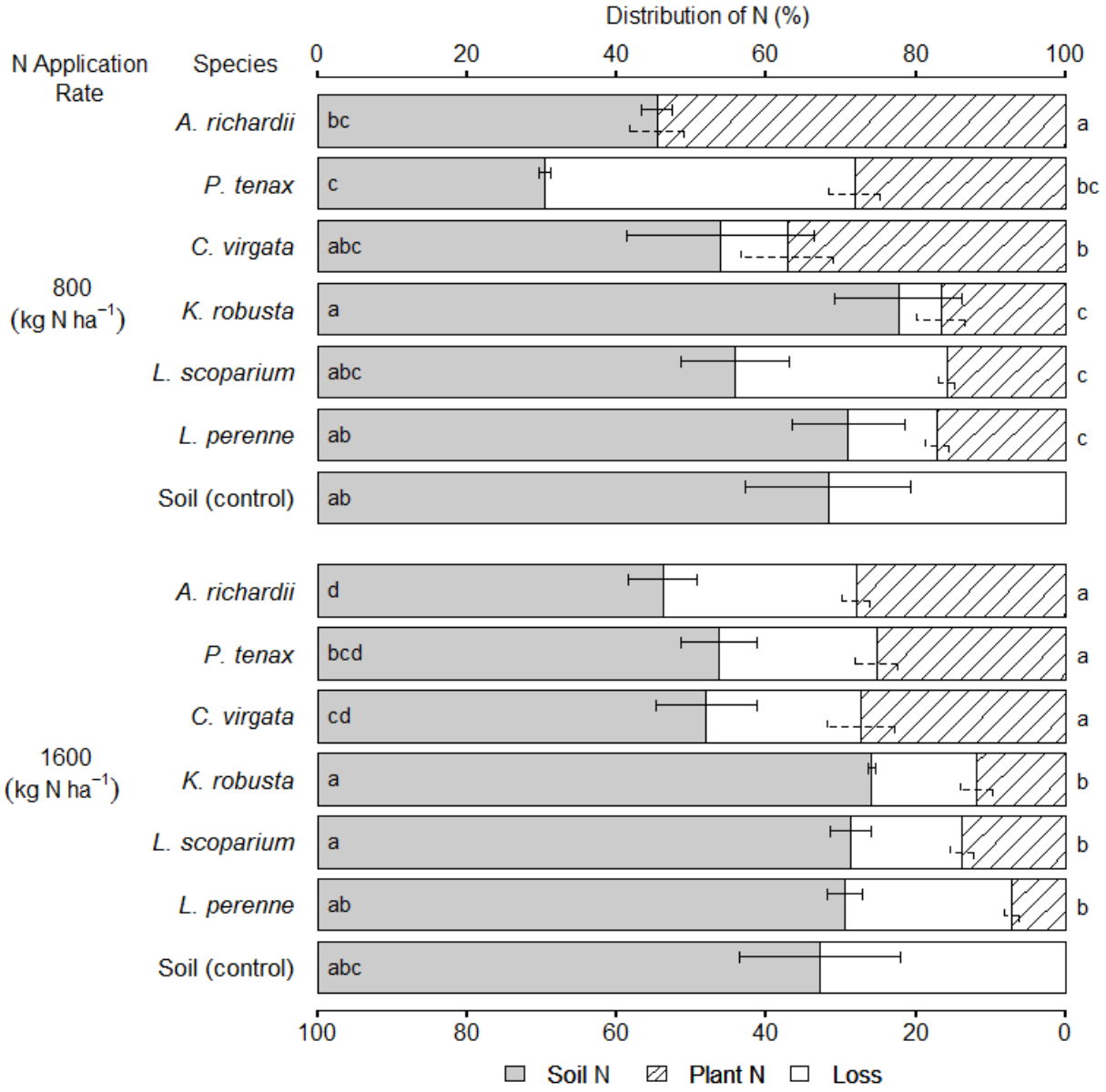


Figure 5.5 The distribution of added N between plant and soil in Trial One. Data is for N applied at rates of 800 and 1600 kg N ha^{-1} to five native species, *L. perenne* and unplanted soil (control). Data for 200 kg N ha^{-1} not shown. "Soil N" is the mean (±SE) percent of added N remaining in the rhizosphere soil at the end of the trial, read on the upper axis. "Plant N" is the mean (±SE) percent of added N accumulated in plant materials, read on the lower axis. Both "Soil N" and "Plant N" were calculated by subtracting the mean values from the 0 kg N ha^{-1} (control) treatment to account for background soil N and plant N uptake. "Loss" denotes the percentage of added N that cannot be accounted for. Within each N treatment (group of bars) means which share letters are not significantly different.

As more applied N was taken up by the plant, less N remained in the soil. The percentage of applied N accumulated by plants had a significant inverse relationship (p<0.001) with the amount of applied N remaining in the soil, at 800 and 1600 kg ha^{-1} N (Figure B.2). Plant species had a significant effect (p<0.05) on the percentage of applied N remaining in the soil (total N in mass of dry soil in pot after subtracting the 0 kg N ha^{-1} mean). N application rate had no effect on N remaining in soil, but there was a significant (p<0.05) species by N interaction. At 1600 kg N ha^{-1} the native monocotyledons had significantly less (p<0.01) applied N in soil than the native dicotyledons (Soil N, Figure 5.5). The amount of applied N that was unexplained (Loss, Figure 5.5) in the distribution was similar between the 800 and 1600 kg N ha^{-1} treatments (mean of 18 and 22 % respectively).

Root biomass N relationships

As native plant root biomass increased, the amount of added N accumulated in above-ground plant material significantly increased (for each N treatment, no root biomass available for *L.* perenne, Figure 5.6). Root biomass explained between 43 and 56 % percent of the variation in plant uptake of applied N. There were also significant positive (p<0.001) relationships between dry above-ground biomass and the uptake of applied N at each N level (data not shown).

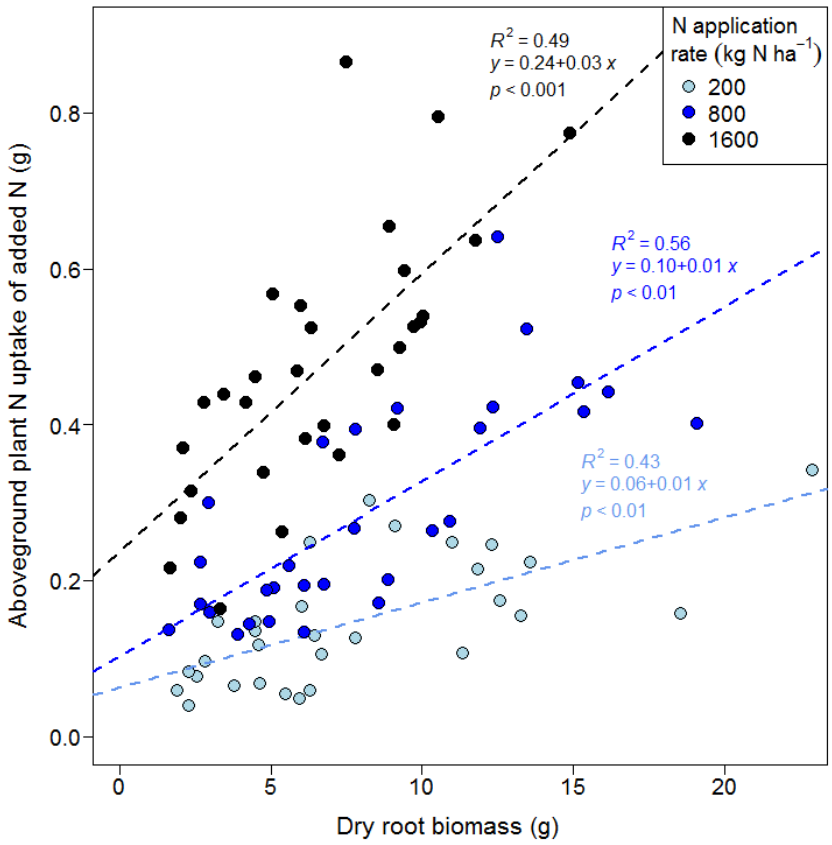


Figure 5.6 The relationship between root biomass (dry) and the amount of added N accumulated by plants in Trial One. Data are for N applied to five native species at rates equivalent to 200, 800 and 1600 kg N ha^{-1}. Regression lines, equations, R^2 and p values are shown for each N application rate.

In addition, within each N treatment level (200-1600 kg N ha^{-1}), as root biomass increased the concentration of soil NO_3^--N significantly decreased (Figure 5.7). The relationship was the strongest at 200 kg N ha^{-1} explaining 50 % of variation in NO_3^--N (Figure 5.7). Similar relationships existed for total soil N (Figure B.3) and NH_4^+-N (Figure B.4). Root biomass explained 21-43 % and 28-34 % of variation in these parameters respectively (for the 200-1600 kg N ha^{-1} treatments). There were no root-soil N relationships within the control treatment level. As for root biomass, an increase in above-ground biomass corresponded to a significant decrease (p<0.001) in available soil N (NO_3^--N and NH_4^+-N) for the 200-1600 kg N ha^{-1} treatments (data not shown). Above-ground biomass explained less variation in NO_3^--N (16-41 %) and NH_4^+-N (12-22 %) than root biomass. There was no relationship between above-ground biomass and total soil N.

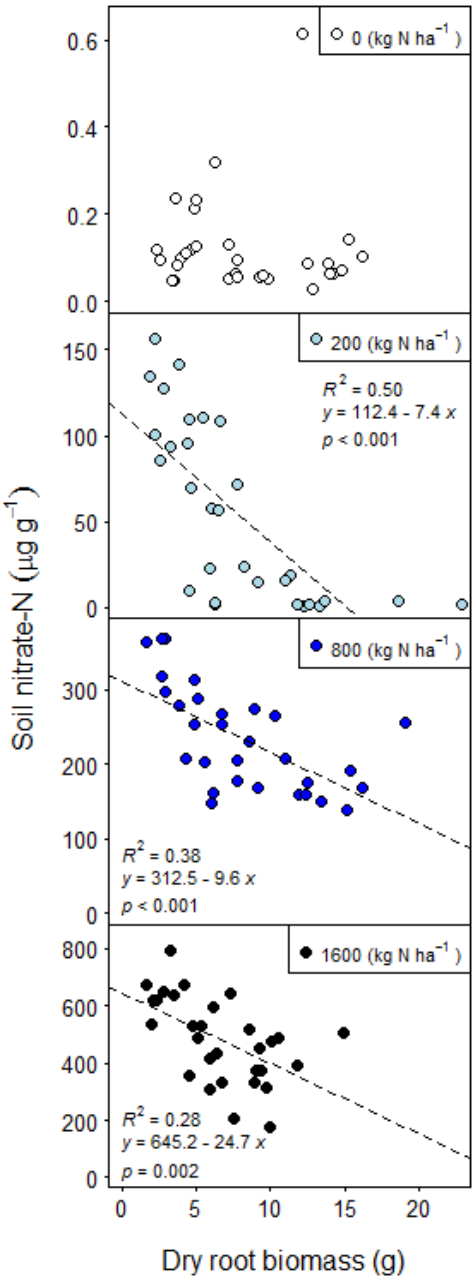


Figure 5.7 The relationship between root dry biomass and soil NO_3^--N concentration in Trial One. Data are for N application to five native species at four N application rates, 0-1600 kg N ha^{-1}. Regression lines, equations, R^2 and p values are shown for each N application rate. No regression line is shown for 0 kg N ha^{-1} as there was no significant relationship.

5.4 Discussion

5.4.1 Limited native species growth response to nitrogen

Growth response to added nitrogen

Nitrogen fertilisation achieved limited or no additional plant productivity for the majority of the native species in this experiment. Of the native species in Trial One, only *C. virgata* had a significant growth response. Above-ground biomass and growth rate (tiller production) increased in line with N application rate (44 % more biomass at 1600 kg N ha^{-1}). While in Trial Two, above-ground biomass increased significantly following application of 200 kg N ha^{-1} to the native monocotyledons *C. virgata* (10 %), *P. tenax* (28 %) and *C. australis* (17 %). *C. virgata* had a greater biomass response (20 %) to 200 kg N ha^{-1} in Trial One. The growth of *L. perenne* consistently increased 15 % when treated with 200 kg ha^{-1} in both trials. No further increased in growth was observed at higher N application rates (Trial One). These findings provide some support for the hypothesis that native plants are poorly adapted to being productive at high N supply (Craine and Lee 2003), compared to exotics, such as *L. perenne*, that have evolutionary history in high fertility soils. In general, the native monocotyledons were more responsive to N than the dicotyledons. Species that were unresponsive may respond at lower N levels than those tested here, which are more likely to be experienced in native soils. Many studies report increases in pasture production with N application (Di et al. 1998a, Di and Cameron 2002a, Monaghan et al. 2005, Moir et al. 2013). Typically there is a linear response up to 200-400 kg N ha^{-1} (Sun et al. 2008). The non-liner response in Trial One is consistent with this, given range of the N treatments. Moir et al. (2013) report increases in *L. perenne* yield up to 700 kg N ha^{-1}. In Trial One, 800 kg N ha^{-1} was excess to the growth requirements of *L. perenne* and growth declined at 1600 kg N ha^{-1}. This negative response was possibly due to root "scorching" damage, previously reported for pasture receiving high N loads (Richards and Wolton 1975, Saarijärvi and Virkajärvi 2009). Overall, root biomass decreased at 1600 kg N ha^{-1} (Trial One), but the roots of native species remained healthy and were able to tolerate high N concentrations. The significant reduced investment in roots by *A. richardii* indicates potential N saturation, as it was unnecessary for roots to forage for more N.

No further increase in biomass production occurred in response to N in limed soil, or when P and S were applied in combination with N (Trial Two). This suggests that these native species are well suited to growth on the acidic Templeton silt loam soil, which is low in available P and S. Such conditions are common to New Zealand soils (McLaren and Cameron 1996). Further, the results of Trial Two confirm that these conditions were not limiting the growth response to N in Trial One. The average Olsen P in samples taken from New Zealand native forest sites was lower than in the soil used in these trials (Ministry for the Environment 2007) and Trial Two confirms P supply was sufficient without fertilisation. Many New Zealand native trees have mycorrhizal associations thought to maximise their access to soil P and facilitate growth on low fertility soils (Wardle 1985).

Consistent with the present study, limited previous work involving the experimental native species has largely found no increase in growth following soil fertility manipulation. Pre-seeding site fertilisation (N and P) did not increase the growth of *L. scoparium* (Ledgard and Davis 2004) and *Kunzea* spp. were more successful on low-fertility un-amended sites (Stevenson and Smale 2005). No growth response was observed for fertilised *P. tenax* seedlings (Ogle 1996), although, a review by McGruddy (2006) details variable responses. No comparable studies have investigated *C. virgata*. The response of other native species to N is variable. The growth of *Coprosma robusta* and *Nothofagus fusca* improved following N addition (Langer et al. 1999), while native podocarp species responded positively in some instances (Hawkins and Sweet 1989), but not others (Guest 1985). Despite the limited growth response, New Zealand native species were able to tolerate and continue healthy growth under N loading (1600 kg N ha^{-1}) in excess of that typically experienced in pastures, and which caused a decline in the growth of *L. perenne*.

Inter-species differences in biomass and growth form

Large differences in plant species biomass occurred in both experiments. The cumulative biomass *L. perenne* produced during each trial was less than the harvested biomass of the native species. Scaling up of the mean *L. perenne* production to a growth period of the native species, one year (30 g dry matter, Trial One) and two years (32 g, Trial Two), provides an alternative comparison. Although likely an overestimate, due to reduced winter growth, this calculation suggests *L. perenne* would produce more biomass than native species in the first year of growth, whereas, when equated to two years' growth, native species produced similar or more biomass. The rapid growth of young native monocotyledons compared to dicotyledons (Trial One) was also observed by Marden and Phillips (2009). Yet in Trial Two, native dicotyledons, *S. microphylla*, and *K. robusta*, surpassed the biomass production of *A. richadii* and *P. tenax*. In the case of *A. richadii*, poor seedling health during the early trial was likely a contributing factor. The mean above-ground biomass and root to shoot ratio for one year old *L. scoparium* (Trial One) was similar to that recorded previously for containerised plants (Marden et al. 2005). Although, Marden et al. (2005) found two year old *C. australis* produced double the biomass of those in the present trial, suggesting these plants may have been restricted by the time spent in the 0.5 L pots prior to Trial Two.

Consistent with field observations (Marden and Phillips 2009), the fine, fibrous root systems of the native monocotyledons in these trials were more extensive than the woody dicotyledonous root systems. Monocotyledonous herbaceous species typically invest a greater proportion of their total biomass in roots than dicotyledonous species of the same inherent growth rate (Garnier 1991). A taproot-like structure is evident in the 2 year old *C. australis*, although less prominent than for plants of this age in field conditions (Czernin and Phillips 2005). Based on root biomass production of *L. perenne* cultivars grown in a sandy loam soil, receiving 0 and 300 kg N ha^{-1} (Moir et al. 2013), root

biomass may be estimated at approximately 1.5 (control) and 2.5 g (200 kg N ha^{-1}) per pot, substantially less than for the native species.

5.4.2 Soil fertility status

The extent of plant growth response to N fertilisation depends, in part, upon whether the initial soil fertility is sufficient for growth. Guideline soil fertility values do not exist for growing New Zealand native species. Total soil N prior to Trial One (0.27 %) was below the guideline range for adequate growth of pastures (0.35-0.65 %) and at the minimum for forestry (0.2-0.6 %) (Hill and Sparling 2009). Nitrogen addition in Trial One raised the total N concentration in post-harvest soil to within the adequate range for forestry and to the minimum for pastures. The concentration of plant available N (mineral N) was low in pre-trial soil. The mineral N fraction of total soil N was higher for pots that received N (3-30 %) than for the control treatment (0.1 %) and more mineral N was present as NO_3^--N. Therefore, plants in N treated pots were well supplied with plant available N. Mean NH_4^+-N concentrations (800 kg N ha^{-1}) in Trial One were comparable those recorded following N application at a similar rate to another Templeton silt loam soil (Taghizadeh-Toosi et al. 2011). Nitrate-N concentrations were higher in the present study, likely due to reduced leaching compared with field conditions. Total N was not measured post-N application in Trial Two. The application of 200 kg N ha^{-1} resulted in a 10 fold increase in soil NO_3^--N concentration, but a decrease in NH_4^+-N (in contrast to the increase in Trial One). Mean NO_3^--N and NH_4^+-N were 50 and 10 % (respectively) the concentrations of Trial One, following application of 200 kg N ha^{-1}. Trial Two proceeded over a longer period, thus it is likely that more NH_4^+-N was nitrified.

Prior to fertilisation, Olsen P of the Gammack Estate soil (16 µg g^{-1}) was below the guideline range for pasture (20-50) and at the minimum for forestry (10-100) (Hill and Sparling 2009). Total sulphur and sulphate-sulphur were also deficient (Hill Laboratories 2014). Following fertilisation (100 kg P ha^{-1}), Olsen P was raised around 10 units above the control, to within pasture guidelines. The pH of Gammack Estate soil was suboptimal for pasture production (Hill and Sparling 2009). Soil pH decreased following N addition in both trials, likely due to nitrification of NH_4^+-N. Hydrolysis of urea initially produces hydroxide ions, although more hydrogen ions are produced through nitrification per unit of urea fertiliser, acidifying the soil (Bolan et al. 2004). In Trial Two, lime addition buffered the impact of N on soil pH. Soil pH decreased in pots which received N, but not to the same extent as in Trial One, following N application at the same rate.

5.4.3 Plant nitrogen uptake varies with species morphology and nitrogen application rate (Trial One)

Nitrogen concentration of plant tissues

The foliar N concentration of native species and *L. perenne* increased in response to N application in Trial One. A similar response has been reported in pasture (Keating and O'Kiely 2000, Moir et al. 2013), forestry (Crane and Banks 1992, Zhang and Allen 1996), orchard (Raese 1997, Zatylny and St-Pierre 2006) and wild (Berendse et al. 1994, Craine et al. 2001) species. No data exists for native plant foliar N concentrations following fertilisation. Control treatment concentrations are at the low-end of reported field values for unfertilised evergreen native species (c. 0.2-3.0 %, Wardle 2002, unpublished data of G Hall in McGlone et al. 2004, Bellingham et al. 2013, Hahner et al. 2014), potentially as a result of the low fertility soil. The exotic pasture species *L. perenne* had higher mean foliar N concentrations than native species in control soil and at every N application rate. Elevated foliar N is among traits often found in species with natural distributions on fertile soils. While for plants adapted to low fertility conditions, long-lived low N foliage is a potential adaptation to conserve N (Hobbie 1992, van der Krift and Berendse 2001, Craine 2009). A comparison of native and exotic grass species in New Zealand found native species had significantly lower foliar and root N concentrations (Craine and Lee 2003).

Nitrogen accumulated in the foliage of native species following fertilisation, reaching more than double the concentrations previously reported for these species in the field (Lambert et al. 1989c, Ross et al. 2009, Hahner et al. 2014 and Chapter 4). Variation in foliar N between populations of native *Nothofagus* (Adams 1976) and *Chionochloa* (Wardle 2002) species has been associated with soil fertility. Although foliar N did not vary between native restoration sites differing in soil N availability (Chapter 4). Of the native species, *K. robusta* and *M. scoparium* had higher foliar N concentrations than the native monocotyledons, a pattern consistent with the field data. These species had linear increases in foliar N in response to increasing N application rates, while for *A. richardii* and *C. virgata*, foliar N increased less above 800 kg N ha^{-1}. *L. perenne* was N-deficient in control treatments (<4 % N, McLaren and Cameron 1996), but 200 kg N ha^{-1} was sufficient to correct this deficiency. Nitrogen addition raised the foliar N concentrations of *L. perenne* above those found experimentally at similar N rates (Crush et al. 2005, Crush et al. 2007, Moir et al. 2013), potentially due to increased N leaching losses in the lysimeters and columns used, compared to the conditions of the present trial.

Nitrogen was more concentrated (approximately double) in the physiologically active leaf tissues of the native species, compared with the woody stems (dicotyledons) and roots. Root N concentrations in control treatments were similar to those of native grass species in the field (Craine and Lee 2003). Although roots of *L. perenne* were not sampled in this study, previously reported root N concentrations (control conditions) are higher than for the native species in Trial One (Moir et al. 2013). Nitrogen concentrations in plant materials of *K. robusta* and *L. scoparium* were similar,

consistent with previous findings (Ross et al. 2009). The increase in N concentration of stem and root material following fertilisation was similar to foliar material. Similar general patterns exist for the fine roots of exotic forest species (Hendricks et al. 1993).

The lack of a relationship between foliar N concentration and biomass production (except *C. virgata*), is further indication that these native species were relatively unresponsive (in terms of growth) to N addition at elevated rates. Nitrogen accumulation in plant tissues occurred, without promoting immediate additional growth, suggesting that this is luxury N consumption (Chapin 1980, Millard 1988). Moir et al. (2013) report luxury N uptake for exotic pasture species at 700 kg N ha^{-1}. For *L. perenne* in the present study, luxury N uptake occurs at 800 kg N ha^{-1} and above. The capacity for luxury uptake has been suggested as an adaptation to low soil fertility, allowing rapid nutrient uptake during nutrient pulses and storage in plant tissues to sustain growth when nutrients are unavailable (Chapin 1980). The native species studied in this experiment are colonisers of disturbed and infertile sites; the capacity for luxury N uptake may support their growth in these situations.

Nitrogen uptake influenced by plant morphology

Plant biomass and the proportion of leafy biomass determined differences in N uptake between species in Trial One. The native monocotyledons took up more N (*C. virgata* the most) than the dicotyledons and *L. perenne*. High N uptake by the native monocotyledons potentially contributed to their generally increased growth response compared to the native dicotyledons. Despite higher foliar N concentrations, *L. scoparium* and *K. robusta* had lower overall N uptake, due to their lower biomass and portion of this (c. 40 %) that was low-N woody material. Strong relationships exist between N uptake and growth rate for exotic pasture grasses (Crush et al. 2005, Moir et al. 2013, Malcolm et al. 2014), riparian species (Tufekcioglu et al. 2003), wetland plants used for wastewater treatment (Tanner 1996) and trees grown as short rotation crops (Guo et al. 2002, Tzanakakis et al. 2009, Pandey et al. 2011). Inter-species variation in cool season growth also influences annual N uptake (Crush et al. 2005, Malcolm et al. 2014), but was not considered in this experiment. As a consequence of lower biomass and N concentration, N uptake by native plant roots was low compared to above-ground components, as commonly reported for exotic trees (Pallardy 2010) and grasses (Crush et al. 2005, Moir et al. 2013). Scant data exists for N uptake by native species and none for studies involving N application. Wardle (2002) determined that total N stored in native podocarp-broadleaf and *Pinus radiata* forests was similar, and greater than in low-biomass *Chionochloa* spp. grasslands. Low N uptake by *L. perenne* compared to the native species in Trial One was the result of low biomass production. However, annual biomass production in the field would be superior to the native species (full soil coverage possible for *L. perenne*, lower planting density for natives), with potential for greater N uptake.

At the level of individual species, foliar N concentration was important in determining the plant N uptake response to increasing N application rates for native species. Uptake increased linearly for *L. scoparium, K. robusta* and *P. tenax*, with increasing foliar N concentrations resulting in a net rise in N uptake, despite the lack of growth response. Additionally, the curvilinear N uptake responses of *C. virgata* and *A. richardii* more closely match the foliar N, than the biomass responses for these species. However, for *L. perenne*, the plateau and decline in biomass at upper N application rates, lead to the plateau in N uptake above 200 kg N ha^{-1} (despite foliar N continuing to increase). Linear increases in N uptake by pasture species have been reported up to rates of 700-1000 kg N ha^{-1} for *L. perenne* (Di and Cameron 2007, Moir et al. 2013).

The uptake of applied N (following subtraction of uptake by controls) was the most efficient at 200 kg N ha^{-1} (Figure 5.5). Given that N uptake efficiency was lower at 800 kg ha N^{-1}, it is likely that maximum uptake efficiency occurred below 200 kg N ha^{-1}, lower than maximal rates identified for exotic pasture species (Di and Cameron 2007, Moir et al. 2013). The amount of added N remaining in soil was linearly related to uptake efficiency, as observed by Moir et al. (2013), where higher N uptake by grassland species resulted in less N leaching from soil. Nitrogen uptake efficiency was greatest for the native monocotyledons in Trial One and less N remained in associated soil. The relatively large (c. 20 %) portion of applied N unaccounted for at the end of the trial may have been lost through denitrification to gaseous N, particularly from waterlogged soils following irrigation. Denitrification losses of up to 25 % are reported following fertiliser application (Bolan et al. 2004). Loss through ammonium volatilisation is unlikely due to the low soil pH (Bolan et al. 2004). Native species with high N uptake efficiency warrant further study, as their strategic planting has the potential to reduce NO_3^--N leaching from agricultural landscapes. In particular, *C. virgata*, which also had a significant growth response to N and is already used in riparian planting throughout New Zealand. This species is likely to establish quickly in high-N agricultural soils and is a good candidates for plantings designed to protect water quality.

Uptake of applied N into above-ground plant materials increased in line with increasing root biomass for the native species within each N treatment (*L. perenne* roots not investigated). For these young native plants, increased root biomass was associated with the presence of more fine roots, <1 mm diameter (rather than large roots) and corresponded to decreased total and available N in rhizosphere soil. These relationships suggest that root systems that are more extensive allow for greater interception and absorption of added N, with the potential to reduce NO_3^--N leaching from soil. As the bulk nutrients enter the plant via root and/or mycorrhizal uptake, allocation to root biomass is an important determinant of nutrient acquisition capacity (Aerts and Chapin 2000). Root depth (Webb et al. 1997), density (Tufekcioglu et al. 1998, Dunbabin et al. 2003) and metabolic activity (Malcolm et al. 2014) have been associated with inter-species differences in N uptake. The extensive

fine root system of the monocotyledons may provide increased access to soil N, facilitating high N uptake and a subsequent growth response, particularly for *C. virgata*. However, in the present study, root biomass and above-ground biomass are correlated, confounding the roles of root acquisition and leafy biomass growth in N uptake. The relationships between plant N uptake, root systems and soil N require further investigation, through comparison of species with contrasting root systems, but similar morphology and growth rate.

5.4.4 Experimental limitations and suggested further work

New Zealand native species had a limited growth response to N fertilisation in these trials. Future work should also consider lower N concentrations, as a growth response may occur for native plants at levels more representative of native environments. Although plants were not root-bound when harvested following Trial One or Two, the pot size used (2.5 L, restricted by greenhouse space) had potential to limit the growth response to N. Previous research indicates that roots of native riparian species have limited spread during the first 1 (0.1 m) and 2 (0.2 m) years of growth (Marden et al. 2005). Thus the pots used here (0.16 m diameter, 0.18 m depth) are likely to be suitable for 1 year old plants, but marginally too small for 2 year olds, potentially restricting growth. Additionally, natural variation in the size and condition of the plants prior to the experiments may have obscured differences in biomass at harvest. If this were the case, the repeated measurement of plant attributes during Trial One would have identified differences in growth rate. A more accurate measure of plant growth responses would be possible through the destructive harvest of a subset of plants, at several time points. Further trials with larger plants in larger containers (or in the field) would build on the findings of this experiment.

5.5 Conclusions

Overall, these experiments have demonstrated that young plants of selected New Zealand native species are relatively unresponsive to added N in terms of biomass production compared to *L. perenne*. However, native species are able to assimilate large amounts of applied N into foliar materials. The main findings of this research are:

- This experimental work has shown that native species are tolerant of, but not particularly responsive to, elevated soil N and P. Greater occupancy of soil by the roots of native species (compared to *L. perenne*) may provide an opportunity to use these plants to mediate soluble N fluxes in the rhizosphere.

- Soil fertility (total N and Olsen P) of the Templeton silt loam soil was increased, through fertiliser addition, but had little effect on native plant productivity.

- The overall growth response of native species to added N and P was limited and lends some support to the hypothesis that native species are poorly adapted to elevated N supply. *L. perenne* had increased growth in response to 200 kg N ha^{-1} and had higher foliar N concentrations than native species, traits typical of species adapted to high fertility environments.

- Of the native species, *C. virgata* was responsive in both trials and to high N levels (1600 kg N ha^{-1}). Selected other monocotyledons had a growth response in Trial Two. These species have potential to be competitive with exotic species, establish quickly in high-N agricultural soils and are good candidates for plantings designed to protect water quality.

- Plant biomass and above-ground morphology determined inter-species differences in N uptake. The leafy, high biomass producing native monocotyledons had higher N uptake efficiency than the native woody species and *L. perenne* (over the course of Trial One). Although extrapolated annual N uptake by *L. perenne* is likely to be greater due to the increased planting density possible for this species.

- Nitrogen accumulated in foliar tissues in response to increasing N application rates, in excess to the immediate growth requirements of most species, suggesting that this is luxury N uptake. Luxury uptake may be an adaptation of native species to the low fertility soils of New Zealand.

Chapter 6

Suppression of nitrous oxide production by *Kunzea robusta* (kānuka)

6.1 Introduction

Anthropogenic emissions of nitrous oxide (N_2O) are environmentally important because N_2O is a potent greenhouse gas with a global warming potential 298 times that of carbon dioxide (van Zwieten et al. 2009). N_2O makes up approximately 7 % of New Zealand's greenhouse gas emissions, contributed almost entirely from agricultural soils (Ministry for the Environment 2012). To reduce New Zealand's greenhouse gas emissions there is a need to find ways to mitigate agricultural N_2O emissions (Clark et al. 2011). The loss of nitrogen (N) in the form of N_2O also represents an important economic loss to the agricultural industry (van Zwieten et al. 2009).

Animal excreta, is a major source of N deposited to grassland in New Zealand. Additional sources include N fertiliser and N-fixation by leguminous species incorporated into pastures (Di and Cameron 2002b, Bolan et al. 2004). Dairy shed effluent (DSE) is a mixture of urine, dung, and washing water from the milking platform, which is rich in nutrients (Longhurst et al. 2000). The concentration of total N (269 mg L^{-1}) in DSE is high, consisting largely of organic N (219 mg L^{-1}) (mean of effluent studies reviewed by Longhurst et al. 2000). Land application (via irrigation) of DSE is common in New Zealand and aims to recycle the nutrients and use the soil/pasture system to absorb, filter and breakdown the effluent, reducing nutrient loads in drainage waters (Houlbrooke et al. 2004).

Nitrogen inputs to soil, predominately as urea-N (in urine and fertilisers) initially lead to the creation of a significant pool of ammonium (NH_4^+), which is relatively immobile in soil (McLaren and Cameron 1996). Nitrification of this pool, by autotrophic bacteria and archaea in aerobic conditions, creates nitrate (NO_3^-). Nitrogen oxides including N_2O are produced via nitrifier-denitrification or denitrification of these inorganic-N pools (Bolan et al. 2004). Nitrate is the substrate for denitrification and gaseous loss of N from the soil N cycle. This is in addition to the detrimental effects of NO_3^- leaching losses to waterways, contributing to their eutrophication (Cameron et al. 2013). In New Zealand, most regional councils limit the DSE application rate to 200 kg ha^{-1} yr^{-1} in order to minimise NO_3^- leaching (Di and Cameron 2002b). Despite advances in the management of soil N on farms, leaching and gaseous N losses following DSE application are widespread (Di and Cameron 2002b, Houlbrooke et al. 2004). In the Canterbury Plains region, NO_3^--N leaching losses from dairy pastures range from 20-80 kg N $ha^{-1}yr^{-1}$ (Lilburne et al. 2010).

Dairy products are New Zealand's largest export commodity. Currently, large-scale land conversion of sheep and beef farms to more intensively managed dairy farms, involves clearing of established trees in windbreaks and hedgerows, to make way for centre-pivot irrigation schemes. Little native vegetation remains in New Zealand's agricultural landscapes; trees and shrubs that are present are typically introduced species (Norton and Miller 2000). Conversion of sheep pasture and plantation forest to dairy farms provides an opportunity to re-introduce New Zealand native plants, in line with recent interest in increasing native biodiversity in farming landscapes (Meurk and Swaffield 2000, Meurk and Hall 2006). Planting paddock margins with low-stature native species allows irrigators to pass-over freely, while a range of native species may be incorporated on stream margins and paddocks corners where irrigators do not reach. Native plants have the potential to alter the soil N cycle compared to pasture or unvegetated land, through plant uptake, leaf litter addition and root exudation, which may affect NO_3^- leaching or N_2O emissions. The application of DSE to land planted with native species, as an alternative to pasture, may result in reduced N losses and environmental protection. Increased tree planting and more effective management of animal wastes have been suggested as ways in which greenhouse gas emissions can be reduced (Ministry for the Environment 2011). Establishing native plants may help achieve this.

Some plants are known to release allelochemicals from their root systems or from degrading leaf litter, which are potentially capable of inhibiting the soil nitrification process (Bremner and McCarty 1993, Paavolainen et al. 1998, Fillery 2007). Inhibiting the function of nitrifying bacteria can significantly reduce the NO_3^- pool available for N leaching or denitrification to N_2O gas (Subbarao et al. 2006, Subbarao et al. 2012a). Dicyandiamide (DCD), a commercial synthetic nitrification inhibitor, can significantly reduce N losses (Di and Cameron 2007, Zaman et al. 2009, Di et al. 2010b). Biological nitrification inhibition compounds have been found associated with plants such as the tropical grass, *Brachiaria humidicola* (Gopalakrishnan et al. 2009) and Sorghum, *Sorghum bicolor* (Zakir et al. 2008). However, New Zealand's native plants have received little attention regarding their influence over the soil N cycle.

New Zealand native trees in the Myrtaceae family, particularly *Leptospermum scoparium* (mānuka) and *Kunzea* spp. (kānuka) produce alleochemicals, such as methylglyoxal, leptospermone and α-pinene (Maddocks-Jennings et al. 2005). *L. scoparium* and *Kunzea* are early successional species, which rapidly establish on disturbed sites and often form long-term canopy communities on drier soils. Monterpenes, such as α-pinene, have been shown to inhibit nitrification in the soil of Californian Redwood forests (Ward et al. 1997). While leptospermone in leaf litter extracts of other Myrtaceae also inhibits nitrification (Boquel and Suavin 1972, Haile et al. 2006). The honey (Allen et al. 1991, Lu et al. 2013, Lu et al. 2014) and essential oils (Lis-Balchin et al. 1995, Porter and Wilkins 1999, Maddocks-Jennings et al. 2005) of *L. scoparium* and *Kunzea* spp. have proven antibacterial properties.

The antibacterial activity of *L. scoparium* and *Kunzea* products indicates the potential of these species to inhibit biological nitrification in surrounding soil. Anti-bacterial agents may enter soil through rhizodeposition from roots or continual leaf fall followed by degradation (Prosser et al. 2014). Water extracts of *L. scoparium* leaves and roots were found to significantly reduce the growth of bacterial pathogens typically found in biosolids (Prosser et al. 2014). The effect of *L. scoparium* and *Kunzea* spp. on soil microorganisms has not been investigated in-situ. Soil bacteria drive much of the soil N cycle. If the antimicrobial properties of these species extend to the soil, potential inhibition of nitrifying bacteria (such as *Nitrosomonas* or *Nitrobacter* species) would reduce the amount of NO_3^- in the soil, in turn reducing denitrification to N_2O and NO_3^- leaching. Inhibition of denitrifying bacteria also has the potential to reduce the amount of N_2O emitted, although may also inhibit the conversion of N_2O to N_2 gas. It is hypothesised that native *Kunzea* spp. could affect N cycling and reduce N_2O emissions.

The aim of this research was to determine, using an established method (closed-chambers), the effect of *Kunzea robusta* on N_2O fluxes from soil compared with bare (unvegetated) soil in the field, when treated with DSE.

6.2 Methods

6.2.1 Site description

Kunzea robusta is the focus of the present research, in Canterbury, New Zealand. Historically, *Kunzea* scrublands covered parts of the lowland Canterbury Plains (Wardle 2002, Meurk 2008). *L. scoparium* is uncommon in the Canterbury lowlands, mainly due to mānuka blight disease, which devastated this species regionally (van Epenhuijsen et al. 2000). *K. robusta* is a widespread member of the *Kunzea* genus, endemic to New Zealand. A recent revision of the *Kunzea* genus has identified several distinct species, previously described as *Kunzea ericoides* (de Lange 2014). *K. robusta* is present on Bank Peninsula and its seed propagated for use in restoration plantings on the Canterbury Plains.

The experimental site was in a corner of the Lincoln University Dairy Farm, Canterbury, New Zealand (43°38'38.07" S, 172°26'1.96" E), where New Zealand native vegetation was replanted in 2008 (Figure 6.1). The soil at the site is a Templeton silt loam (Immature Pallic, Hewitt 1998, Udic Haplustept, Soil Survey Staff 2014) developed from alluvium. This is an agriculturally important soil, covering around 10 % of the intermediate terraces of the Canterbury Plains (Molloy 1998). Knowles et al.(2011) described the chemical properties of Templeton silt loam soil at the Lincoln University Dairy Farm (Table 6.1). The restoration site was a fenced triangular area (c. 1800 m^2) beyond the reach of the centre-pivot irrigator (Figure 6.1b). Drainage ditches on two sides and a dairy paddock on the third,

bordered the site. The dairy farm was converted from former dry sheep pasture in 2001. The site was retired from dairy grazing in early 2008 (sprayed, ripped and rolled in preparation) and planted with one year old native seedlings in spring 2008. Full Glyphosate weed control during the first two seasons had maintained a largely bare soil surface between plantings. The native plants were 5 years old at the time of this experiment (established at the site for four years). The *K. robusta* trees were approximately 2 m in stature.

Table 6.1 Chemical properties for the Templeton silt loam soil at the Lincoln University Dairy Farm and of dairy shed effluent. Data are mean values with standard error (SE). Soil values are in µg g^{-1} unless otherwise stated (n=3, data from Knowles et al. 2011). For DSE all nutrient concentrations are in mg L^{-1}. The pH, total C and N are of the DSE collected from the Lincoln University Diary Farm (n=10), while the remainder are mean mineral concentrations reported for DSE in New Zealand (SE not calculated, data from Longhurst et al. 2000).

	Templeton silt loam soil		Dairy shed effluent
pH	5.6		7.5 [1]
CEC (cmolc kg^{-1})	12.4	(0.5)	
C (%)	2.4	(0.1)	1070 (95) [1]
N (%)	0.28	(0.01)	450 (45) [1]
P	518	(25)	69
S	193	(15)	65
Ca	3005	(101)	177
Mg	855	(11)	39
K	1401	(119)	370
Na	136	(4)	54
Cd	0.4	(0.1)	
Cr	11.6	(0.4)	
Cu	4.5	(0.1)	
Pb	12.0	(0.1)	
Zn	43	(1)	

[1] Values for the DSE used in this study.

Figure 6.1 The location of the experimental site. (a) The study site at the Lincoln University Dairy Farm, near Lincoln, Canterbury, New Zealand (Image sourced from Topo50 Map BX23 Lincoln, Crown Copyright Reserved). (b) The native plantation where the experiment was conducted, in the corner of the dairy paddock (Image sourced from Google Earth, Image © 2014 DigitalGlobe, 17/02/2014, 43◦38'38.5" S, 172◦26'02.29 E, elevation 17m). Circles represent control sampling locations (areas of unvegetated soil) and diamonds represent *K. robusta* sampling locations.

6.2.2 Treatments and Experimental Design

Nitrous oxide emissions were determined using a closed-chamber method similar to that described by Hutchinson and Mosier (1981), as used by Di and Cameron (2012) and Taghizadeh-Toosi et al. (2011). On 26 July 2012, headspace chamber bases (0.48 m diameter, stainless steel) were inserted 0.05-0.10 m into the soil. These contained an annular water trough. During gas sampling events, insulated, stainless steel headspace covers, with 0.10 m high walls, created an approximately 25 L headspace when placed on the bases (individual headspace varied slightly and was recorded for calculations). The headspace cover sat on the annular water-filled trough, creating a gas-tight seal. Chambers had two holes in the top, one with a cut down 30 mL plastic vial with a screw lid (removed then replaced after placing the cover to equilibrate pressure), the other with a rubber septum in which to insert the sampling needle and valve. Located immediately adjacent to each gas sampling chamber was a destructive soil sampling plot, consisting of a metal ring (0.48 m diameter, without water trough) inserted 0.05-0.10 m into the soil (Figure 6.2, Plate 6.1).

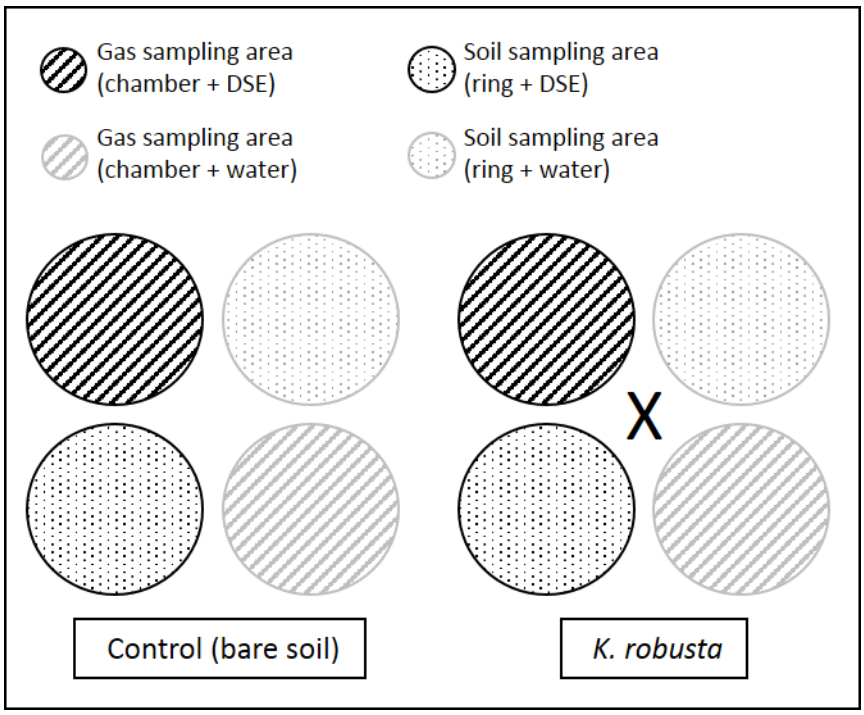


Figure 6.2 Field experiment layout. The arrangement of gas sampling chambers and soil sampling plots in control (bare soil) and *K. robusta* location. The X represents the stem of *K. robusta*.

Five replicate *K. robusta* locations (trees of approximately the same size) and control locations (areas of bare soil with no native plants within a 2 m diameter) were selected, relatively spaced out, within the restoration area. Each of these locations consisted of two, paired gas sampling/soil sampling plots (Figure 6.2, Plate 6.1). Dairy shed effluent was applied to one pair of plots and water to the other, at each *K. robusta* and control location, in order to account for differences in conditions between individual locations. Untreated tap water was used in this experiment. Plots were established radially (after randomly assigning the position of the first plot) around the stems of *K. robusta* (ring edge 0.2 m away from stem) or central point in control locations. The plots were separated by at least 0.2 m, to avoid seepage of treatments between plots. Gas sampling plots were established on opposite sides, with soil sampling plots adjacent. The distribution of treatments (DSE and water) to plots pairs was assigned randomly. In some cases plots in control locations could not be arranged radially due the shape of the available bare soil, in this case plots were placed to maximise distance from surrounding native plants (Plate 6.1a).

Treatments, DSE and tap water (as a control), were applied to the soil surface within the headspace chamber bases and soil sampling rings on the morning of 7 August 2012. Dairy shed effluent was collected from the storage pond that services the milking platform at the Lincoln University Dairy Farm. The dairy cows had been grazing perennial ryegrass/white clover (*Lolium perenne*/*Trifolium repens*) pasture. The DSE was collected on 9 September 2011, homogenised and stored at 5 °C prior

use in this experiment. Total carbon (C), N and pH were analysed for the Lincoln University Dairy Farm DSE (Table 6.1). Table 6.1 also provides means concentrations of selected other elements as reported for DSE in New Zealand (reviewed by Longhurst et al. 2000). Dairy shed effluent was applied at a rate of 50 kg N ha^{-1}, to reflect the maximum amount (of the yearly application total) which is typically applied by farmers in a single application (Houlbrooke et al. 2004). The DSE contained 450 mg N L^{-1} (Table 6.1), thus requiring 2 L of per sampling ring (0.18 m^2), an equal volume of tap water applied to the control plots.

Plate 6.1 Layout of gas sampling chambers and adjacent soil sampling areas. (a) control (bare soil) and (b) *K. robusta* locations.

6.2.3 Field Sampling, Analyses and Micrometeorological Measurements

Gas samples were taken the day prior to treatment application on 6 August 2012 (termed Day 0), then in the afternoon of 7 August 2012 (the day of treatment application, Day 1), and again on the days following, the 8, 9, 15, 22, and 29 August 2012 (Day 2, 3, 9, 16 and 23). Gas sampling was carried out at 1.30 pm each day. On each gas sampling occasion at 0, 15, and 30 minutes, after positioning the headspace cover, headspace N_2O samples (10 mL) were taken manually using a glass syringe fitted with three-way taps. Samples were compressed into 6 mL Exetainer tubes (Labco Ltd., High Wycombe, UK). The headspace temperature was measured at time of gas collection under an additional chamber placed in a representative location.

Immediately prior to analysis, gas samples were brought to ambient atmospheric pressure. Nitrous oxide concentration was determined using gas chromatography (GC) (SRI 8610 gas chromatograph; SRI Instruments, CA, USA) fitted with a ^{63}Ni electron capture detector (ECD) and linked to an autosampler (Gilson 222 XL; Gilson Inc., WI, USA). PeakSimple (SRI Instruments, CA, USA) was the

software used to control and monitor the ECD. Equations derived from Muller (1995) were used to calculate the N_2O flux (μg N_2O-N m^{-2} h^{-1}) from the concentration (μL L^{-1}) given after GC analysis (Equation 6.1). These values were converted to daily flux per hectare. Cumulative N_2O emissions were calculated by integrating the calculated daily N_2O fluxes and linearly interpolating between measurement points for each plot.

Equation 6.1 Equations derived from Muller (1995) were used to calculate the N_2O flux (μg N_2O-N m^{-2} h^{-1}) from the concentration (μL L^{-1}).

Equation A, when $(C_1\text{-}C_0)/(C_2\text{-}C_1) \leq 1$

$$\frac{[(C_1 - C_1)V \times P]M_{N2}}{[R(T_K + T_{\circ C})]A \times T_2}$$

Equation B, when $(C_1\text{-}C_0)/(C_2\text{-}C_1) > 1$

$$\frac{V(C_1\text{-}C_0)^2}{(2C_1\text{-}C_2\text{-}C_0)} \ln\left[\frac{C_1\text{-}C_2}{C_2\text{-}C_1}\right] \frac{P\, M_{N2}}{[R\,(T_K + T_{\circ C})]A \times t_1}$$

where:

C_0, C_1, C_2	= N_2O concentration [μL L^{-1}] at times t_0, t_1, t_2 respectively
P	= atmospheric pressure [atm] = 1
V	= chamber volume [L]
R	= gas constant [L atm K^{-1} mol^{-1}] = 0.08205746
T_K	= absolute temperature at 0°C [K] = 273.15
T_oC	= air temperature [°C]
A	= soil surface area [m^2]
t_2	= total cover period [h]
t_1	= $t_2/2$ [h]
M_{N2}	= molecular weight of N_2O-N [g mol^{-1}] = 28.01344

Soil cores (25 mm diam. × 75 mm depth) were taken on the morning of 6, 8, 9, 15, 22, and 29 of August 2012 (Days 0, 2, 3, 9, 16 and 23) to monitor soil gravimetric moisture content, pH and inorganic-N concentrations. On the 6 August, prior to treatment application, three soil cores were taken adjacent to the soil sampling plot, directly outside the ring, to ensure no leaching via preferential flow down the core holes occurred when the treatments were applied to the soil surface. On the following sampling days, three soil cores were taken at random from each soil sampling plot. Soil was immediately homogenised, sieved (4 mm) and refrigerated until analysis that afternoon. A subsample of soil was dried at 105°C for 24 h to determine soil gravimetric moisture content. Soil pH was measured on field moist soil in suspension with water (10 g of soil to 25 mL of water) (S20 SevenEasy™ pH; Mettler-Toledo, Switzerland) (Blakemore et al. 1987). Another 4 g subsample of field moist soil

was shaken with 40 mL of 2 M KCl for 1 hour, centrifuged (10 min at 2000 rmp) and then filtered (Whatman No. 41), (Blakemore et al. 1987). The KCl extracts were frozen until they could be analysed by Flow Injection Analyser (FIA) (FOSS FIAstar 5000 triple channel with SoFIA software version 1.30; Foss Tecator, Hoganas, Sweden) for ammonium-N (NH_4^+-N) and nitrate-N (NO^{3-}-N).

Meteorological data (air temperature and rainfall) and the soil temperature (0.10 m depth) corresponding to the duration of the study were obtained from the NIWA Broadfield Climate Station (43°37'34.4" S, 172°28'13.4 E), located approximately 3.5 km northwest of the research plot. A cylinder (55 ml, 25 mm diameter) was placed randomly within each *K. robusta* and control sampling location (between two sampling rings) to act as a rain gauge.

6.2.4 Statistical analysis

N_2O flux data (daily and cumulative) were log transformed to give normal distribution and analysed for significance using a special case of repeated measures analysis of variance (ANOVA) using plot type (*K. robusta* and control), treatment (DSE and water) and time (Day) as factors. A split-split plot design was used in the analysis, as within whole plots (the 10 sampling locations, 5 *K. robusta* and 5 control) treatments (DSE and water) were randomly assigned to split-plots (paired gas/soil sampling rings). Within split-plots, sampling occurred on multiple dates (split-split plots). Three-way interactions (non-significant in all analyses) were removed to simplify the models and as the interpretation of such effects is complex. Mean and standard errors for pH measurements were calculated by conversion to the equivalent hydrogen ion concentrations and back calculation to pH. Hydrogen ion concentration, soil gravimetric moisture content, NO_3^--N and NH_4^+-N data were also analysed for significance using repeated measures ANOVA with a split-split plot design. Following the identification of significant interaction of species or treatment with time, data were compared on individual sampling days using ANOVA with a split-plot design, to identify on which days differences between group means occurred. Where significant differences were found Fisher's protected least significant differences (LSD) test was used to identify differences among means (p<0.05). A two-sample t test was used to compare total rainfall collected in rain gauges at *K. robusta* and control locations. All analyses were conducted using R version 3.0.1 (R Development Core Team, 2010, R Foundation for Statistical Computing, Vienna, Austria, http://www.r-project.org/) using base software and the "agricolae" package to perform the post-hoc testing (de Mendiburu 2014).

6.3 Results

6.3.1 Nitrous oxide fluxes

Figure 6.3a shows that *K. robusta* significantly reduced N_2O flux following DSE application. Plot type (p<0.05), treatment (p<0.01) and time (sampling date, p<0.05) significantly affected daily N_2O flux. The effect of treatment on N_2O flux changed over the course of the trial (treatment x time interaction, p<0.05). Prior to treatment application (on Day 0) there were no significant differences in mean N_2O flux between plots (Figure 6.3a). Following DSE application, N_2O fluxes were raised in control-DSE plots compared to *K. robusta*-DSE and all water plots (Figure 6.3a), from Days 1-3 (p<0.001) and on Day 9 (p<0.05). N_2O flux from *K. robusta* plots also rose following DSE application and was significantly higher than *K. robusta*-water plots on Day 1 (p<0.001). There were no significant differences in mean N_2O flux between plots on Day 16 and 23. Control-water plots showed a gradual increase in N_2O flux following treatment application, peaking on Day 9.

The cumulative N_2O flux was significantly higher (p<0.001) from control-DSE plots than *K. robusta* plots and water treated plots (totalling 676 g of N_2O-N ha^{-1} or 1.35% of N applied on Day 23) (Figure 6.3b). Plot type (p<0.01), treatment (p<0.05), time (p<0.001) and the plot type x treatment interaction (p<0.01) had a significant effects on mean cumulative N_2O-N flux (Figure 6.3b). Cumulative N_2O flux was similar for control-water plots (152 g of N_2O-N ha^{-1}), *K. robusta*-DSE plots (133 g of N_2O-N ha^{-1} or 0.27 % of applied N) and *K. robusta* -water plots (95 g of N_2O-N ha^{-1}) (Figure 6.3b). When expressed as an emission factor (N_2O-N from the DSE treatment minus N_2O-N from the control, divided by the amount of N applied), the control and *K. robusta* plots had mean emission factors of 1.05 and 0.07 % respectively, for DSE application.

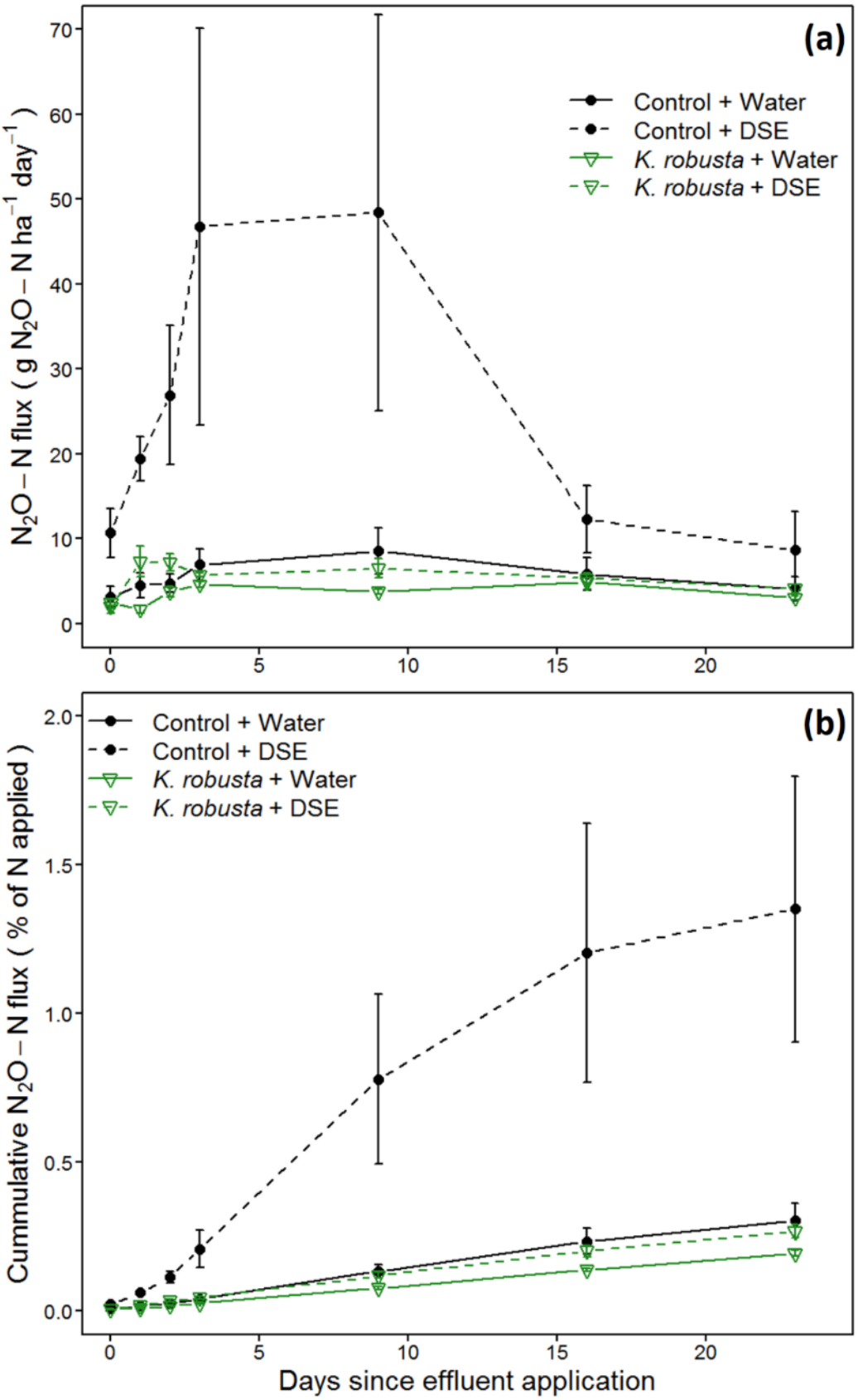


Figure 6.3 Daily and cumulative flux of N_2O from *K. robusta* and control (bare soil) plots following the application of DSE and water. (a) Mean (±SE) daily N_2O-N flux (n=5) and (b) mean (±SE) cumulative flux of N_2O-N from DSE treated soils, as a percent of the total N applied to the plots. Water treatments are also plotted to show control emissions (n=5). Day 0 precedes treatment application, which occurred on the morning of Day 1.

6.3.2 Soil analyses and meteorological measurements

K. robusta plots had significantly lower (p<0.05) mean soil gravimetric moisture content than the control plots (Figure 6.4). Soil moisture content did not vary due to treatment (DSE or water) or time. A total of 104 mm of rain fell during the experimental period, consisting of one substantial rainfall event (75 mm, 8-10 days following treatment application) and two smaller events (Figure 6.4). Significantly less total rainfall collected in rain gauges in *K. robusta* locations (mean 44 mL) than in control locations (64 mL) (T_5=-3.61, p<0.05). The mean daily air temperature ranged from 6.7 to 14.9 °C (Figure 6.4). A spike in air temperature near the end of the experiment is consistent with fine periods entering the spring and corresponds to a decrease in soil moisture between Days 16 and 23.

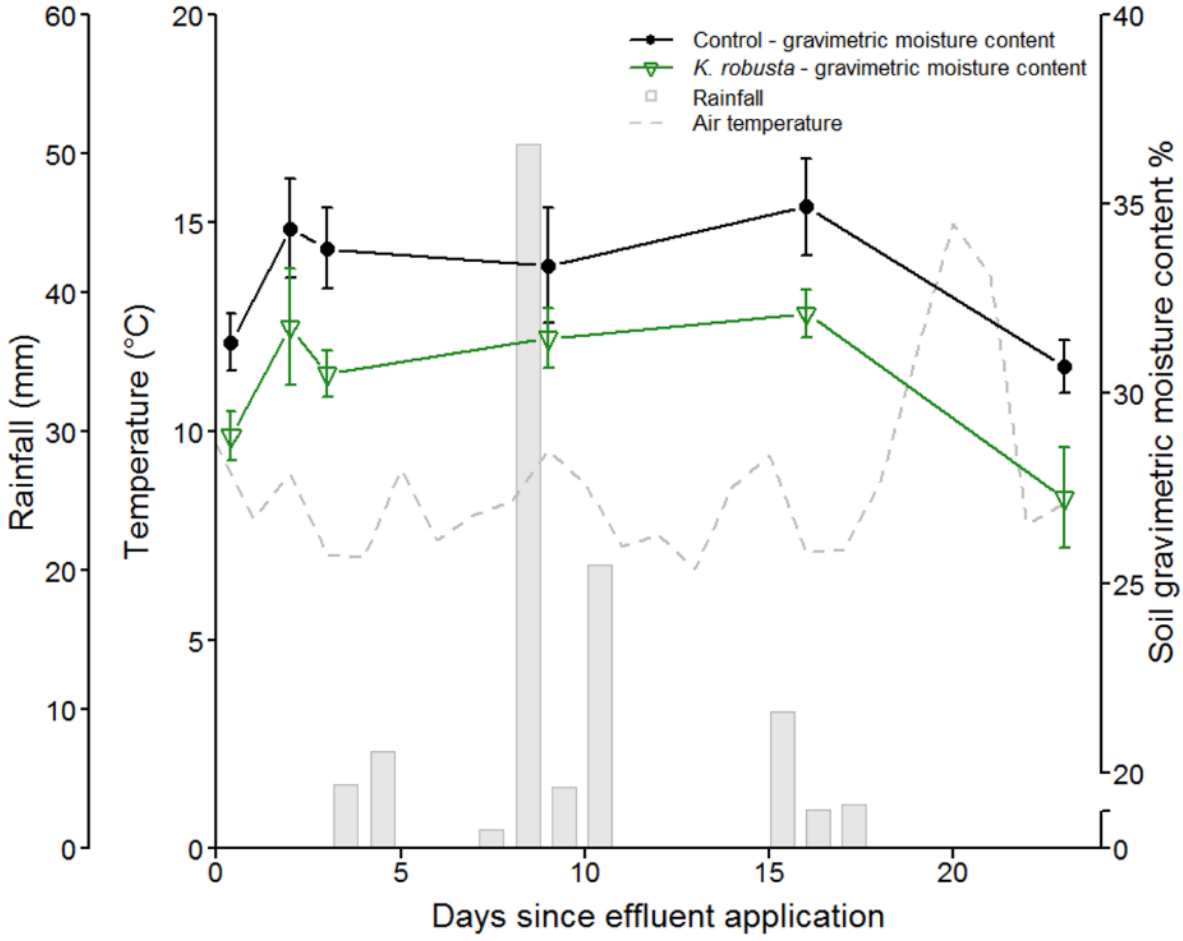


Figure 6.4 Soil moisture and meteorological measurements over the 24 day experimental period. Data for total daily rainfall and mean daily air temperatures were from a meteorological station 3.5 km from the experiment site. Mean (±SE) gravimetric water content for control and *K. robusta* plots were determined from in situ soil sampling (0-75 mm depth) at the field site (n=5). Data are for water treated plots, effluent plots not shown (no effect of treatment type). Day 0 precedes treatment application, which occurred on the morning of Day 1.

Soil pH was significantly higher (p<0.05) in control plots than *K. robusta* plots, throughout the experiment (Figure 6.5). There was no significant effect of treatment or time on soil pH.

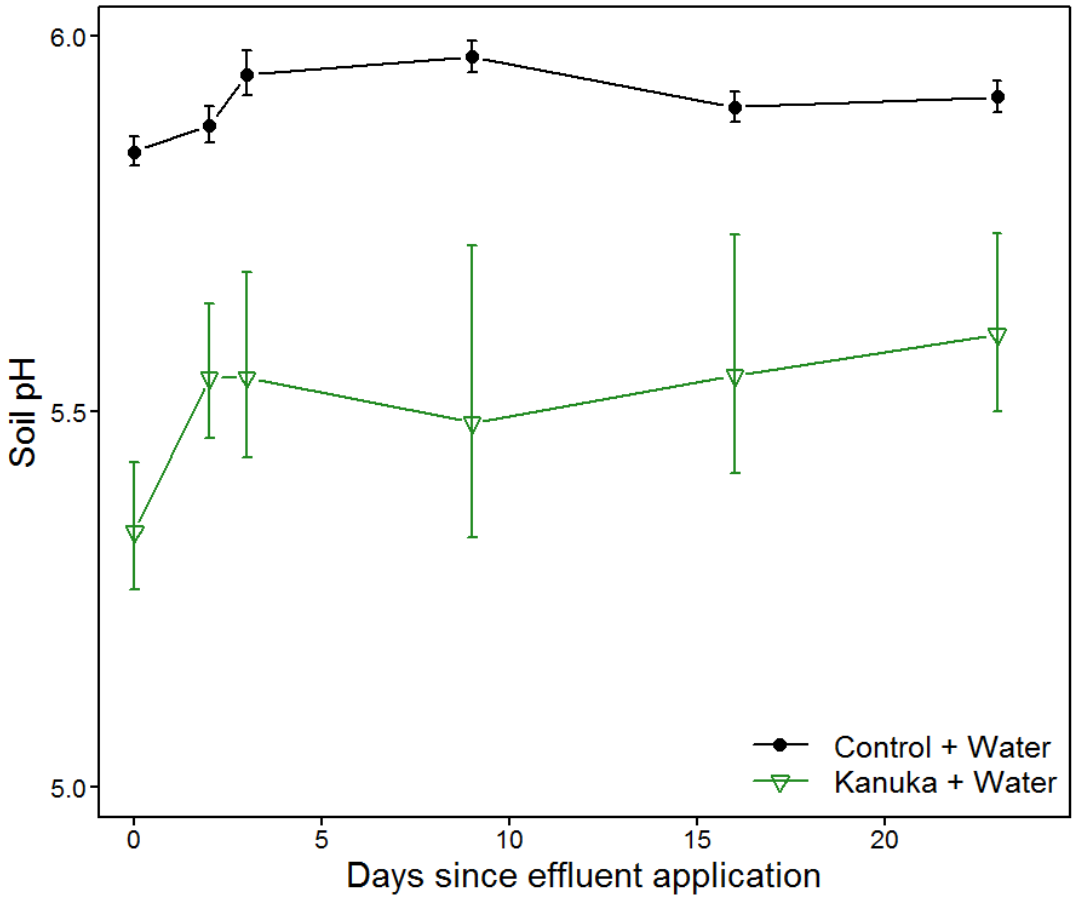


Figure 6.5 Mean (±SE) soil pH for control and *K. robusta* plots, determined using fresh soil (0-75 mm depth) sampled in situ at the field site (n=5). Data are for water treated plots, effluent plots not shown (no effect of treatment type). Day 0 precedes treatment application, which occurred on the morning of Day 1.

The mean soil NO_3^--N concentration was significantly higher (p<0.01) in *K. robusta* plots than control plots during the experiment (Figure 6.6a). Treatment and time had no effect on NO_3^--N concentrations. Mean NH_4^+-N concentrations were significantly higher (p<0.05) in DSE plots than water plots, but did not differ between *K. robusta* and control plots (Figure 6.6b). Soil NH_4^+-N concentrations varied significantly through time (p<0.001) and there was a significant treatment x time interaction (p<0.01). Prior to treatment application, NH_4^+-N did not vary between plots (Figure 6.6b). Immediately following treatment application, NH_4^+-N concentrations were raised in DSE plots (c. 15 µg g^{-1}) and water plots (c. 5 µg g^{-1}). On Day 3, NH_4^+-N was significantly higher (p<0.01) in DSE plots than the respective water plots. NH_4^+-N concentrations increased in water treated plots on Day 16 and did not vary between plot types on Day 16 or 23.

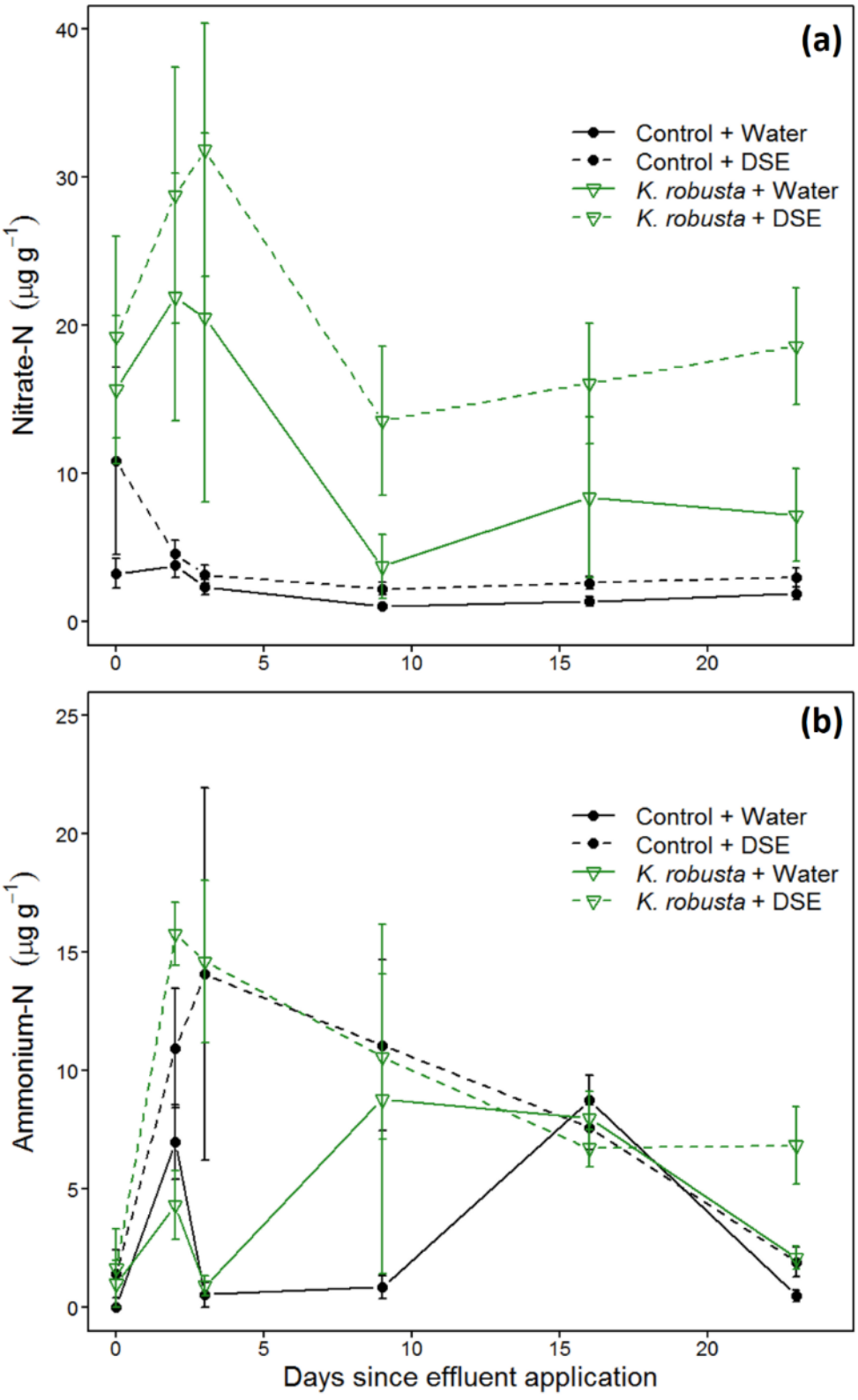


Figure 6.6 Mean (±SE) soil NO_3^--N (a) and NH_4^+-N (b) concentration over time, following the application of dairy-shed effluent and water to control and *K. robusta* plots (n=5).

6.4 Discussion

6.4.1 Suppression of nitrous oxide emissions by *K. robusta*

The presence of *K. robusta* plants reduced mean cumulative N_2O emissions following effluent application by approximately 80 % relative to the control (bare soil). Mean cumulative emissions from DSE-treated *K. robusta* plots were comparable to those from water-treated control plots. Mean cumulative N_2O emissions from control-DSE plots in the present experiment (267 g ha^{-1}) were much lower than those reported by Cameron et al. (2002) for pasture on the same soil type during a similar spring period (2810 g ha^{-1}), but were comparable to autumn data (208 g ha^{-1}). The emission factor for DSE applied to control soil (1.05 %) in this trial was close to the IPCC default value for the emission factor of land-applied effluent (1.25 %) and the New Zealand country-specific emission factor for animal excreta returned during grazing (1 %) (de Klein and Ledgard 2005, de Klein et al. 2006). Both lower (Bhandral et al. 2007) and higher (Cameron et al. 2002, Bhandral et al. 2010) emission factors for DSE have been reported in previous studies.

The N_2O emission factor for effluent applied below *K. robusta* (0.07 %) was significnatly lower than reported values for effluent applied to pasture. No other studies to date have investigated N_2O emissions from soil planted with New Zealand native plants receiving land applied DSE. Mean cumulative N_2O emissions from the control-water, *K. robusta*-water and *K. robusta*-DSE plots (32-54 g ha^{-1}) are comparable to emissions from water-irrigated exotic ryegrass on the same soil type over a similar period (Cameron et al. 2002). Reported N_2O emission factors for forested riparian soil receiving high N loads were generally similar that of *K. robusta* soil in this study (Weller et al. 1994, Jacinthe et al. 1998, Groffman et al. 2000), although were much higher in some instances on poorly drained soil (Jacinthe et al. 1998). In this study the N_2O emission factor under *K. robusta* is 92 % lower than that the control soil suggesting reduced denitrification is occurring in this soil. As a point of comparison, studies in New Zealand have shown that the nitrification inhibitor DCD can be potentially reduce N_2O emissions from urine patches by up to 70 % (reviewed by Luo et al. 2010). A few New Zealand studies have compared soil N cycling and N_2O emissions of shrubland to pastures (Price et al. 2010, Hedley et al. 2013), but none involving external N sources. Internationally, riparian denitrification studies report variable emission factors for grassed and vegetated areas receiving high N groundwater (Groffman et al. 1991, Hefting et al. 2003).

The time between DSE application and the measured peak in N_2O production (9 and 2 days in control and *K. robusta* plots respectively) was longer than that reported in several other studies (Barton and Schipper 2001, Bhandral et al. 2010). Emissions returned to background levels (16 days) over a similar period to that found for pasture by Bhandral et al. (2010). In contrast, shorter periods of

elevated N_2O flux have been reported in pasture studies on the same soil type (Cameron et al. 2002) and with the same N application rate (Barton and Schipper 2001) as the present study.

Mean background N_2O emission rates from all *K. robusta* plots pre-application (equivalent to 2.3 kg N ha^{-1} yr^{-1}) and from water-treated *K. robusta* plots throughout the trial (3.5 kg N ha^{-1} yr^{-1}) were higher than the few recorded emissions from soil under *Kunzea* spp. elsewhere in New Zealand. N_2O fluxes from a silt loam soil under regenerating *K. robusta*, 35 km from the Lincoln University Dairy Farm, were largely below detection limits (Price et al. 2010). Estimated annual emissions from mixed *L. scoparium* and *K. ericoides* forest on Pumice and Brown soils in North Island, New Zealand (0.30 kg N ha^{-1} yr^{-1}) were also lower than the present study (Hedley et al. 2013). The discrepancy between background emissions previously reported under *Kunzea* spp. and those of the present study is likely due to previous land use at the site. Intensive dairy farming at experimental site prior to planting with native species has elevated soil NO_3^--N levels, providing substrate for denitrification to N_2O. Background N_2O emissions under *K. robusta* in this study were comparable to the mean losses reported from unfertilised agricultural soil in New Zealand (3.2 kg N ha^{-1} yr^{-1}) (Parfitt et al. 2006), but higher than estimated losses from native forest soils (c. 1 kg N ha^{-1} yr^{-1}) (Barton et al. 1999, Saggar et al. 2008).

6.4.2 Soil conditions as potential drivers of nitrous oxide emissions

Soil gravimetric moisture contents were consistent with spring soil conditions in the moderately well drained Templeton silt loam soil (Di and Cameron 2006). The use of water as a treatment-control was effective, with no differences in soil moisture between DSE and water treatments for each plot type. Mean moisture content was 10% higher in control (bare soil) plots than *K. robusta* plots during the experiment. This is likely due to transpiration of *K. robusta* or the umbrella effect of the dense canopy of fine branches and leaves, which resulted in less rainfall incident on the soil. Stands of naturally regenerating *Kunzea* spp. are known to reach 100% canopy closure in 3 years (Phillips et al. 2000). Despite a significant rainfall event during the experiment (c. 10 % of annual rainfall on the Canterbury Plains), soil moisture did not increase correspondingly in *K. robusta* or control plots. However, it is possible that the sampling dates missed a short peak in soil moisture.

The lower soil moisture status may have contributed to the reduced N_2O emissions found beneath *K. robusta* compared with control soil in this study. Denitrification rates increase with increasing soil moisture, particularly at or above field capacity, as the soil becomes anaerobic, which favours denitrification (Saggar et al. 2009). Soil moisture in control plots (33.3 %) was close to the previously recorded field capacity for this soil type (Di and Cameron 2006), thus there is potential for high denitrification rates. Improved soil aeration (through the presence of roots and leaf litter) below *K. robusta* may further decrease N_2O emissions, due to reduced activity of N_2O-reductase in aerobic

environments (Bakken and Dorsch 2007). Soil moisture has been cited as a major driver of N_2O emissions from pastures (Ledgard et al. 1999, Luo et al. 2000a) and strong climatic control (temperature and moisture) on N_2O emissions has been found in *Kunzea* forest soil (Hedley et al. 2013). Soil moisture is considerably less in this region in the dry summer months (Webb 2008), therefore rates of N_2O production are likely to be lower than those calculated here for the wet spring period.

Mean soil pH was 0.3 units higher in control plots than in *K. robusta* plots in this study. The lower pH under *K. robusta* (before and during the trial) may be caused by the presence of organic acids from root exudates which are known to acidify rhizosphere soil (Salisbury and Ross 1992), compared to the unvegetated control soil. Denitrification is reduced in acidic conditions (Van der Weerden et al. 1999). Reduced denitrification under *K. robusta* due to lower pH may partially explain the reduced N_2O emissions compared to control soil. Previous authors have reported increase in soil pH following DSE application, due to hydrolysis of the urea (Schipper et al. 1996, Taghizadeh-Toosi et al. 2011). However, in the present study this was not the case, soil pH was similar in water and DSE treated plots of each type and did not change significantly through time.

The ten-fold increase in NH_4^+-N concentrations immediately after DSE application (in both *K. robusta* and control plots) resulted from the hydrolysis of effluent-derived urea, with the smaller increase in NH_4^+-N concentrations in the water treatments possibly due to the mineralisation of soil organic matter. Loss of NH_4^+-N as NH_3 through ammonia volatilisation, often reported immediately following the application of DSE or urine, is unlikely to have occurred due to the low soil pH (Bolan et al. 2004). A rapid increase in NH_4^+-N concentration and subsequent decline over a similar period were seen following DSE application to a silt loam soil in New Zealand (Bhandral et al. 2010). Plant uptake, immobilisation by soil microbes or oxidisation to NO_3^--N may reduce the available urea derived NH_4^+-N pool in soil over time. Accelerated mineralisation of soil organic matter following rainfall events may potentially explain NH_4^+-N fluctuations in control plots during the experiment.

Prior to effluent application, NH_4^+-N concentrations were similar in *K. robusta* and control soil, whereas NO_3^--N concentrations were 60 % lower in control soil than *K. robusta* (81% lower when averaged across the whole trial). High NO_3^--N in *K. robusta* soil may be a result of increased NO_3^- retention with control soil, through either decreased leaching or increased nitrification. Elevated NO_3^--N concentrations in soil under certain native species (compared with ryegrass) has been reported earlier in this thesis (Figure 4.4a) and by Hahner et al. (2014). In contrast, low NO_3^--N production was found in pumice soil forested with *Kunzea* spp. compared to pastures and angiosperm–conifer forests on similar soil (Ross et al. 2009). In contrast to other studies (Di et al. 1998b, Bhandral et al. 2010), this study found no significant increase in soil NO_3^--N following DSE application. A lag in NO_3^- production

has been observed, following the peak in NH_4^+ concentrations (Bhandral et al. 2010), thus it is possible the NO_3^--N peak was missed due to sparse sampling as the experiment progressed.

6.4.3 Potential mechanisms reducing nitrous oxide emissions under *K. robusta*

The comparatively higher NO_3^--N concentrations under *K. robusta* in this trial implies that the reduced N_2O emissions are not likely the result of inhibition of nitrifying bacteria. Low NO_3^--N levels have been reported for pumice soils under mixed *L. scoparium-Kunzea* forest compared with other vegetation types (Ross et al. 2009). However, in this case inhibition of nitrification by allelochemicals of these species was considered unlikely, based on appreciable NO_3^--N production under *L. scoparium* and *Kunzea* spp. on non-volcanic soils (Ross et al. 2009).

Despite higher availability of NO_3^--N substrate for denitrification, *K. robusta* soil treated with DSE emitted less N_2O compared to control soil, indicating that bacteria involved in the denitrification pathways are being inhibited or reduced. Inhibition of denitrifying bacteria has the potential to reduce the amount of N_2O emitted, though may also inhibit the conversion of N_2O to N_2 gas. No studies have specifically examined the effect of *Kunzea* spp. on soil denitrifying bacteria. Ross et al. (2009) found that *L. scoparium* and *Kunzea* spp. had no adverse effect on total soil microbial biomass, while another study found that water extracts of *L. scoparium* plant materials had a significant impact on bacteria commonly found in human biosolids (Prosser et al. 2014). If the presence of *K. robusta* inhibits denitrifying bacteria, several possible pathways may lead to this:

(i) Antimicrobial agents from *K. robusta* may enter the soil through rhizodeposition or the degradation of plant material and inhibit the growth or activity of denitrifying bacteria. Water extracts of *L. scoparium* roots significantly reduced bacteria growth, however leaf extracts were far more active, even at low concentrations (Prosser et al. 2014). Leaf-fall is common in *L. scoparium* year round and Prosser et al. (2014) infer this is the most likely pathway for antimicrobial agents from *L. scoparium* to accumulate in soil. Root exudated leptospermone has been shown to remain in soil for considerable lengths of time (Cornes 2005), thus potentially allelochemicals released through biomass degradation would behave in a similar way. In the present study, although the *K. robusta* plants are young, leaf litter has already built up on the soil surface, potentially decomposing.

(ii) The dry aerated soil conditions under *K. robusta* plants may not be suitable for the production of N_2O via denitrification pathways, which require high soil moisture and low rates of oxygen diffusion (Bolan et al. 2004). In this case, soil at the naturally dry sites which *Kunzea* spp. often dominate may be responsible for the low reported rates of N_2O production under this species (Price et al. 2010, Hedley et al. 2013). Additionally, the low soil pH under *K. robusta* is also sub-optimal for denitrifcation. These soil conditions are likely not unique to *K. robusta*. Low soil

moisture may occur under any species with a dense canopy or high water uptake and the root exudates of many plant species may acidify soil.

(iii) The microbial community under *K. robusta* may differ from control soil in this study. Through the release of organic C compounds, plant roots are able to modify physico-chemistry and influence the abundance and functional diversity of both soil fauna and microbes (Bardgett and Wardle 2010). Denitrifying bacteria exhibit a variety of reduction pathways, some bacteria produce only N_2, while others a mixture of N_2O and N_2, and some only N_2O (Stouthamer 1988). A bacterial community dominated by less N_2O producing denitrifying bacteria would result in reduced N_2O emissions.

6.4.4 Implications for on-farm planting

The large reduction in N_2O emissions under *K. robusta* observed following DSE application suggests that the disposal of excess effluent onto areas of *K. robusta* incorporated into dairy farms may result in lower N_2O emissions than conventional land application. If litter decomposition is responsible for reduced emissions then rotational cropping and mulching of this species may increase the attenuation of alleochemicals in soil (Prosser et al. 2014). This has implications in terms of the Emissions Trading Scheme (Ministry for the Environment 2011) and the provision of an incentive to increase the planting of native vegetation on New Zealand dairy farms. Moist riparian zones receive large amounts of N, thus are potential hotspots for N_2O production in agricultural landscapes (Groffman et al. 2000). Planting *K. robusta* in riparian zones may mitigate this source of N_2O, however this typically dryland species may not be well suited to moist riparian soils. *K. robusta* could be planted or allowed to regenerate within farm paddocks to encourage stock to shelter and camp in these locations. Deposition of urine onto *K. robusta* soils would reduce N_2O emissions that follow such high N loading. Although suppression of N_2O emissions is desirable, reduced denitrification may raise soil NO_3^--N concentrations. The potential adverse effects of increased NO_3^--N on freshwater quality need weighing against the benefits of reduced N_2O emissions. Further research is needed to determine the balance of N loss pathways following DSE application to *K. robusta*.

Additional benefits of planting *Kunzea* spp. on farms could include; methane uptake through enhanced soil methanotrophic activity (Price et al. 2010); erosion control aided by rapid canopy closure (Phillips et al. 2000, Saggar et al. 2008); C sequestration (Trotter et al. 2005); production of high value honey (Stephens et al. 2010); pathogen reduction during the disposal of human or animal wastes (Prosser et al. 2014). If establishment can be achieved on otherwise unusable land (due to contamination or degradation), where effluents, bio-solids or other bio-wastes can be recycled, then this system has added potential to generate an economic return (Prosser et al. 2014).

6.5 Conclusions

This experiment has identified significant suppression of N_2O by *K. robusta* flux following application of DSE, when compared to unvegetated soil. Following on from this:

- The current extensive conversion of agricultural land to dairy farms on the Canterbury Plains, offers the potential to re-introduce *Kunzea* spp. and contribute to the high value honey industry. *Kunzea* spp. dominated plant communities represent the native vegetation of the Canterbury Plains. The irrigation of DSE to land planted with *K. robusta* may provide an alternative to pasture application, potentially with comparatively less N_2O emissions, although subsequent NO_3^- leaching also needs consideration.

- Despite higher levels of NO_3^--N in *K. robusta* soil, reduced N_2O flux was observed, indicating possible inhibition of denitrification. Potential mechanisms involved include suppression of denitrifying bacteria by *K. robusta* allelochemicals or constraints on the denitrification process in *K. robusta* soil. This study does not have the scope to draw conclusions on the likely mechanisms behind reduced N_2O emissions under *K. robusta*.

- The environmental benefits of reduced N_2O emissions warrant a closer look at the effect of *Kunzea* spp. on the soil N cycle. Further field research should incorporate comparison to other native species, to determine if this effect is unique to *K. robusta*. ^{15}N tracer studies involving plants grown in controlled conditions would be valuable to determine mechanisms involved. Furthermore, simultaneous investigation of gaseous and leaching N losses is necessary, as a reduction in N_2O emissions may lead to pollution swapping, through increased NO_3^- leaching.

Chapter 7

Upscaling and practical application of the findings

7.1 Introduction

The strategic incorporation of New Zealand native plants into farmland offers the potential to reduce nitrogen (N) losses through retention in soil and plant uptake, whilst increasing native flora and fauna in biodiversity-depauperate landscapes. Despite recent advances in the management of N (applied as fertilisers and effluents) on farms, widespread nitrate (NO_3^-) leaching contributes to poor water quality in New Zealand (Smith et al. 1993, Larned et al. 2004). Gaseous losses of nitrous oxide (N_2O) from soil are also of concern. Although only representing a small portion of applied N (typically <3 %), N_2O is a potent greenhouse gas (Cameron et al. 2013). Regional councils throughout New Zealand are developing regulatory limits to control the amount of NO_3^--N leaching from farmland. In the Canterbury Plains region, current and planned extensive land conversion to high intensity dairy farming is likely to further increase NO_3^--N concentrations in shallow groundwater (Bidwell et al. 2009). Modelling has indicated that a 40 % reduction in leaching rates from all land uses on the Canterbury Plains will be necessary to meet new standards (Canterbury Water 2009).

There have been no attempts to measure or model the possible farm-scale N uptake, and subsequent reduction in N losses, which is possible through incorporation of native species. The copious literature regarding temperate pasture species shows that these grasses remove large amounts (up to 300 kg N ha^{-1} yr^{-1}) of N applied to farmland via plant uptake (Di and Cameron 2002b). Clearly, when harvested in cut-and-carry systems this amount of N may be removed from the soil-plant environment (Di et al. 1998a). However, in grazed pastures a large proportion (60-90 %) of N ingested by stock is returned to soil as urine and dung, with only a small portion removed as animal products (Haynes and Williams 1993). The N loading under urine patches is around 1000 kg N ha^{-1} (Di and Cameron 2002b) and is likely to be higher in locations where stock camp. This heterogeneous matrix of N returns (c. 20-30 % if grazed area) augments N leaching losses resulting from fertiliser application. As a result, NO_3^--N leaching losses from grazed dairy pastures on the Canterbury Plains range from 20-80 kg N ha^{-1} yr^{-1} depending on soil type and management practices (Lilburne et al. 2010).

Nitrogen accumulated by native herbaceous and woody species is retained in plant biomass for varying periods of time, with a portion subsequently returned to soil through litter fall, or removed through grazing or harvest. New Zealand native shrubs and trees have deeper more extensive root systems than pasture species (Marden et al. 2005) and have potential to access and extract N from a greater depths in the soil profile. As such, NO_3^- leaching may be mitigated by both native plant N uptake

and reduced water flux through their rhizosphere soil. Plant transpiration and foliar interception of rainfall are likely to reduce downward transport of water compared to pastoral soils. In addition, native species have unique rhizosphere soil chemistry that may influence soil microbiology and N cycling (Chapter 4, Chapter 6). Nitrate leaching rates from below undisturbed native forest are typically much lower than for agricultural land (Davis 2014), estimated at <0.1 kg N ha^{-1} yr^{-1} for native remnants on the Canterbury Plains (Lilburne et al. 2010).

New Zealand native plants certainly have the potential to extract N from farm soils given their tolerance to elevated N and propensity for high foliar N uptake (Chapter 5). Extrapolations to annual uptake in Chapter 5 indicated that pasture is likely to be removed more N from soil than native species. However, N is potentially retained longer in the biomass of native species than in pasture under grazed conditions. This temporary immobilisation of N, as well as altered rhizosphere N cycling, may slow the N flux across riparian zones compared to rapid N cycling in grazed pastures. Application of effluents to land planted with native species may reduce ensuing NO_3^- leaching, whilst revenue gain is possible through commercial plant products. For example, the honey produced by *Leptospermum scoparium* forms an industry worth more than NZD 75 million per annum (Landcorp). Investment in a growing natural products industry has developed novel uses for the fibre, seed and gels of *Phormium tenax* (McGruddy 2006, Wehi and Clarkson 2007). Planting native species in an agricultural matrix, may also be considered in terms of reducing N_2O emissions associated with effluent application to land (Chapter 6) and sequestering atmospheric carbon (Trotter et al. 2005).

Disposal of dairy shed effluent (DSE) through irrigation to pastures is common in New Zealand (Houlbrooke et al. 2004). Whilst providing an effective source of plant nutrients, this raises the risk of NO_3^- leaching to groundwater when N is in excess of pasture growth requirements and percolates below the root zone (Di and Cameron 2002b). In short-rotation forests used for land treatment of effluents, the nutrients incorporated in above-ground biomass are harvested and processed for commercial uses (Moffat et al. 2001, Tzanakakis et al. 2009). In New Zealand, field and lysimeter based reserach has focused on exotic trees, such as *Eucalyptus*, *Pinus* and *Populus* spp. (Sims and Riddell-Black 1998, Roygard et al. 2001, Guo et al. 2002, Nicholas 2003). These studies report increased yields following effluent application and large amounts of nutrient storage, particularly for species with high growth rates. Variation in foliar N concentration may additionally contribute to species-specific N uptake rates. New Zealand native trees are slower growing than exotic species (Phillips et al. 2011a). However, native riparian and colonising species establish quickly and have considerable growth within 3-5 years of planting (Marden et al. 2005). Native species may have potential for use as crops to extract N from farm soils. Prosser et al. (2014) identified the potential for application of human biosolids to land planted with *L. scoparium*, as extracts of the roots and leaves proved effective inhibitors of

pathogenic bacteria. No studies to date have considered the potential for large-scale application of effluents to native species.

The present chapter aims to calculate potential farm-scale N uptake possible through planting selected native species. Estimated uptake by *L. perenne* is provided for comparison. The empirical results of Chapter 5 and biomass productivity values from literature are used to model N uptake. The results are discussed in light of potential N losses from several possible on-farm situations, drawing on the findings of Chapter 4 and Chapter 6.

7.2 Calculations - Upscaling plant nitrogen uptake

Hypothetical annual amounts of N uptake into above-ground plant material were calculated per hectare for land planted with the New Zealand native dicotyledons (*L. scoparium* and *Kunzea robusta*), monocotyledons (*Carex virgata*, *P. tenax* and *Austroderia richardii*) and the exotic pasture species *L. perenne*. Nitrogen uptake was calculated using a combined approach which incorporated annual biomass production rates from literature and the experimentally determined growth and N uptake response of each species to specified N addition rates (200, 800 and 1600 kg N ha^{-1}, Chapter 5, Trial One).

7.2.1 Species-specific biomass production rates

Native species

Biomass production rates from literature were used for the modelling as experimentally determined rates were for 1 year old containerised plants, thus were not suitable for estimating production in field conditions. Species-specific allometric equations for estimating biomass exist for a range of New Zealand native species (Coomes et al. 2002, Beets et al. 2012, Mason et al. 2014, Schwendenmann and Mitchell 2014), but not the monocotyledons investigated in this thesis.

Biomass production rates were based on replicated data for plants grown in similar conditions (one plant per m^2) to most restoration sites (Marden et al. 2005, Marden and Phillips 2009). Although these native plant trials (see Plate 3.13) were conducted in Gisborne (North Island, New Zealand), growth rates were considered a suitable general estimate and representative of differences between species. A non-linear biomass production response was observed during early growth of New Zealand native plants (Marden et al. 2005). Therefore, estimates were based on annual growth, averaged over 5 years (*L. scoparium* and *K. robusta*) and 3 years (*C. virgata*, *P. tenax* and *A. richardii*) since germination (Table 7.1). These time-averaged estimates do not necessarily reflect growth in any particular year and may not be accurate for older plants. In lieu of suitable literature data, the annual production of

K. robusta was based on the biomass of closely related species *L. scoparium*. Data were available for *Austroderia toetoe* and *Carex secta* (Marden and Phillips 2009). However, this was not suitable to estimate growth of related species, *A. richardii* and *C. virgata*, due to a lack of replication and the comparatively larger mature plant size of *A. toetoe* and *C. secta* (Edgar and Connor 2000). Therefore, production rates for *A. richardii* and *C. virgata* were based on *P. tenax*, an herbaceous monocotyledon of comparable morphology that had similar growth for year old plants (Chapter 5, Figure 5.1).

Table 7.1 Calculation of annual above-ground biomass production (dry matter) per hectare for five native species and *L. perenne* based on biomass reported in literature. Biomass are for plants grown without fertiliser addition. The annual production rates calculated are used for the control (0 kg N ha^{-1} yr^{-1}) treatment in N uptake modelling.

Species	Literature based above-ground biomass			Annual production per plant (kg yr^{-1})	Annual production per hectare (t ha^{-1} yr^{-1}) [1]
	Biomass (kg)	Age (yr)	Reference		
L. scoparium	0.75	5	(Marden et al. 2005, n=5)	0.25	2.5
K. robusta			Based on *L. scoparium*	0.25	2.5
C. virgata			Based on *P. tenax*	1.10	10.1
P. tenax	3.3	3	(Marden and Phillips 2009, n=3)	1.10	10.1
A. richardii			Based on *P. tenax*	1.10	10.1
L. perenne	10 t ha^{-1} yr^{-1}		(Dairy NZ Ltd. 2011)		10

[1] Based on a native plant density typical of riparian and restoration planting, 1 plant per 1 m^2 (Environment Canterbury 2011)

Annual biomass production per hectare for the control (0 kg N ha^{-1}) treatments were based on the literature estimates (Table 7.1). Biomass production rates for plants which received N were estimated by adjusting control treatment rates, using the incremental change in biomass data from Chapter 5 (Trial One, Figure 5.1). To obtain biomass production rates for the N treatments, the control treatment rates were multiplied by the proportional change between the control and each N treatment (200, 800 and 1600 kg N ha^{-1}), for each species.

Lolium perenne

Growth of South Island pastures, without N fertiliser, range from 10 to 16 t ha^{-1} yr^{-1} dry matter, across a range of soil types and climates (Dairy NZ Ltd. 2011). As Trial One (Chapter 5) was conducted using a low fertility soil, the lower end of this range (10 t ha^{-1} yr^{-1}) was used to estimate biomass production for the control treatments of *L. perenne*. The cumulative biomass produced by control pots of *L. perenne* during Trial One (equivalent to approximately 15 t ha^{-1} yr^{-1}) was not used in the present calculations, as it does not accurately reflect field conditions or seasonal variations. Biomass

production for N treatments was estimated using the proportional changes in *L. perenne* biomass data from Trial One (as for the native species).

7.2.2 Nitrogen uptake

Nitrogen uptake was calculated by multiplying the estimated annual biomass production (dry matter per hectare) for each species, at each N treatment level, by the N concentration of above-ground biomass for plants within the associated species x N combination in Trial One (Chapter 5). Treatment means and standard errors were then calculated (n=6). The mean from the control treatment of each species was then subtracted from each value where N was applied, to account for the background uptake of N from the soil. The resulting values represent the amount of added N taken up by plants at each N application rate and were also converted to a percentage uptake of applied N, or uptake efficiency.

7.3 Nitrogen uptake through biomass production

Calculated N uptake for *L. perenne* was approximately 10 times higher than the native dicotyledons and double that of the native monocotyledons, when treated with 200 and 800 kg N ha^{-1} yr^{-1} (Figure 7.1). Differences in N uptake are generally related to biomass production rates. The high biomass producing native monocotyledons were calculated to take up more applied N than the slow-growing woody dicotyledons (Figure 7.1). Strong relationships between species biomass and N uptake have been similarly reported for exotic tree species, treated with effluents (Guo et al. 2002, Nicholas 2003). Calculations for *L. perenne* and the native monocotyledons were based on similar biomass production rates (c. 10 t ha^{-1} yr^{-1} for controls). However, upscaling showed that *L. perenne* would initially accumulate more N, due to this species' greater proportion of N in biomass (Chapter 5, Table B.1). At 1600 kg N ha^{-1} yr^{-1}, N uptake by C*. virgata* and *P. tenax* equated to that of *L. perenne* (Figure 7.1), due to the decline in *L. perenne* production experienced at this rate in Trial One (Chapter 5, Figure 5.1). Native species with enhanced biomass production in soils with elevated N levels, such *C. virgata* (Chapter 5, Figure 5.1), would remove the most N. *Carex* species are commonly used in riparian restoration due to their rapid establishment (Plate 7.1a and b). These findings suggest *C. virgata* is likely to have an important role in mitigating N flux from agricultural land.

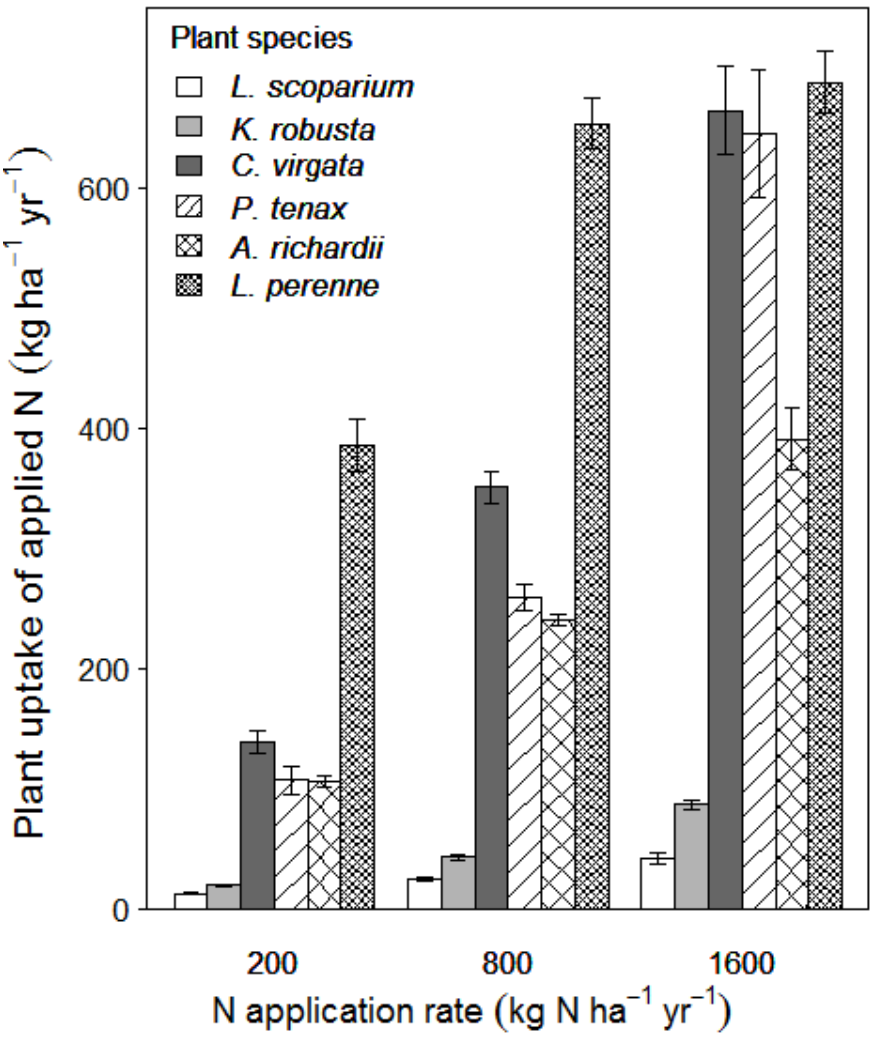


Figure 7.1 Calculated mean (±SE) yearly uptake of applied N per hectare (three application rates 200, 800 and1600 kg N ha^{-1} equivalent) into above-ground plant material for five New Zealand native species and *L. perenne*. Based on a planting density of 1 plant per 1 m^2 for native plants.

The calculated annual N recovery in harvested *L. perenne* was higher than values reported for field and lysimeter studies at similar application rates (Di et al. 1998a, Di and Cameron 2002a, Moir et al. 2003). Nitrogen losses through leaching in previous studies may contribute to this discrepancy, as the present values are based on the closed-system (no leaching) conditions of Trial One (Chapter 5). Although calculated N uptake was higher for *L. perenne* than the native species, uptake can only be considered a net N removal if foliage is harvested (Di and Cameron 2002b). Based on the present calculations a fenced grass riparian zone that is mown regularly (clippings removed) would remove more N than native plants. However, in grazed pastures the net N uptake (removal from soil) is likely to be lower than the values calculated, due to rapid N turnover. Native plants would take up less N, but this would potentially be retained for longer than *L. perenne* in a grazed system, although leaf fall in *L. scoparium* and *K. robusta* is considerable and deposition of the high-N foliage may reduce net N uptake considerably. Litter fall has been compared amongst native forest species (Bellingham et al.

2013) but data is lacking for riparian species. The periodic harvest of native plants such as *P. tenax* would constitute a net N removal. In locations where N flux occurs as lateral subsurface flows, the deep roots of native plants may access N at greater depths than *L. perenne*. Thus, the effect of native plants on total NO_3^- leaching losses may be unrelated to their comparative uptake abilities.

In terms of large-scale uptake of applied N by native plants, no data are available for comparison. As for *L. perenne*, the values estimated here for native plants are likely to be an overestimate due to increased leaching in field conditions. Background N uptake rates of unfertilised mature *L. scoparium* and *Kunzea* spp. (Scott et al. 2000) were less than those calculated in the present model (N uptake for control treatments, data not shown). This discrepancy is potentially due to the lower contribution of high-N foliar material (3-5 % total biomass) of the mature trees (Scott et al. 2000), compared to the young plants on which the present calculations were based (c. 60 %). Several studies have identified uptake of around 50 kg N ha^{-1} yr^{-1} from applied effluent by *Eucalyptus* and *Pinus* spp. in New Zealand (Tomer et al. 2000, Guo et al. 2002). N removal by the native monocotyledons in the present study surpassed this rate.

The N uptake calculations rely on the accuracy of the biomass production rates extrapolated from literature. The production estimate used for *K. robusta* and *L. scoparium* (Marden et al. 2005) was lower than reported growth rates of mature plants (Scott et al. 2000, Trotter et al. 2005). Although more biomass may be produced for older trees, N uptake may not increase accordingly, as the proportion of low-N, woody material increases with age (Scott et al. 2000). Historical records of *P. tenax* biomass production are wide ranging. A conservative estimate (6 t ha^{-1} yr^{-1}) is similar to that used in the present calculations, although values up to 20 t ha^{-1} yr^{-1} have been reported (McGruddy 2006). No field data are available for comparison of the rates used for *C. virgata* and *A. richardii*. In the field, mature plants of *P. tenax* are typically larger (3 m height) than *A. richardii* (2 m) and *C. virgata* (1 m) (Edgar and Connor 2000). Therefore, it is likely that production rates used for the monocotyledons do not reflect field conditions. *P. tenax* is expected to outperform the other native monocotyledons in terms of biomass production and N uptake.

7.4 Nitrogen uptake efficiency, nitrous oxide emissions and nitrate leaching

For all species studied N uptake efficiency was greatest at 200 kg N ha^{-1} (Figure 7.2). Among species, N uptake efficiency was the highest for *L. perenne* and greater than that found in previous pasture studies (10-50 %, Di et al. 1998a, Di and Cameron 2002a, Moir et al. 2003). In those lysimeter and field-based studies, N losses through volatilisation and leaching prior to plant uptake may have contributed to reported inefficiencies. Nitrogen uptake efficiency was calculated at above 100 % for *L. perenne* at

200 kg N ha^{-1} yr^{-1} in the present study. *L. perenne* would require N at a higher rate to sustain soil fertility under the conditions assumed from Trial One (Chapter 5). In the native species, calculated N uptake efficiency was around 10 times higher for the monocotyledons than dicotyledons (maximum efficiency; *C. virgata*, 69 % at 200 kg N ha^{-1} yr^{-1}), due to the increased biomass production. Following effluent application, N uptake efficiency of around 10 % has been reported for *Eucalyptus* (Gielen et al. 1999) and *Pinus sp.* in New Zealand (Tomer et al. 2000). These efficiencies are similar to those calculated for the native woody species in the present study.

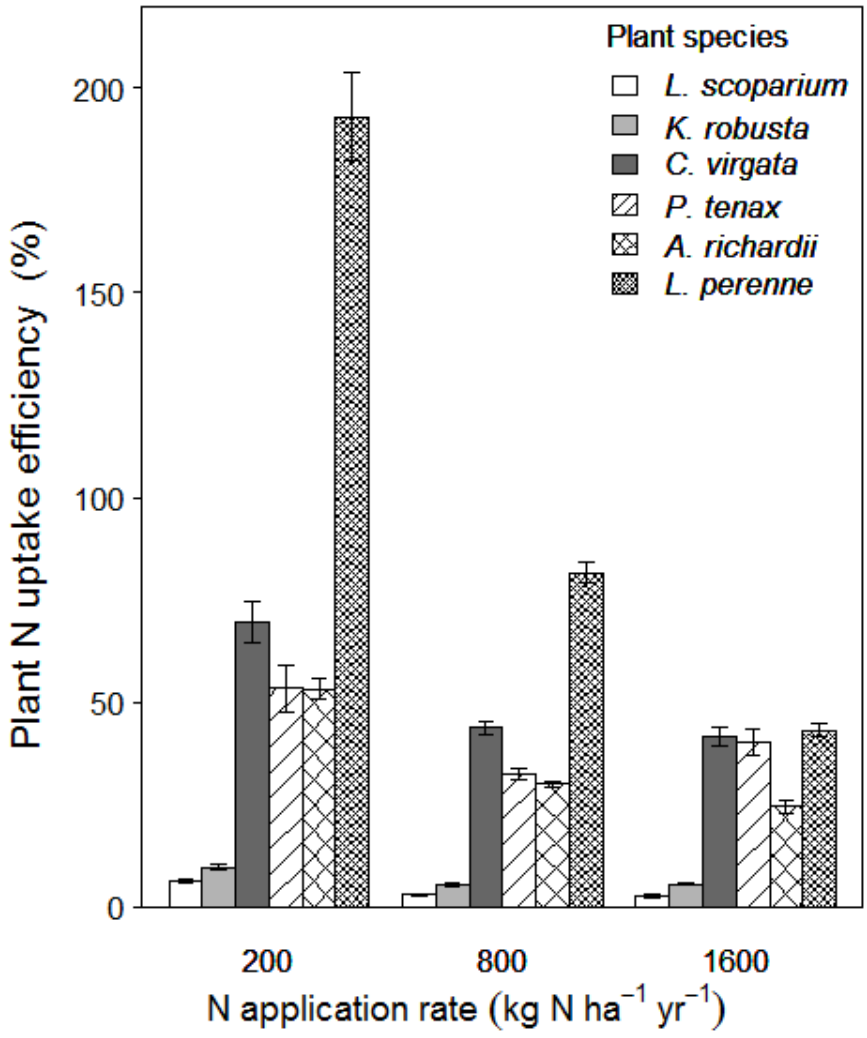


Figure 7.2 Uptake efficiency (±SE) of N applied at three application rates (200, 800 and 1600 kg N ha^{-1} yr^{-1} equivalent) for five New Zealand native species and *L. perenne*. Uptake efficiency represents the percentage of N applied to plants that would be incorporated into plant tissues at a farm-scale.

The potential reduction in N losses as NO_3^--N and N_2O from a grazed dairy paddock (1 hectare) were considered, following the set-aside of a 2 m fenced border (8 % of land) planted with either native monocotyledons or dicotyledons (Table 7.2, Plate 7.1b). In New Zealand, 200 kg N ha^{-1} yr^{-1} is the upper

limit for fertiliser application to grazed dairy pastures set by many local authorities (Di and Cameron 2002b). Nitrate-N leaching losses for the dairy-grazed area were taken from literature for soil of the Templeton series (similar to that used in Trial One) receiving fertiliser N at a rate of 200 kg N ha^{-1} yr^{-1} (Silva et al. 1999). This value takes into account N returns through urine. A reduced N loading on the planted area was assumed at 50 kg N ha^{-1}, considering that some N may be received as fertiliser or effluent overspray, or leaching laterally from surrounding land to down-slope paddock borders (Table 7.2). Based on the N uptake efficiencies calculated at 200 kg N ha^{-1} (Figure 7.2), 100 % efficiency is assumed for native monocotyledons receiving 50 kg N ha^{-1} and a scaled efficiency of 30 % for dicotyledons. The New Zealand country specific emission factor for fertiliser applied N was used to estimate N_2O-N losses from the pasture area (de Klein et al. 2001). The potential reduction in N_2O-N emissions from planting the set-aside land with *K. robusta* was calculated using the emission factor for DSE applied N for this species (Chapter 6). This assumed to be suitable for fertiliser N as well.

Table 7.2 Potential reductions in N losses from one hectare of a grazed dairy system through the incorporation of native monocotyledons and dicotyledons in a 2 m fenced border (8 % of land). The model assumed fertiliser N applied at 200 kg N ha yr^{-1} to the grazed pasture, while the native border receives N at 50 kg N ha yr^{-1}.

	Calculation	N flux (kg N yr^{-1})
NO_3^--N leaching from grazed pasture at 200 kg N yr^{-1}:	(Silva et al. 1999)	**54**
N uptake by natives planted on 8 % of land at 50 kg N yr^{-1}:		
Monocotyledons (100 % efficiency)	50 x 1.00 x 0.08	4.0
Dicotyledons (30 % efficiency)	50 x 0.3 x 0.08	1.2
NO_3^--N leaching from grazed pasture on 92 % of land:	54 x 0.92	49.7
NO_3^--N leaching from combined pasture -native plants:		
Monocotyledons [a]	49.7 + (4.0 - 4.0)	**49.7**
Dicotyledons [a]	49.7 + (4.0 - 2.8)	**52.5**
N_2O-N emissions:		
All land in grazed pasture at 200 kg N yr^{-1} [b]	200 x 0.0125 x 1.0	**2.5**
Combined pasture-native plants (*K. robusta*) [c]	(2.5 x 0.92) + (50 x 0.0007 x 0.08)	**2.3**

[a] 50 kg N yr^{-1} applied to 0.08 ha = 4 kg of potentially leachable N, leaching reduced by plant uptake

[b] Emission factor for fertilised pasture 1.25 % (de Klein et al. 2001)

[c] Emission factor for *K. robusta* 0.07 % (Chapter 6)

A reduction in NO_3^--N leaching resulted from reducing the grazed-fertilised land area through fencing (Table 7.2). At the assumed N loading of 50 kg N ha^{-1}, no additional NO_3^--N leaching occurred from the fenced-off area when planted with native monocotyledons. Native monocotyledons made greater contribution to reducing NO_3^--N leaching (8 %) from the hectare paddock, than the native dicotyledons (3 %) under which some NO_3^--N leaching occurred (Table 7.2). Although, as discussed previously, N returns through litter fall or stock browsing on the native vegetation may raise NO_3^--N leaching above calculated rates. Nevertheless, high biomass producing native monocotyledons appear to be the best choice for reducing NO_3^--N leaching losses. Harvest of the planted border would constitute a permanent removal of N if the biomass were used for purposes outside the farm system, such as in a fibre production industry (McGruddy 2006). In the context of mass a balance of N, there is every likelihood that N uptake by native monocotyledons would play a substantial role in N management.

Calculated annual NO_3^--N leaching was by reduced 4 kg from the one hectare paddock under the monocotyledon border-planting scenario (Table 7.2). If repeated across multiple hectares of farmland these species have the potential to reduce total farm leaching losses in line with new limits set by local authorities. A single row of planted natives along fence lines (1 m border, Plate 7.1f) would reduce leaching losses by half this rate. The reductions in farm outputs associated with the loss of land may be offset by the ability to maintain stocking limits on land retained in pastures and remain with the local authority NO_3^--N limits. Mitigating farm-scale NO_3^--N leaching through native plantings provides an alternative to reducing farm-wide stocking rates. Native plantings additionally offer conservation benefits and potentially ecosystem services of value to the farmer, such as increased crop pollination (Sandhu et al. 2008). Society is becoming less accepting of the negative impacts of farming. Reduced NO_3^--N leaching through the incorporation of culturally appealing native plants onto farms could improve public perceptions of the dairy industry in New Zealand (Baskaran et al. 2009).

A 8 % reduction in N lost as N_2O was achieved through planting a 2 m border with *K. robusta* (Table 7.2). In terms of kilograms of N, this was small compared to reductions in NO_3^--N leaching losses. Despite this, the environmental cost per unit of N is higher for N_2O-N than NO_3^--N, thus a small reduction may still be of great benefit (Eory et al. 2013). *Kunzea* spp. frequently colonise marginal farmland and stock often camp beneath these trees for shelter (Plate 7.1e). Through continual urine deposition, the N loading and potential for N_2O emissions is likely to be high in stock camp soil. Increased NO_3^--N leaching may occur in these location, due to low N uptake by the native dicotyledons compared to pasture. However, suppression of the extensive N_2O emissions that follow urine deposition may outweigh the NO_3^--N losses in terms of environmental benefit. *K. robusta* could be allowed to regenerate within farm paddocks (Plate 7.1e) or planted in paddock corners to encourage stock to shelter and deposit urine beneath, thereby reducing farm-scale greenhouse gas emissions.

This may be of financial importance in light of the potential for future emissions trading schemes (Ministry for the Environment 2011).

In addition to their propensity for high N uptake, the rhizosphere soil conditions associated with native species require consideration. The modelling results showed *A. richardii* has the potential to extract large amounts of applied N from soil, yet field investigation of this species planted at restoration sites found higher rhizosphere soil NO_3^--N concentrations, compared to the other native species (Figure 4.4, Chapter 4). The cause of the elevated NO_3^--N in the soil beneath this species is unclear, but this may lead to higher NO_3^--N leaching rates than under the other monocotyledons. Thus, *A. richardii* is potentially less suited for plantings designed to reduce N losses. The rapid biomass production (Marden et al. 2005) and elevated foliar N concentrations (Figure 4.3, Chapter 4) of *Plagianthus regius* offer potential to reduce NO_3^--N leaching losses through plant uptake, but winter-leaf loss in this deciduous species is likely to return a considerable amount of assimilated N to the soil. Elevated soil NO_3^--N was also recorded beneath *P. regius* (Figure 4.4, Chapter 4). Understanding the balance between plant N uptake, NO_3^--N leaching and N_2O-N emissions is crucial. Further study is needed to accurately estimate potential reductions in farm N losses through planting set-aside land.

7.5 Potential for effluent application to stands of native species

Typically, N uptake by pasture or forest species results in decreased N leaching beyond the root zone to ground waters (Roygard et al. 2001, Moir et al. 2013). However, when external N inputs exceed plant accumulation, NO_3^--N leaching losses occur (Sims and Riddell-Black 1998, Di and Cameron 2002b). Disposal of DSE to land planted with native species may result in lower NO_3^--N leaching than irrigation to grazed pastures (Plate 7.1c and d). Based on the calculated N uptake efficiencies, application of 200 kg N ha^{-1} yr^{-1} (or higher) is likely to cause significant NO_3^--N leaching. However, a lower application rate of around 100 kg N ha^{-1} yr^{-1}, to land planted with native monocotyledons, is potentially sustainable, without adverse N leaching. A suitable application rate on to *K. robusta* and *L. scoparium* would be lower still (< 50 kg N ha^{-1} yr^{-1}), due to the lower N uptake efficiencies of these species. Based on the average N uptake efficiencies of native monocotyledons and dicotyledons at 200 kg N ha^{-1} yr^{-1}, a scaled-back application rate was calculated at which 100 % N uptake would be possible. The land area required for a dairy farm to recover the annual N produced and stored in an effluent pond as plant biomass was calculated for the native monocotyledons and dicotyledons (Table 7.3). The annual pond discharge value was calculated by Roygard et al. (2001) from average reported discharge rates and N concentrations of ponds in New Zealand.

Table 7.3 Calculation of the land area required for the annual disposal of a dairy farm effluent pond to land planted with either native monocotyledons or dicotyledons. Application rates are based on plant N uptake efficiencies and are those at which no additional NO_3^--N leaching is likely to occur. Calculations follow those of Roygard et al. (2001).

Annual dairy pond N discharge:	607 kg N yr^{-1}
100 % efficient N uptake possible at application rates of:	
Monocotyledons	116 kg N ha^{-1} yr^{-1}
Dicotyledons	15 kg N ha^{-1} yr^{-1}
Land area for effluent application:	
Monocotyledons	5.2 ha
Dicotyledons	38.0 ha
Land area for re-planting post-harvest:	
Monocotyledons	1.7 ha
Dicotyledons	12.6 ha
Total land area required:	
Monocotyledons	7.0 ha
Dicotyledons	50.5 ha

An area of 5.2 ha would be required to apply the entire pond discharge to land planted with native monocotyledons at a rate of 115 kg N ha^{-1} yr^{-1} (Table 7.3). If plants were established as three blocks, using a 3 year growth rotation, one block could be harvested per year. Previous studies have identified the importance withholding DES application for 1 year post-harvest, as N uptake is low for young plants when roots do not fully occupy the site (Roygard et al. 2001, Guo et al. 2002). To avoid this, an extra block of land is planted, one third the size of that required per year (Roygard et al. 2001). This block would not receive DSE in the first year following planting (Roygard et al. 2001). Therefore, a total of 7.0 ha would be required to grow native monocotyledons in a short rotation system for the total disposal of DSE (Table 7.3). However, continual harvest of substantial amounts of fibre from *P. tenax* is possible without effecting regeneration (McGruddy 2006), thus no additional land may be necessary for this species. Roygard et al. (2001) calculated a lesser area (5.4 ha) would be required for total N recovery using *Eucalyptus* spp. A higher application rate (150 kg N ha^{-1} yr^{-1}) was possible to this species without adverse N leaching. However, Roygard et al. (2001) did not include a control treatment from which to compare background N uptake and leaching rates.

The amount of land required for disposal to the woody dicotyledons is far greater, due to the lower sustainable application rate (Table 7.3) and it is unlikely that a farmer would consider setting aside this extent of land. Yet, the N_2O emissions from land planted with *K robusta* are likely to be significantly less than following effluent application to grazed pastures at the same rate (Table 7.4).

However, if denitrification is suppressed by *K. robusta* and *L. scoprium* allelochemicals (Chapter 6), a corresponding increase in soil NO_3^--N concentrations may cause NO_3^--N leaching below these species, even at low application rates. Research to determine N_2O emissions from soil beneath native monocotyledons would allow comparison of the full environmental impact of effluent application to such plantings.

Table 7.4 Rates of N_2O emission following dairy effluent application to grazed pasture and land planted with *K robusta*. The emission factor for grazed pasture is based on de Klein et al. (2001), while the emission factor for *K. robusta* was calculated in Chapter 6.

	kg ha^{-1} yr^{-1}
Sustainable N application rate to *K. robusta*:	15
N_2O-N losses from application to:	
Grazed pastures (1.25 %)	0.37
K. robusta (0.07 %)	0.01

Based on the calculations from the present study, application of more than 200 kg N ha^{-1} yr^{-1} to *L. perenne* may be possible without adverse NO_3^--N leaching. Based on this rate, 3.0 ha or less would be required to dispose of the dairy farm effluent pond to pastures. However, previous study has shown considerable NO_3^--N leaching (47 kg N ha^{-1} yr^{-1}) does occur when effluent is applied at this rate to dairy-grazed pastures (Silva et al. 1999). New Zealand native species are deeper rooted (Marden et al. 2005, Marden and Phillips 2009) than *L. perenne* (Haynes and Williams 1993), potentially removing N leaching through the lower soil profile. Yet, this may only be effective for older native plants, with roots that fully occupy soil at the site. Through complete ground coverage, roots of *L. perenne* will rapidly occupy the site, but not to the depth of native species. Native species with rapid lateral root spread may be the most effective at capturing N leaching following effluent spraying. In addition, fast canopy closure would reduce water infiltration rates and potentially NO_3^--N leaching. Rooting depth may be more important where N is percolating in deeper drainage layers. The monocotyledons investigated here have substantially greater total root length than *K. robusta* and *L. scoparium* (Marden and Phillips 2009). This correlated with individual plant N uptake efficiency in Trial One (Chapter 5), and may facilitate greater capture of applied N in the field. The native monocotyledon, *Cordyline australis*, is also deep rooting and obtains fast root site occupancy (Phillips et al. 2011a), thus may be suitable for N uptake and interception, but was not considered in the present modelling.

7.6 Implications for on-farm planting and ecosystem-services

The area required for uptake of the entire dairy effluent pond (following Roygard et al. 2001) by native monocotyledons is large (although less than for dicotyledons) and may require removing an impractical amount of land from productive pastures. Additionally, application of DSE to pastures reduces fertiliser costs through the recycling of nutrients. More possible, is the application of any DSE remaining, in excess of pasture growth requirements, to smaller native plantings.

Additional cost benefits may encourage land set aside for planting with native species. For example, planting *L. scoparium* and *K. robusta* in conjunction with honey production may be financially viable, due to the high market value of these species products (Stephens et al. 2010). This warrants further investigation into the effects of elevated N on flowering and nectar properties. Stock are known to browse several native species, including *P. tenax*, *C. australis* and *C. robusta* (Wardle 2002). If harvested, such species may be used as supplementary forage over winter (Lambert et al. 1989a), which may provide trace elements (accumulated in excess of pasture grasses) to nutrient deficient stock (Hahner et al. 2014), thereby reducing other farm costs. For example zinc is deficient in New Zealand soils and is associated with facial eczema in sheep, this may be acquired through *P. tenuifolium* foliage which is high in zinc (Hahner et al. 2014). Other research has suggested that native scrubland (*Kunzea* spp. and *L. scoparium*) may accumulate as much C in biomass as exotic forestry plantations, thereby providing an economic offset for fossil-fuel emissions in emissions trading schemes (Scott et al. 2000, Whitehead et al. 2004, Trotter et al. 2005)

In terms of the native monocotyledons investigated in this research, *P. tenax* has perhaps the most potential to mitigate N fluxes, in light of its larger mature size and the high NO_3^--N concentrations associated with *A. richardii*. The integration of *P. tenax*, which has a wide range of potential uses, into land management systems has received considerable attention (McGruddy 2006). Harvest of entire *P. tenax* plants for commercial use would remove substantial amounts of N from farm soils. The regular harvest of *P. tenax* seed heads and leaves from riparian zones would also make a modest contribution to N removal (McGruddy 2006). The application of modern technologies to this fibre aims to provide viable and environmentally-friendly commercial uses (McGruddy 2006). The harvest of *P. tenax* may be used to supply fibre, gel, seed oil and other extractives to growing industries, which make use of traditional craft and medicine knowledge (McGruddy 2006). If stock were given access to stands of *P. tenax* (Plate 7.1d), this species may form a dietary addition which is lower in N than pasture species and reduce overall N content in urine. However, there is some concern among farmers that the dense bases of *P. tenax* may harbour rats which are vectors for disease (Ngai Tahu Farming Ltd., personal communication).

Plate 7.1 Potential scenarios in which native plants could be incorporated into farmland. Riparian planting bordering a small stream (a). A 2 m fenced paddock border, which includes a drainage ditch (b). Planted area in the corner of a dairy farm where the centre pivot irrigator does not reach (c). A planted block of *P. tenax* to which effluent could be applied, stock have access to browse the foliage (d). *Kunzea* sp. regenerating within farm paddocks, used for shelter by stock (e). A 1 m fenced planted border surrounding paddocks (f). Photos were taken: near Leeston (a) (courtesy of Michael Simmler), at the Lincoln Univeristy Dairy Farm (b,c and f), near the Selwyn Huts (d) and at Mt. Fyffe, Kaikoura (e) (courtesy of Obed Lense).

The potential of each native species to accumulate N in plant material (or rhizosphere associated soil) should be considered in conjunction with the ecological and cultural roles of that species. For riparian planting, it is important to consider the C:N ratio of leaf litter as the decomposition rate will affect nutrient cycling and may alter aquatic food-webs (Quinn et al. 2000). In regions depauperate in native vegetation such as the Canterbury Plains, the choice of fruiting native species

would provide food supply to support returning populations of native birds, while those species with uses in Māori culture may be best situated in locations with public access, to encourage harvest.

7.7 Conclusions

- Annual N uptake rates and N uptake efficiencies were higher for *L. perenne* than the native species. A fenced and mown grassed area on farmland is likely to removed more N through plant uptake than native species.

- High biomass-producing native monocotyledons are able to take up around 10 times more applied N from farm soils than woody dicotyledons. Modelling suggests that compared to land fully grazed with dairy cows, up to an 8 % reduction NO_3^--N leaching losses may be achieved through planting a 2 m border surrounding every hectare of paddock. This may lower total farm leaching losses in line with new regulatory limits.

- Irrigation of DSE onto native monocotyledons provides an alternative option to application to grazed pastures. Minimal NO_3^- leaching is possible if N is applied at around 100 kg ha^{-1} yr^{-1}. *P. tenax* is a promising candidate for growth as a short rotation crop, paired with effluent disposal, due to its high N uptake and the potential value of its fibres and other plant products. *A. richardii* is a poor choice due to high rhizosphere NO_3^- concentrations, increasing the potential for N leaching.

- While *K. robusta* and *L. scoparium* remove relatively small amounts of N from soil water or applied effluents, these species have the potential to reduce N_2O emissions. This may make a significant contribution to reducing on farm greenhouse gas emissions if *K. robusta* or *L. scoparium* were used for shelter by stock.

Chapter 8

Conclusions and future research

The main conclusions of this research are synthesised in the following sections with respect to the four objectives of the study (Introduction, Page 5). Then, a summary of the applications of the research and recommendations for future study is presented, prior to a closing statement.

8.1 Variation in foliar and rhizosphere soil nitrogen among New Zealand species and comparison to *Lolium perenne*

Foliar nitrogen status

- Field study at two planted restoration sites on the Canterbury Plains identified a consistent pattern of foliar N concentrations in native species. Soil total N and nitrate (NO_3^-) were considerably higher at one site which had previously been used as a dairy farm. The foliar N status of *Lolium perenne* (perennial ryegrass) was elevated at the ex-dairy farm site but was similar in native plants at both restoration sites. However, when NO_3^- concentrations were elevated to even higher concentrations through fertilisation in the pot experiment young native plants had higher concentrations of foliar N.
- Deciduous native tree species, *Plagianthus regius* (ribbowood) and N-fixing *Sophora microphylla* (kōwhai) had consistently higher foliar N than evergreen native species and *L. perenne* at the restoration sites. *L. perenne* had similar concentrations of foliar N to the majority of native species at the field sites, but *L. perenne* grown in pots had higher foliar N than young native plants established on the same low-fertility soil.

Rhizosphere nitrogen status

- At the two restoration sites there was substantial variation in the NO_3^- status and chemistry of native plant rhizosphere soil between species. Native species may alter the conditions for soil N cycling and subsequently influence the flux of N through soils, and differentially to *L. perenne*.

- The rhizosphere soil of the native tussock grass *Austroderia richardii* (toetoe) and woody *P. regius* had consistently raised NO_3^- concentrations compared to *L. perenne* at the field sites. Nitrate concentrations in soil extracts and pore water samples under *A. richardii* were consistently higher than for the other native species.

- There was little variation in total N concentrations of rhizosphere soil between species at the field sites. Rhizospheres of some species mobilised more total N into mineral form, as found in *A. richardii* and *P. regius*. Elevated rhizosphere soil NO_3^- status may be due to inputs of large amounts of N-rich foliage to the soil surface, followed by N leaching or decomposition, or enhanced root exudation from these extensively rooted species. However, elevated NO_3^- was not observed under other species, such as *Phormium tenax* (flax) and *Cordyline australis* (cabbage tree), with similarly extensive root systems.

8.2 The growth and uptake response of selected native species and *Lolium perenne* to elevated levels of nitrogen

- Native species were more tolerant than *L. perenne* to N loading at the highest rate (1600 kg N ha^{-1}) in the greenhouse experiment. It was thought that the larger roots of native species provided more resistance to the toxic effects of such high N rates.

- There was little growth response to added N for native species in pot trials. This supports previous findings and background theory that natives are well adapted to low nutrient soil and grow well without fertilisers. Of the native species, only the monocotyledon *C. virgata* was consistently responsive to added N (up to rates of 1600 kg N ha^{-1}). This tussock species naturally grows in wet soils and riparian zones. Other monocotyledons, *C. australis* and *P. tenax* had a marginal response to 200 kg N ha^{-1} in one trial. There was little response above 200 kg N ha^{-1} for *L. perenne* in pot trials and it appears this may be the upper limit to achieve a growth response.

- Despite a lack of growth response, native plants showed a propensity for luxury N uptake when supplied with high concentrations of plant available N. Nitrogen accumulated in foliar tissues to higher concentrations than recorded at the un-fertilised field sites.

- Leafy, high biomass producing native monocotyledons accumulated more N in plant tissues than native woody dicotyledons and *L. perenne.* Annual N uptake by *L. perenne* is likely to be greater than for one year old native plants.

- Nitrogen accumulation in plant tissues was negatively correlated with the amount of total and available N remaining in soil, indicating the potential to reduce NO_3^- leaching from soil planted with native species of N uptake, such as the monocotyledons.

- *C. virgata* was the best native plant in terms of N uptake and subsequent mitigation of NO_3^- leaching, due to its high N uptake, growth response to N and extensive fibrous root system.

8.3 The potential of New Zealand native species *Kunzea robusta* to alter the soil nitrogen cycle and reduce nitrous oxide emissions

- Nitrous oxide (N_2O) emissions were significantly suppressed under *Kunzea robusta* (kānuka) compared to control plots (unvegetated soil) following application of dairy shed effluent (DSE) to the soil surface. The N_2O-N emission factor for DSE applied to *K. robusta* soil was 0.07 % of N compared to 1.05 % in the control soil.

- Nitrate-N concentrations were elevated beneath *K. robusta* compared to control soils, both before and after DSE was applied, providing substrate for denitrification. This suggests that inhibition of the denitrification process is leading to reduced N_2O emissions. This may be due to the suppression of the denitrifying bacterial community by antimicrobial plant compounds of *K. robusta* or altered soil conditions beneath *K. robusta* (low soil moisture and pH) limiting the denitrification process.

- This research has implications for reduced greenhouse gas emissions during land treatment of DSE or other high-N wastes. However, suppression of N_2O emissions may lead to pollution swapping, due to leaching of increased concentrations of NO_3^- that remain in soil.

8.4 Farm-scale nitrogen uptake and practical application to native plantings (nitrate leaching and nitrous oxide emissions)

- At a farm scale, the N uptake efficiency was higher for *L. perenne* than the native species, due to high biomass production and more accumulation of N in foliage. A fenced and mown grassed farm area is likely to remove more N through plant uptake than native species. The benefits of native plants is only apparent in terms of their effects on soils.

- Modelling suggests that native monocotyledons may take up large amounts of applied N from farmland and could remove N with 100 % efficiency at application rates of around 100 kg N ha^{-1}. Planting native monocotyledons in a 2 m border surrounding every hectare of paddock may reduce NO_3^--N leaching losses up to 8 % compared to land fully grazed with dairy cows. This may be significant in terms of lowering total farm leaching losses in line with regulatory limits.

- Irrigation of DSE to native plantings is proposed as an alternative to pastures. The area of farmland required to sustainably (without NO_3^- leaching) apply an annual load of DSE to native monocotyledons is large. However, is potentially viable if economic offsets are gained from the set-aside land. *P. tenax* is suggested as a suitable species for growth as a short-rotation

crop paired with effluent disposal, due to the likely reduction in NO_3^- leaching and potential for commercial use of its fibrous leaves and seed oils.

- The slow-growing woody species *K. robusta* and *Leptospermum scoparium*, had less potential to remove N from soil. Nitrate leaching is likely to occur below these species, even at low N application rates. However, there is potential for reduced N_2O emissions from soils under these species. If used for shelter by stock, *K. robusta* and *Leptospermum scoparium* may suppress urine-N N_2O emissions and would be likely to make a significant contribution to reducing farm-scale greenhouse gas emissions.

8.5 Applications of this research

Local authorities in New Zealand are currently setting restrictions on NO_3^- losses from farmland to improve freshwater quality. Additionally, the potential future implementation of an emissions trading scheme will put pressure on farmers to reduce greenhouse gas losses, such as N_2O. The findings of the present research have relevance to assessing the potential of native species to mitigate environmentally damaging N fluxes from agricultural land. Information regarding the performance of native plants in high N environments will facilitate the strategic incorporation of these species into farming systems. A range of native species are shown to be tolerant to elevated soil N and are suitable for planting on N-loaded soils. Carefully considered species-selection in native plantings may beneficially alter environmental outcomes.

It is clear that NO_3^- leaching and N_2O losses are likely to be lower from land planted with native species compared with grazed fertilised pastures. Nitrogen accumulation in the leaves of native monocotyledons, in particular *P. tenax* and *C virgata*, is likely to provide an additional offset against NO_3^- leaching. These species are recommended for planting; on sloping riparian banks; the down-slope margins of hill country paddocks; hollows or depressions; and in locations likely to receive effluent or fertiliser overspray from adjoining paddocks. Conversely, caution should be taken when considering *A. richardii* and *P. regius* for native plantings aiming to mitigate N flux. The high background NO_3^--N concentrations in the rhizosphere soil of these species may result in higher leaching rates than under other natives. *A. richardii* and *P. regius* are currently widely used in riparian and restoration projects. Further study is needed to determine field NO_3^- leaching rates below these species and ascertain if their planting should be recommended.

Although *L. perenne* is likely to extract more N from farm soils into its foliage than native species, substantial NO_3^- leaching and N_2O emissions typically occurs following fertiliser of effluent applications to grazed pastures, due in part to N returns in animal excreta. The extensive root systems

of the native monocotyledons have potential to capture N leaching below the shallow rooting depth of *L. perenne*. Disposal of excess DSE to planted blocks of these species is a potentially sustainable and economically viable alternative to pasture application. Nitrogen uptake by native monocotyledons can be considered as a sink for excess N produced by the dairy system, similar to carbon sinks. If the plant products are suitable for growth and harvest in a short rotation production system, N uptake would also represent a net removal from the farm. In particular, *P. tenax* shows promise due to its rapid biomass production, extensive root system, high N uptake efficiency and multitude of potential uses for its plant products.

It is important to consider both N_2O emissions and NO_3^- leaching losses simultaneously in planting decisions. Alterations to the N cycle associated with *K. robusta* affirm the possibility of species-specific plant effects on soil. Combined with the findings of previous reserach regarding bacterial inhibition, *K. robusta* and related species *L. scoparium* may be used to mitigate both N_2O emissions and pathogenic contamination following land application of effluents or human biosolids.

8.6 Recommendations for further research

Further research, involving study sites across a wider range of soil fertility and types would help to elucidate the causal mechanisms that determine rhizosphere N mobility associated with New Zealand native species. In addition to direct N depletion through plant uptake, complex factors such as transpiration, leaf litter decomposition and soil leaching rates control rhizosphere N cycling. Further measurement of these contributing factors is required. Soil hydrology strongly influences NO_3^- leaching, and therefore future studies should pay attention to preferential water flow through root channels left by decaying roots and earthworm burrows. The roles of canopy rainfall interception and plant transpiration in reduced water flux under native species also deserve more attention. The vertical horizontal, surface and subsurface flow pathways of water and leachates from dairy systems clearly would play an important role in the effectiveness of native plant barriers.

The pot trials allowed the flux of N to be controlled and N uptake carefully measured but were limited in application, as they are unlikely to accurately reflect field conditions. Further trials in large lysimeters or in the field would provide more realistic N mass balance budgets. ^{15}N tracer studies would allow the path of added N through the soil-plant system to be more precisely determined. Additionally this would permit accurate measurement of fractions of applied N that are leached, emitted as gases, incorporated into plant materials or retained in soils. Studies in riparian zones are also vital, due the complexity of hydrological processes and specific soil conditions. Increased understanding of the

response of native plants to elevated soil phosphorus is also necessary to determine their suitability for agricultural plantings and potential to mitigate P transport in runoff.

Further research into the likely mechanisms behind reduced N_2O emissions under *K. robusta* is warranted due to large environmental impact of this gas and present economic interest in *L. scoparium* and *Kunzea* spp. Moreover, this part of the thesis research requires further repetition and extension. Comparison of *K. robusta* to other native and exotic tree species will aid in determining if the suppression effect is species specific. A direct comparison to emissions from *L. perenne*, to which DSE is typically applied, is also important. Other native species known to contain bioactive plant compounds, such as *P. tenax* and *L. scoparium*, could also be investigated in this regard.

8.7 Concluding remarks

Native species are tolerant of agriculturally elevated levels of N. Although farm-scale N uptake into above-ground plant tissues is low for native species compared to *L. perenne*, N cycling in the soil beneath native species is likely to differ from grazed pasture. Planting farmland with native species is likely to reduce NO_3^- leaching and N_2O losses. This research has identified large inter-species differences in terms of the interaction of native species with soil N. Native monocotyledons are able to accumulate large amounts of agricultural N and are the best of the native species at reducing NO_3^- leaching losses, while *K. robusta* is able to suppress soil N_2O emissions. Carefully considered species-selection in native plantings on farms may alter environmental outcomes. Furthermore, the incorporation of New Zealand native plants into agricultural landscapes increases native biodiversity and provides additional ecosystem services to farmers and wider society.

Appendix A

Soil profile descriptions

This appendix includes supplementary data for Chapter 3. A full pedological description is given (Table A.1) for profiles shown in Plate A.1. Profiles are the rhizosphere and adjoining walls of soil pits excavated adjacent to six New Zealand native species at the Lincoln University Dairy Farm site.

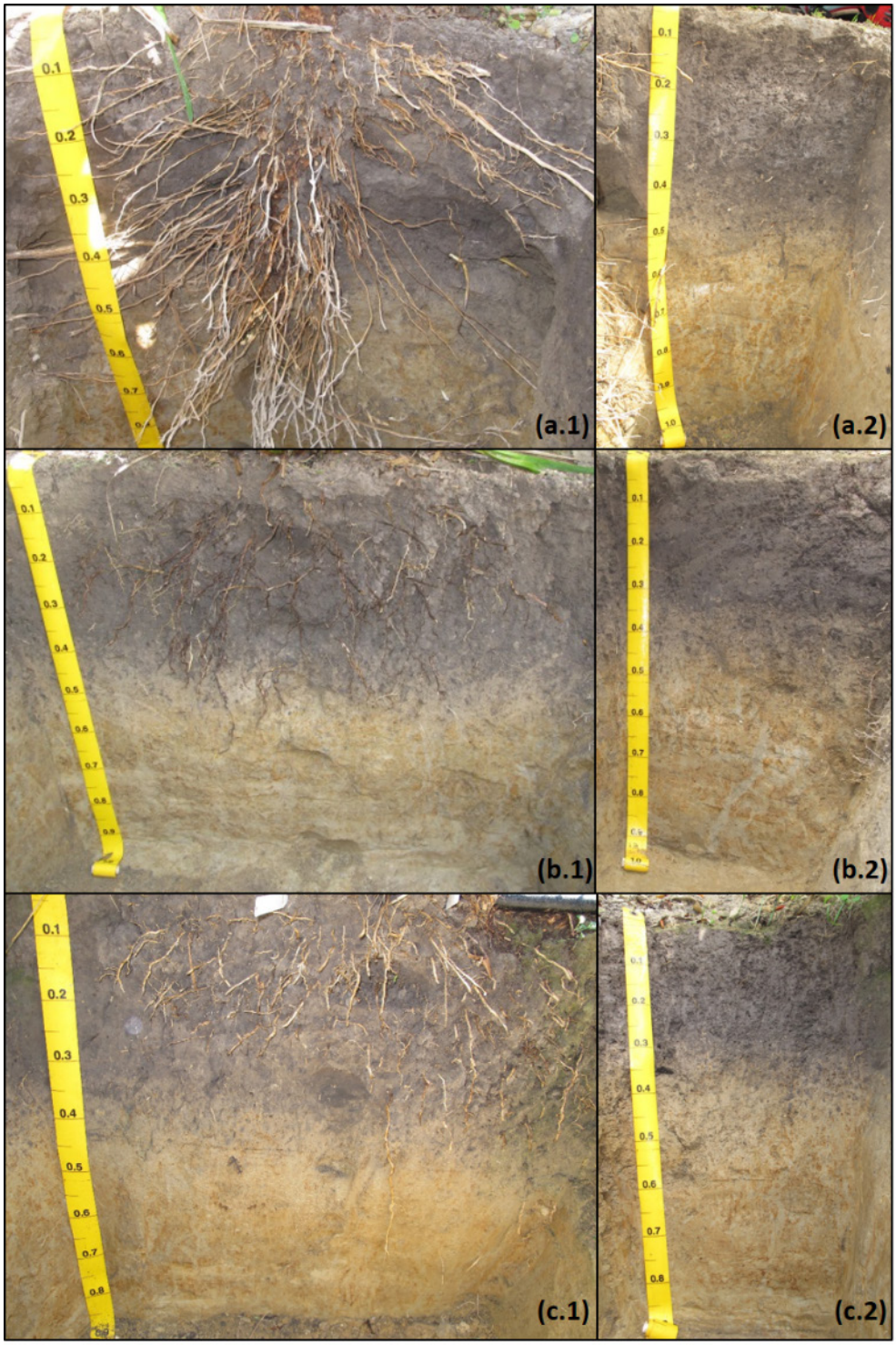


Plate A.1 Rhizosphere soil profiles (full caption over page)

Plate A.1 **The rhizosphere (principal wall) (1) and adjoining wall (2) of soil pits adjacent to six native species, at the Lincoln University Dairy Farm site. Species are *Cordyline australis* (a), *Phormium tenax* (b), *Austroderia richardii* (c), *Coprosma robusta* (d), *Kunzea robusta* (e) and *Pittosporum tenuifolium* (f). The site is Templeton silt loam soil (Hewitt 1998) (Typic halustept soil, Soil Survey Staff 2014), 43°38'25.95" S, 172°26'34.37" E (Figure 4.1). See Table A.1 for a detailed pedological description.**

Table A.1 Soil profile description of the rhizosphere (principal wall) and adjoining wall of soil pits adjacent to six native species, at the Lincoln University Dairy Farm site. The site is Templeton silt loam soil (Hewitt 1998) (Typic halustept soil, Soil Survey Staff 2014), 43◦38'25.95" S, 172◦26'34.37" E (Figure 4.1). Grey cells highlight points of difference between the rhizosphere and adjoining walls profiles.

Species profile		Soil horizon	Depth (cm)	Boundary	Colour	Texture	Consistence	Structure	Roots	Concretions	Mottles	Worm Castings
Cordyline australis	Rhizosphere (a.1)	Ah	0 - 38-43	wavy distinct	10YR 3/2	silt loam	slightly firm, friable, slightly sticky, non-plastic,	strongly, medium, crumb	very coarse 5%, fine 10%			v. fine, v. few
		Bw	38-45 - 45-50	wavy distinct	2.5Y 4/3	silt loam	weak, friable/brittle, slightly sticky, non-plastic	moderate, medium, blocky/platy	very coarse 5%, fine 10%	7.5YR 3/4, prominent, fine, v. few		v. fine, v. few
		Bw(g)	45-50 - 89	smooth distinct	2.5Y 5/3	clay loam	slightly firm, brittle, moderately sticky, plastic, consistence,	strong, coarse, angular-blocky	very coarse 5%, fine 10%		7.5 YR 5/6, prominent, fine-medium, abundant	
		Bg	89		2.5Y 5/3	silt loam	weak, friable, non-sticky, non-plastic	weak/moderate, medium, blocky	fine 10%		7.5 YR 4/6, distinct/prominent, medium, abundant	
	Adjoining wall (a.2)	Ah	0 – 43	smooth distinct	10YR 3/2	silt loam	slightly firm, friable, slightly sticky, non-plastic,	strongly, medium, crumb	v. fine 2%, extremely fine 1%, microfine 5%			v. fine, v. few
		Bw	43 - 45-49	smooth distinct	2.5Y 4/3	silt loam	weak, friable/brittle, slightly sticky, non-plastic	moderate, medium, blocky/platy	v. fine 2%, microfine 2%	7.5YR 3/4, prominent, fine, v. few		v. fine, v. few
		Bw(g)	45-49 - 89	smooth distinct	2.5Y 5/3	clay loam	slightly firm, brittle, moderately sticky, plastic, consistence,	strong, coarse, angular-blocky	v. fine 1%, micro-fine 1%		7.5 YR 5/6, prominent, fine-medium, abundant	v. fine, v. few
		Bg	89		2.5Y 5/3	silt loam	weak, friable, non-sticky, non-plastic	weak/moderate, medium, blocky	v. fine <1%		7.5 YR 4/6, distinct/prominent, medium, abundant	
	(b.1)	Ah	0 - 38-40	wavy distinct	10YR 3/2	silt loam	weak, friable, slightly sticky, plastic	strong, medium, crumb	v. fine 10%, microfine 5%			
		Bw	38-40 - 45-55	wavy distinct	2.5Y 5/3	silt loam	weak, friable, slightly sticky, plastic	moderate, coarse blocky	v. fine 2%, microfine 2%	5YR 3/4, prominent, fine, v. few		v. fine, few

Species profile		Soil horizon	Depth (cm)	Boundary	Colour	Texture	Consistence	Structure	Roots	Concretions	Mottles	Worm Castings
Phormium tenax	Rhizosphere	Bw(g)	45-55 - 80	smooth wavy	2.5Y 5/3	clay loam	slightly firm, friable , moderately sticky, plastic	strongly, coarse, blocky	v. fine 2%, microfine 2%	7.5 YR 4/6, prominent, v. fine, few	7.5 YR 4/6, prominent, v. fine, v. few	
		Bg	80		2.5Y 5/4	silt loam	weak, friable, non-sticky, non-plastic	weak, coarse, blocky	v. fine 2%, microfine 2%		7.5 YR 4/6, prominent, v. fine, v. few	
	Adjoining wall (b.2)	Ah	0 - 35	smooth distinct	10YR 3/2	silt loam	weak, friable, slightly sticky, plastic	strong, medium, crumb	v. fine 1%, micro-fine 1%			
		Bw	35 - 45-55	wavy distinct	2.5Y 5/3	silt loam	weak, friable, slightly sticky, plastic	moderate, coarse blocky/platy	v. fine 1%, micro-fine 1%	5YR 3/4, prominent, fine, v. few		v. fine, few
		Bw(g)	45-55 - 80	Smooth wavy	2.5Y 5/3	clay loam	slightly firm, friable , moderately sticky, plastic	strongly, coarse, blocky	micro-fine 1%	7.5 YR 4/6, prominent, v. fine, few	7.5 YR 4/6, prominent, v. fine, v. few	
		Bg	80		2.5Y 5/4	silt loam	weak, friable, non-sticky, non-plastic	weak, coarse, blocky			7.5 YR 4/6, prominent, v. fine, v. few	
Austroderia richardii	Rhizosphere (c.1)	Ah	0 - 32	smooth distinct	10YR 3/2	silt loam	slightly firm, friable, slightly sticky, non-plastic	moderate, medium, crumb	v. fine 15%, extremely fine 10%			
		Bw(f)	32 - 45	irregular abrupt	2.5Y 5/4	silt loam	weak, friable, moderately sticky, plastic	moderate, medium, angular-blocky	v. fine 5%	7.5YR 3/4, prominent, v. fine, few	5YR 3/4, prominent, fine, few/common	v. fine, few
		B(g)	45 - 85	smooth distinct	2.5Y 5/3	silt loam	brittle, fracture, moderately sticky, plastic	moderate, medium, angular-blocky	v. fine 2%	5YR 4/6, prominent, v. fine, few	5YR 4/6, prominent, fine, common	
		Cg	85		5Y 5/2	silt loam	weak, friable, non-sticky, non-plastic	weak, coarse, sub-angular blocky			7.5YR 5/8, prominent, fine, abundant	

Species profile		Soil horizon	Depth (cm)	Boundary	Colour	Texture	Consistence	Structure	Roots	Concretions	Mottles	Worm Castings
Austroderia richardii	Adjoining wall (c2)	Ah	0 - 32	smooth distinct	10YR 3/2	silt loam	slightly firm, friable, slightly sticky, non-plastic	moderate, medium, crumb	extremely fine2 %,microfine 1%			
		Bw(f)	32 - 45-60	irregular abrupt	2.5Y 5/4	silt loam	weak, friable, moderately sticky, plastic	moderate, medium, angular-blocky	v. fine 1%, extremely fine2%	7.5YR 3/4, prominent, v. fine, few	5YR 3/4, prominent, fine, few/common	v. fine, few
		B(g)	45-60 - 85	smooth distinct	2.5Y 5/3	silt loam	brittle, fracture, moderately sticky, plastic	moderate, medium, angular-blocky	extremely fine 1%, microfine 1%	5YR 4/6, prominent, v. fine, few	5YR 4/6, prominent, fine, common	
		Cg	85		5Y 5/2	silt loam	weak, friable, non-sticky, non-plastic	weak, coarse, sub-angular blocky			7.5YR 5/8, prominent, fine, abundant	
Coprosma. robusta	Rhizosphere (c.1)	Ah	0 - 35	smooth wavy	10YR 3/2	silt loam	weak, friable, slightly sticky, non-plastic	strong, medium nut	v. coarse 2%, medium 10%, v. fine 10%, extremely fine 5%			very fine, few
		Bw(f)	35 - 45	smooth distinct	2.5Y 5/3	silt loam	gentle, moderately sticky, plastic, friable	moderate, medium, angular blocky	v. coarse 2%, medium 10%, v. fine 10%, extremely fine 5%	5YR 3/4, prominent, fine, v. few		very fine, few
		B(g)	45 - 75	smooth distinct	2.5Y 5/3	silt loam	gentle, non-sticky, non-plastic, friable	weak, medium, sub-angular blocky	extremely fine 2%	5YR 3/4, prominent, coarse, few	5YR 4/6, prominent, fine, few	
		Cg	85		2.5Y 5/3	silt loam	weak, non- sticky, non-plastic, friable	weak/moderate, coarse, angular blocky	extremely fine 2%		5YR 4/6, prominent, fine, few	
	wall (c.2)	Ah	0 - 35	smooth abrupt	10YR 3/2	silt loam	weak, friable, slightly sticky, non-plastic	strong, medium nut	v. fine 1%, extremely fine 2%, microfine 1%			very fine, few
		Bw(f)	35 - 45	smooth distinct	2.5Y 5/3	silt loam	gentle, moderately sticky, plastic, friable	moderate, medium, angular blocky	extremely fine 1%, microfine 1%	5YR 3/4, prominent, fine, v. few		very fine, few

Species profile		Soil horizon	Depth (cm)	Boundary	Colour	Texture	Consistence	Structure	Roots	Concretions	Mottles	Worm Castings
	Adjoining	B(g)	45 – 75	smooth distinct	2.5Y 5/3	silt loam	gentle, non-sticky, non-plastic, friable	weak, medium, sub-angular blocky	v. fine 1%	5YR 3/4, prominent, coarse, few	5YR 4/6, prominent, fine, few	
		Cg	85		2.5Y 5/3	silt loam	weak, non- sticky, non-plastic, friable	weak/moderate, coarse, angular blocky	extremely fine1 %		5YR 4/6, prominent, fine, few	
Kunzea robusta	Rhizosphere (e.1)	Ah	0 - 40-43	wavy distinct	10YR 3/2	silt loam	weak, friable, slightly sticky, non-plastic	strong, coarse, crumb	coarse roots 2 % medium 10%, fine 10 %, extremely fine 5%		7.5 YR 4/6, prominent, fine, abundant	v. fine, few
		Bw(g)	40-43 - 95	smooth distinct	2.5Y 5/3	clay loam	weak, friable, moderately sticky, plastic	strongly, coarse blocky/platy	medium 10% fine 5%, micro-fine 2%	7.5YR 3/4, prominent, very fine, few	7.5 YR 4/6, prominent, fine, abundant	v. fine, few
		Bg	95		2.5Y 5/4	silt loam	weak, friable, non-sticky, non-plastic	moderate, medium, sub-angular blocky	extremely fine 2 %		10YR 4/6, distinct, fine, common	
	Adjoining wall (e.2)	Ah	0 – 40-43	wavy distinct	10YR 3/2	silt loam	weak, friable, slightly sticky, non-plastic	strong, coarse, crumb	v. fine 2%, micro-fine 2%			v. fine, few
		Bw(g)	40-43 - 95	smooth distinct	2.5Y 5/3	clay loam	weak, friable, moderately sticky, plastic	strongly, coarse blocky/platy	v. fine 2%, micro-fine 1%	7.5YR 3/4, prominent, very fine, few	7.5 YR 4/6, prominent, fine, abundant	v. fine, few
		Bg	95		2.5Y 5/4	silt loam	weak, friable, non-sticky, non-plastic	moderate, medium, sub-angular blocky			10YR 4/6, distinct, fine, common	

Species profile		Soil horizon	Depth (cm)	Boundary	Colour	Texture	Consistence	Structure	Roots	Concretions	Mottles	Worm Castings
Pittosporum tenuifolium	Rhizosphere (f.2)	Ah	0 - 30	smooth wavy	10YR 3/2	silt loam	weak, friable, slightly sticky, non-plastic	strong, coarse, crumb	v. coarse 5%, coarse 5%, medium 10%, fine 5%,			
		Bw(f)	30 - 45-50	convolute abrupt	2.5Y 4/2	silt loam	weak, friable, moderately sticky, plastic	moderate, medium, angular blocky	v. coarse 5%, coarse 5%, medium 10%, fine 5%	5YR 3/4, prominent, v. fine, few/common		
		Bg	45-50 - 80	smooth distinct	2.5Y 6/3	silt loam	weak, brittle , non-sticky, non-plastic	moderate, medium, sub-angular blocky	v. fine 2%	7.5YR 3/4, prominent, v. fine, common.	7.5YR 4/6, prominent, v. fine, common	
		Cr	80		2.5Y 6/3	silt loam	weak, friable, non-sticky, non-plastic	moderate/weak , medium sub-angular blocky	microfine 2%		7.5YR 5/6, prominent, fine, common	
	Adjoining wall (f.2)	Ah	0 - 30	abrupt smooth	10YR 3/2	silt loam	weak, friable, moderately sticky, plastic	strong, coarse, crumb	v. fine 1%, extremely fine 2%, micro-fine 2%			
		Bw(f)	30 - 45-50	convolute abrupt	2.5Y 4/2	silt loam	weak, brittle , non-sticky, non-plastic	moderate, medium, angular blocky	v. fine 2%, micro-fine 1%	5YR 3/4, prominent, v. fine, few/common		
		Bg	45-50 - 80	smooth distinct	2.5Y 6/3	silt loam	weak, friable, non-sticky, non-plastic	moderate, medium, sub-angular blocky	v. fine 2%, micro-fine 1%	7.5YR 3/4, prominent, v. fine, common.	7.5YR 4/6, prominent, v. fine, common	
		Cr	80		2.5Y 6/3	silt loam	weak, friable, moderately sticky, plastic	moderate/weak , medium sub-angular blocky	micro-fine1 % %		7.5YR 5/6, prominent, fine, common	

Appendix B

Supplementary information to Chapter 5

Supplementary data for Chapter 5: Response of New Zealand native plants to agriculturally elevated levels of soil nitrogen.

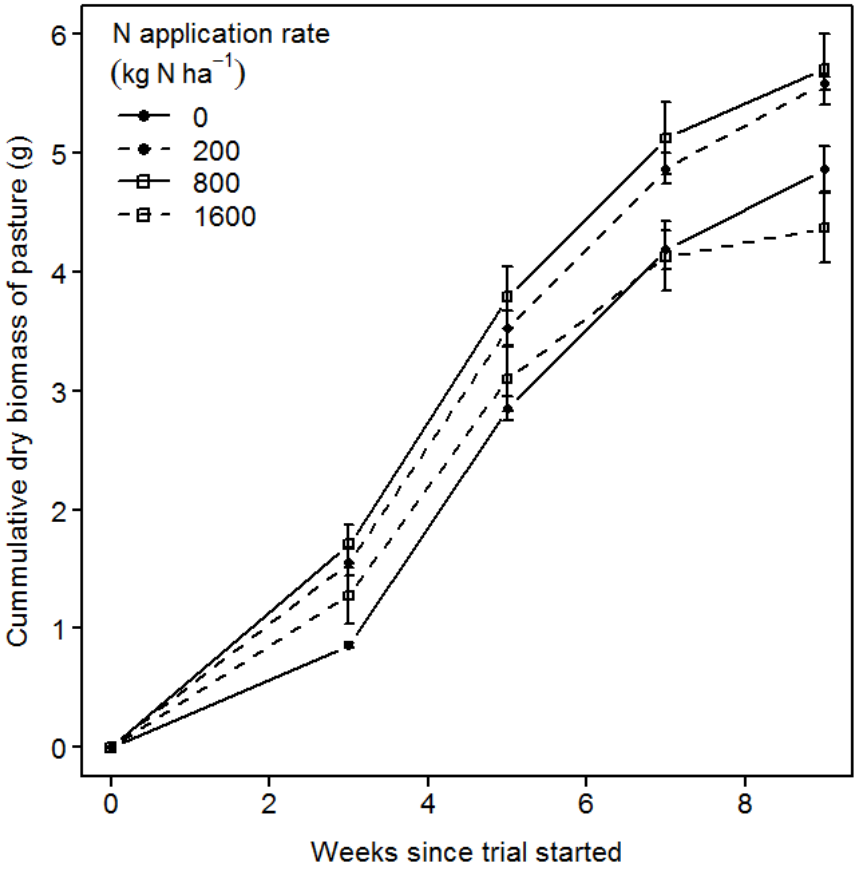


Figure B.1 Mean (± SE) cumulative dry biomass produced by *L. perenne* during Trial One in response to increasing N application rate (ranging 0-1600 kg N ha^{-1} equivalent, n=6).

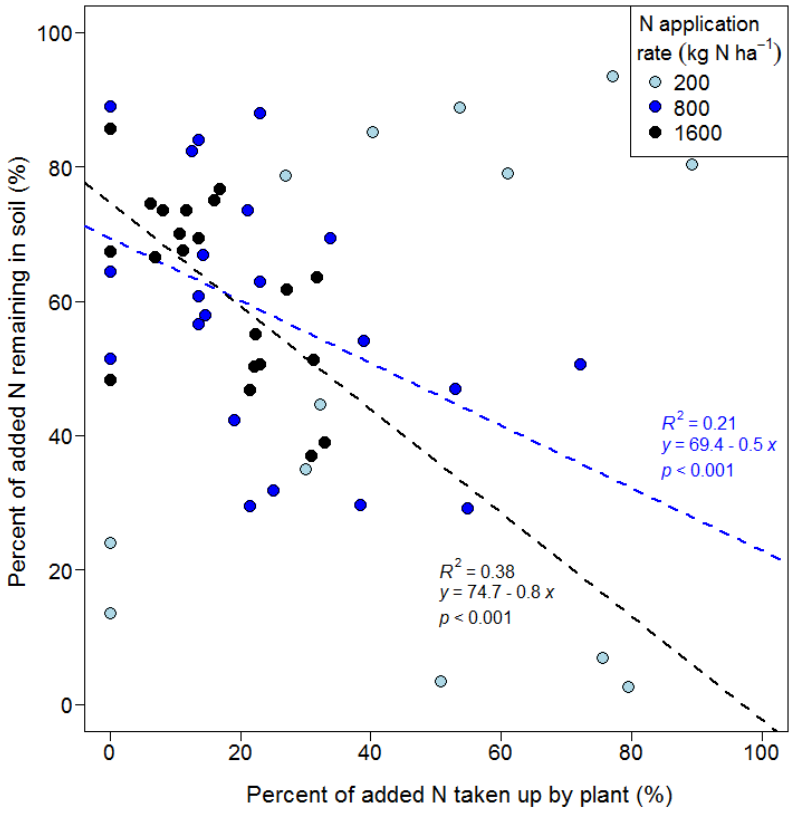


Figure B.2 The relationship between the percent of added N accumulated by plants and percent remaining in the soil in Trial One. Data are for N application to five native species and *L. perenne* at three application rates (200, 800 and 1600 kg N ha^{-1} equivalent). Regression lines, equations, R^2 and p values are shown for each N application rate. No regression line is shown for the 200 kg N ha^{-1}, as the relationship was not significant.

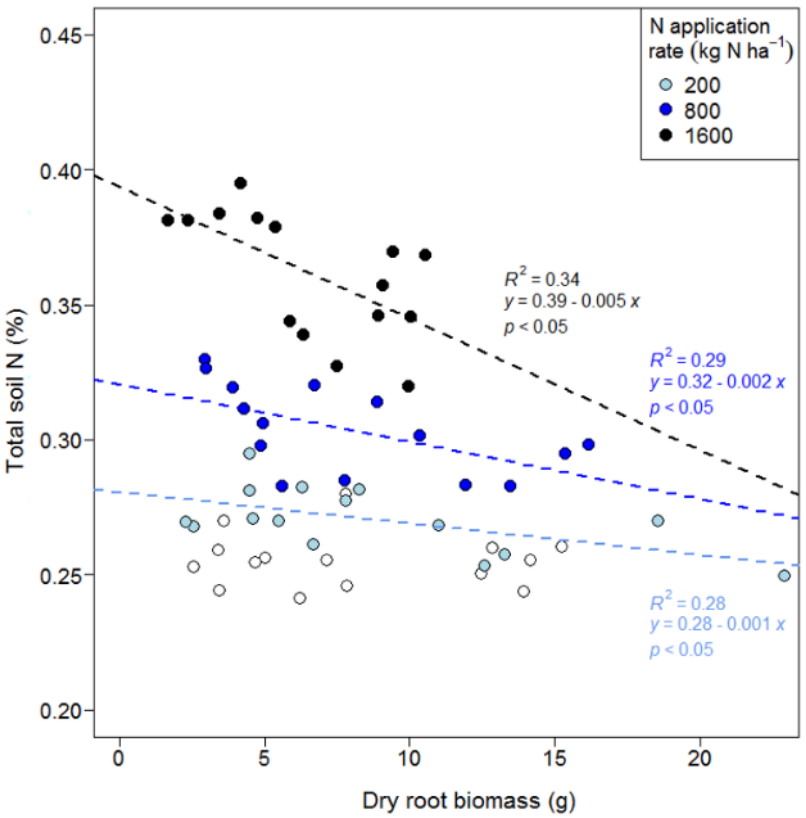


Figure B.3 The relationship between root dry biomass (g) and total N concentration of rhizosphere soil (%) in Trial One. Data are for N application to five native species (four application rates, 0-1600 kg N ha^{-1} equivalent). Regression lines, equations, R^2 and p values are shown for each N application rate. No regression line is shown for 0 kg N ha^{-1} as there was no significant relationship.

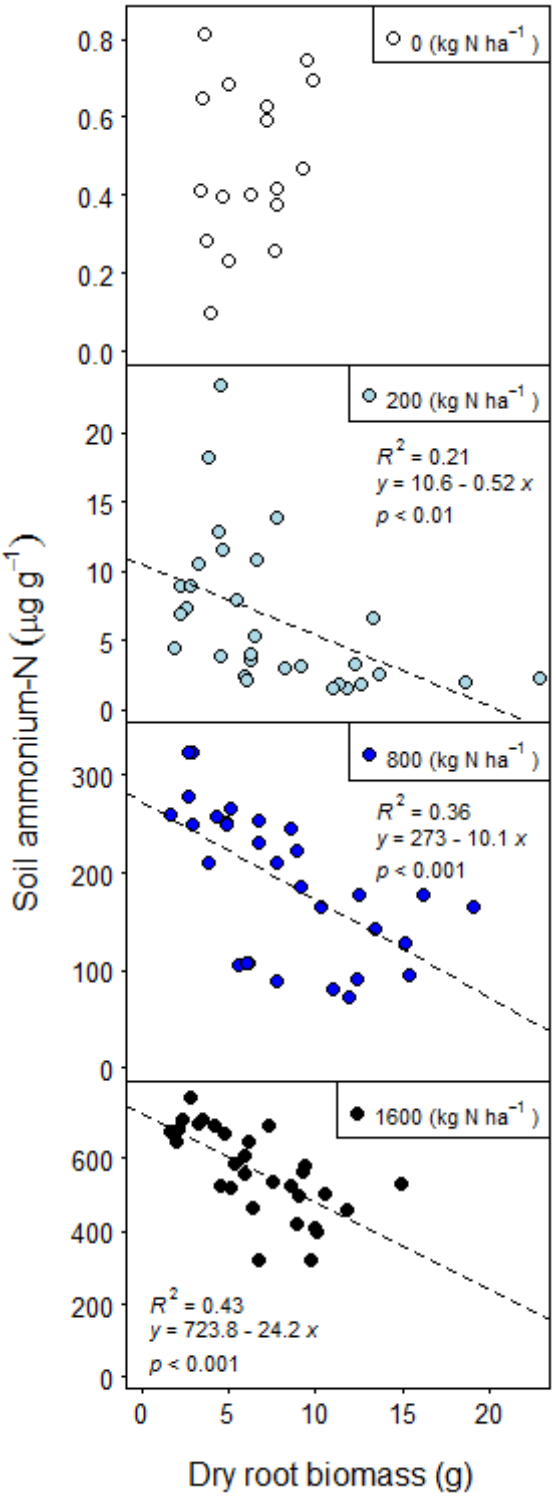


Figure B.4 The relationship between root dry biomass (g) and soil NH_4^+-N concentration in Trial One. Data are for N application to five native species (four application rates, 0-1600 kg N ha^{-1} equivalent). Regression lines, equations, R^2 and *p* values are shown for each N application rate. No regression line is shown for 0 kg N ha^{-1} as there was no significant relationship.

Table B.1 Biomass, foliar N concentrations and N uptake in Trial One. Mean above- (leaves + stems) and below- (roots) ground biomass for native plant species and *L. perenne*, grown under greenhouse conditions in low fertility soil, with increasing rates of N (0-1600 kg N ha^{-1} equivalent) (n=6 for all except soil total N, n=3). *, ** and * indicate the treatment effect being significant at $p<0.001$, $p<0.1$ and $p<0.05$ respectively. Means that share a letter are not significantly different following post-hoc tests. Roots were not analysed for *L. perenne*.**

	Above-ground biomass (g)	Below-ground biomass (g)	Root to shoot ratio	Concentration of foliar N (%)	N uptake (g) (above-ground)	Concentration of root N (%)	N uptake (mg) (below-ground)
Species							
L. scoparium	12.8^{d}	4.87^{c}	0.39^{c}	2.67^{b}	0.27^{c}	1.41^{c}	64.5^{cd}
K. robusta	15.0^{c}	2.96^{d}	0.20^{d}	2.44^{c}	0.29^{c}	1.13^{d}	35.6^{d}
C. virgata	19.2^{ab}	8.20^{b}	0.45^{c}	2.31^{c}	0.46^{a}	1.77^{b}	158^{b}
P. tenax	17.7^{b}	8.97^{b}	0.52^{b}	2.08^{d}	0.38^{b}	1.24^{cd}	109^{bc}
A. richardii	21.1^{a}	13.5^{a}	0.65^{a}	1.93^{d}	0.41^{b}	2.14^{a}	259^{a}
L. perenne	5.13^{e}	NA	NA	5.55^{a}	0.28^{c}	NA	NA
LSD (5%)	1.89	1.30	0.07	0.19	0.04	0.28	49.5
N rate (kg ha^{-1})							
0	13.6^{b}	8.16^{a}	0.53^{a}	1.25^{d}	0.13^{d}	0.67^{c}	54.8^{c}
200	15.4^{a}	7.75^{ab}	0.42^{b}	2.43^{c}	0.29^{c}	1.29^{b}	109^{b}
800	15.3^{a}	8.21^{a}	0.46^{b}	3.33^{b}	0.42^{b}	2.08^{a}	186^{a}
1600	16.3^{a}	6.64^{b}	0.35^{c}	4.32^{a}	0.57^{a}	2.12^{a}	151^{ab}
LSD (5%)	1.55	1.16	0.06	0.15	0.04	0.25	44.2
Significance of effects							
Species	***	***	***	***	***	***	***
N rate	**	*	***	***	***	***	***
Species x N rate	*	*	***	***	***	***	**
Block							

References

Adams, J. A. 1976. Nutrient requirements of four *Nothofagus* species in north Westland, New Zealand, as shown by foliar analysis. New Zealand Journal of Botany **14**:211-223.

Aerts, R. 1995. The advantages of being evergreen. Trends in Ecology and Evolution **10**:402-407.

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**:1-67.

Agricom. 2011. Agrinote: Ceres *ONE*^{50} Perennial Ryegrass. Retrieved 10 October 2014. http://www.agricom.co.nz/assets/files/nz/agrinotes/One50%20Agrinote%20.pdf.

Aldridge, R., and R. J. Jackson. 1968. Interception of rainfall by manuka (*Leptospermum scoparium*) at Taita, New Zealand. New Zealand Journal of Science **11**:301-317.

Allen, K. L., P. C. Molan, and G. M. Reid. 1991. A survey of the antibacterial activity of some New Zealand honeys. Journal of Pharmacy and Pharmacology **43**:817-822.

Allen, R. B., T. R. Partridge, W. G. Lee, and M. Efford. 1992. Ecology of *Kunzea ericoides* (A. Rich.) J. Thompson (kanuka) in east Otago, New Zealand. New Zealand Journal of Botany **30**:135-149.

Amberger, A. 1989. Research on dicyandiamide as a nitrification inhibitor and future outlook. Communications in Soil Science and Plant Analysis **20**:1933-1955.

Atkin, O. K. 1996. Reassessing the nitrogen relations of Arctic plants: A mini-review. Plant, Cell and Environment **19**:695-704.

Atkinson, E. H. 1922. *Phormium tenax*: The New Zealand fibre industry. New Zealand Department of Agriculture Bulletin **95**:1-53.

Atkinson, I. A. E., and E. K. Cameron. 1993. Human influence on the terrestrial biota and biotic communities of New Zealand. Trends in Ecology and Evolution **8**:447-451.

Avery, A. A. 1999. Infantile methemoglobinemia: Reexamining the role of drinking water nitrates. Environmental Health Perspectives **107**:583-586.

Bakken, L., and P. Dorsch. 2007. Nitrous oxide emission and global changes: Modelling approaches. Pages 381-395 *in* H. Bothe, S. J. Ferguson, and W. E. Newton, editors. Biology of the Nitrogen Cycle. Elsevier, Amsterdam, Netherlands.

Band, L. E., C. L. Tague, P. Groffman, and K. Belt. 2001. Forest ecosystem processes at the watershed scale: Hydrological and ecological controls of nitrogen export. Hydrological Processes **15**:2013-2028.

Bardgett, R. D., J. L. Mawdsley, S. Edwards, P. J. Hobbs, J. S. Rodwell, and W. J. Davies. 1999. Plant species and nitrogen effects on soil biological properties of temperate upland grasslands. Functional Ecology **13**:650-660.

Bardgett, R. D., and D. A. Wardle. 2010. Aboveground-belowground linkages: Biotic interactions, ecosystem processes, and global change. Oxford University Press, Oxford, UK.

Barton, L., C. D. McLay, L. A. Schipper, and C. T. Smith. 1999. Annual denitrification rates in agricultural and forest soils: A review. Soil Research **37**:1073-1094.

Barton, L., and L. A. Schipper. 2001. Regulation of nitrous oxide emissions from soils irrigated with dairy farm effluent. Journal of Environmental Quality **30**:1881-1887.

Basher, L. R. 2013. Erosion processes and their control in New Zealand. Pages 363-374 *in* J. R. Dymond, editor. Ecosystem services in New Zealand: conditions and trends. Manaaki Whenua Press, Lincoln, New Zealand.

Baskaran, R., R. Cullen, and S. Colombo. 2009. Estimating values of environmental impacts of dairy farming in New Zealand. New Zealand Journal of Agricultural Research **52**:377-389.

Beets, P. N., M. O. Kimberley, G. R. Oliver, S. H. Pearce, J. D. Graham, and A. Brandon. 2012. Allometric equations for estimating carbon stocks in natural forest in New Zealand. Forests **3**:818-839.

Bellingham, P. J., C. W. Morse, R. P. Buxton, K. I. Bonner, N. W. H. Mason, and D. A. Wardle. 2013. Litterfall, nutrient concentrations and decomposability of litter in a New Zealand temperate montane rain forest. New Zealand Journal of Ecology **37**:162-171.

Bengtson, P., J. Barker, and S. J. Grayston. 2012. Evidence of a strong coupling between root exudation, C and N availability, and stimulated SOM decomposition caused by rhizosphere priming effects. Ecology and Evolution **2**:1843-1852.

Bennett, A. F. 1998. Linkages in the landscape: The role of corridors and connectivity in wildlife conservation. IUCN, Gland, Switzerland.

Berendse, F. 1998. Effects of dominant plant species on soils during succession in nutrient-poor ecosystems. Biogeochemistry **42**:73-88.

Berendse, F., M. Schmitz, and W. de Visser. 1994. Experimental manipulation of succession in heathland ecosystems. Oecologia **100**:38-44.

Bergin, D. O., M. O. Kimberley, and M. Marden. 1995. Protective value of regenerating tea tree stands on erosion-prone hill country, East Coast, North Island, New Zealand. New Zealand Journal of Forestry Science **25**:3-19.

Bhandral, R., N. S. Bolan, and S. Saggar. 2010. Nitrous oxide emission from farm dairy effluent application in grazed grassland. Revista de la ciencia del suelo y nutrición vegetal **10**:22-34.

Bhandral, R., N. S. Bolan, S. Saggar, and M. J. Hedley. 2007. Nitrogen transformation and nitrous oxide emissions from various types of farm effluents. Nutrient Cycling in Agroecosystems **79**:193-208.

Bidwell, V. J., L. Lilburne, M. J. Thorley, and D. Scott. 2009. Nitrate discharge to groundwater from agricultural land use: An initial assessment for the Canterbury Plains. Lincoln Ventures Ltd., Lincoln, New Zealand.

Blakemore, L. C., P. L. Searle, and B. K. Daly. 1987. Methods for Chemical Analysis of Soils. New Zealand Soil Bureau, Lower Hutt, New Zealand.

Bohlen, P. J., R. W. Parmelee, D. A. McCartney, and C. A. Edwards. 1997. Earthworm effects on carbon and nitrogen dynamics of surface litter in corn agroecosystems. Ecological Applications **7**:1341-1349.

Bolan, N. S., S. Saggar, J. F. Luo, R. Bhandral, and J. Singh. 2004. Gaseous emissions of nitrogen from grazed pastures: Processes, measurements and modelling, environmental implications, and mitigation. Advances in Agronomy **84**:37-120.

Bolinder, M. A., D. A. Angers, G. Bélanger, R. Michaud, and M. R. Laverdière. 2002. Root biomass and shoot to root ratios of perennial forage crops in eastern Canada. Canadian Journal of Plant Science **82**:731-737.

Boquel, G., and L. Suavin. 1972. Inhibition of nitrification by aqueous extracts of litter of *Tectona grandis* and *Melaleuca leucadendron*. Revue d'Ecologie et de Biologie du Sol **9**:641-654.

Borken, W., and F. Beese. 2006. Methane and nitrous oxide fluxes of soils in pure and mixed stands of European beech and Norway spruce. European Journal of Soil Science **57**:617-625.

Bremner, J. M., and G. W. McCarty. 1993. Inhibition of nitrification in soil by allelochemicals derived from plants and plant residues. Pages 181-218 *in* J. Bollag and G. Stotzky, editors. Soil biochemistry, New York, USA.

Brockerhoff, E. G., H. Jactel, J. A. Parrotta, C. P. Quine, and J. Sayer. 2008. Plantation forests and biodiversity: oxymoron or opportunity? Biodiversity and Conservation **17**:925-951.

Bronick, C. J., and R. Lal. 2005. Soil structure and management: A review. Geoderma **124**:3-22.

Brzostek, E. R., A. Greco, J. E. Drake, and A. C. Finzi. 2013. Root carbon inputs to the rhizosphere stimulate extracellular enzyme activity and increase nitrogen availability in temperate forest soils. Biogeochemistry **115**:65-76.

Butterbach-Bahl, K., A. Rothe, and H. Papen. 2002. Effect of tree distance on N_2O and CH_4-fluxes from soils in temperate forest ecosystems. Plant and Soil **240**:91-103.

Cameron, K. C., H. J. Di, and J. L. Moir. 2013. Nitrogen losses from the soil/plant system: A review. Annals of Applied Biology **162**:145-173.

Cameron, K. C., H. J. Di, B. P. A. Reijnen, Z. Li, J. M. Russell, and J. W. Barnett. 2002. Fate of nitrogen in dairy factory effluent irrigated onto land. New Zealand Journal of Agricultural Research **45**:207-216.

Canterbury Water. 2009. Canterbury water management strategy – strategic framework. Environment Canterbury, Chistchurch, New Zealand.

Carpenter, S. R., N. F. Caraco, D. L. Correll, R. W. Howarth, A. N. Sharpley, and V. H. Smith. 1998. Nonpoint pollution of surface waters with phosphorus and nitrogen. Ecological Applications **8**:559-568.

Casler, M. D., and R. L. Kallenbach. 2007. Cool-season grasses for humid areas. Page Chapter 12 *in* R. F. Barnes, C. J. Nelson, J. M. Kenneth, and M. Collins, editors. Forages: The science of grassland agriculture. Wiley-Blackwell, New York, USA.

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

Charlton, J. F. L., and A. V. Stewart. 1999. Pasture species and cultivars used in New Zealand – a list. Proceedings of the New Zealand Grassland Association **61**:147-166.

Chen, J., and J. M. Stark. 2000. Plant species effects and carbon and nitrogen cycling in a sagebrush–crested wheatgrass soil. Soil Biology and Biochemistry **32**:47-57.

Christiansen, J. R., and P. Gundersen. 2011. Stand age and tree species affect N_2O and CH_4 exchange from afforested soils. Biogeosciences Discussions **8**:5729–5760.

Clarholm, M. 1985. Interactions of bacteria, protozoa and plants leading to mineralization of soil nitrogen. Soil Biology and Biochemistry **17**:181-187.

Clark, H., F. Kelliher, and C. Pinares-Patino. 2011. Reducing CH_4 emissions from grazing ruminants in New Zealand: Challenges and Opportunities. Asian-Australasian Journal of Animal Sciences **24**:295-302.

Clément, J. C., G. Pinay, and P. Marmonier. 2002. Seasonal dynamics of denitrification along topohydrosequences in three different riparian wetlands. Journal of Environmental Quality **31**:1025-1037.

Cook, J. M., A. F. Mark, and B. F. Shore. 1980. Responses of *Leptospermum scoparium* and *L. ericoides* (Myrtaceae) to waterlogging. New Zealand Journal of Botany **18**:233-246.

Coomes, D. A., R. B. Allen, N. A. Scott, C. Goulding, and P. Beets. 2002. Designing systems to monitor carbon stocks in forests and shrublands. Forest Ecology and Management **164**:89-108.

Cooper, A. B. 1990. Nitrate depletion in the riparian zone and stream channel of a small headwater catchment. Hydrobiologia **202**:13-26.

Cooper, R. C., R. C. Cambie, and B. S. G. 1991. New Zealand's economic native plants. Oxford University Press, Auckland, New Zealand.

Corbett, R. E., H. Young, and R. S. Wilson. 1964. Extractives from the bark of *Olearia paniculata*. Australian Journal of Chemistry **17**:712-714.

Cornelissen, J. H. C., M. J. A. Werger, P. Castro-Diez, J. W. A. Van Rheenen, and A. P. Rowland. 1997. Foliar nutrients in relation to growth, allocation and leaf traits in seedlings of a wide range of woody plant species and types. Oecologia **111**:460-469.

Cornes, D. 2005. Callisto: a very successful maize herbicide inspired by allelochemistry. Pages 569-572 *in* Proceedings of Fourth World Congress on Allelopathy, August 2005, Wagga Wagga, Australia.

Correll, D. L. 1997. Buffer zones and water quality protection: general principles. Pages 7–20 *in* N. E. Haycock, T. P. Burt, K. W. T. Goulding, and G. Pinay, editors. Buffer zones: Their processes and potential in water protection. Proceedings of the international conference on buffer zones, September 1996. Quest Environmental, Harpenden, UK.

Correll, D. L. 2005. Principles of planning and establishment of buffer zones. Ecological Engineering **24**:433-439.

Craine, J. M. 2009. Resource strategies of wild plants. Princeton University Press, Princeton, USA.

Craine, J. M., J. Froehle, D. G. Tilman, D. A. Wedin, and F. S. I. Chapin. 2001. The relationships among root and leaf traits of 76 grassland species and relative abundance along fertility and disturbance gradients. Oikos **93**:274-285.

Craine, J. M., and W. G. Lee. 2003. Covariation in leaf and root traits for native and non-native grasses along an altitudinal gradient in New Zealand. Oecologia **134**:471-478.

Crane, W. J. B., and J. C. G. Banks. 1992. Accumulation and retranslocation of foliar nitrogen in fertilised and irrigated *Pinus radiata*. Forest Ecology and Management **52**:201-223.

Crush, J. R., H. S. Easton, J. E. Waller, D. E. Hume, and M. J. Faville. 2007. Genotypic variation in patterns of root distribution, nitrate interception and response to moisture stress of a perennial ryegrass (*Lolium perenne* L.) mapping population. Grass and Forage Science **62**:265-273.

Crush, J. R., J. E. Waller, and D. A. Care. 2005. Root distribution and nitrate interception in eleven temperate forage grasses. Grass and Forage Science **60**:385-392.

Czernin, A., and C. Phillips. 2005. Below-ground morphology of *Cordyline australis* (New Zealand cabbage tree) and its suitability for river bank stabilisation. New Zealand Journal of Botany **43**:851-864.

Dahm, C. N., N. B. Grimm, P. Marmonier, H. M. Valett, and P. Vervier. 1998. Nutrient dynamics at the interface between surface waters and groundwaters. Freshwater Biology **40**:427-451.

Daily, G. C., S. Alexander, P. R. Ehrlich, L. Goulder, J. Lubchenco, P. A. Matson, H. A. Mooney, S. Postel, S. H. Schneider, and D. Tilman. 1997. Ecosystem services: benefits supplied to human societies by natural ecosystems. Ecological Society of America, Washington, USA.

Dairy NZ Ltd. 2011. Pasture growth data: South Island. Retrieved 5 September 2014. http://www.dairynz.co.nz/media/323786/average_pasture_growth_data_sth_is_oct_2010.pdf.

Davies-Colley, R. J., and R. J. Wilcock. 2004. Water quality and chemistry in running waters. Pages 11.11-11.17 *in* J. Hardling, P. Mosley, C. Pearson, and B. Sorrell, editors. Freshwaters of New Zealand. Caxton Press, Christchurch, New Zealand.

Davis, M. 2014. Nitrogen leaching losses from forests in New Zealand. New Zealand Journal of Forestry Science **44**:1-14.

Davis, M. R. 1990. Chemical composition of soil solutions extracted from New Zealand beech forests and West German beech and spruce forests. Plant and Soil **126**:237-246.

Dawson, J., and R. Lucas. 2011. New Zealand's native trees. Craig Potton Publishing, Nelson, New Zealand.

de Klein, C. A. M., and S. F. Ledgard. 2005. Nitrous Oxide emissions from New Zealand agriculture – Key sources and mitigation strategies. Nutrient Cycling in Agroecosystems **72**:77-85.

de Klein, C. A. M., C. Pinares-Patino, and G. C. Waghorn. 2008. Greenhouse gas emissions. Pages 1-32 *in* R. W. McDowell, editor. Environmental impacts of pasture-based farming. CAB International London, UK.

de Klein, C. A. M., R. R. Sherlock, K. C. Cameron, and T. J. van der Weerden. 2001. Nitrous oxide emissions from agricultural soils in New Zealand—a review of current knowledge and directions for future research. Journal of the Royal Society of New Zealand **31**:543-574.

de Klein, C. A. M., L. C. Smith, and R. M. Monaghan. 2006. Restricted autumn grazing to reduce nitrous oxide emissions from dairy pastures in Southland, New Zealand. Agriculture, Ecosystems and Environment **112**:192-199.

de Lange, P. J. 2014. A revision of the New Zealand *Kunzea ericoides* (Myrtaceae) complex. PhytoKeys **40**:1-185.

de Mendiburu, F. 2014. Agricolae: Statistical procedures for agricultural research. R package version 1.2-1.

DeLuca, T. H., M. D. MacKenzie, and M. J. Gundale. 2009. Biochar effects on soil nutrient transformations. Pages 251-256 *in* J. Lehmann and S. Joseph, editors. Biochar for environmental management: science and technology. Earthscan, London, UK.

Dhondt, K., P. Boeckx, G. Hofman, and O. Van Cleemput. 2004. Temporal and spatial patterns of denitrification enzyme activity and nitrous oxide fluxes in three adjacent vegetated riparian buffer zones. Biology and Fertility of Soils **40**:243-251.

Di, H. J., and K. C. Cameron. 2000. Calculating nitrogen leaching losses and critical nitrogen application rates in dairy pasture systems using a semi-empirical model. New Zealand Journal of Agricultural Research **43**:139-147.

Di, H. J., and K. C. Cameron. 2002a. Nitrate leaching and pasture production from different nitrogen sources on a shallow stoney soil under flood-irrigated dairy pasture. Soil Research **40**:317-334.

Di, H. J., and K. C. Cameron. 2002b. Nitrate leaching in temperate agroecosystems: sources, factors and mitigating strategies. Nutrient Cycling in Agroecosystems **64**:237-256.

Di, H. J., and K. C. Cameron. 2006. Nitrous oxide emissions from two dairy pasture soils as affected by different rates of a fine particle suspension nitrification inhibitor, dicyandiamide. Biology and Fertility of Soils **42**:472-480.

Di, H. J., and K. C. Cameron. 2007. Nitrate leaching losses and pasture yields as affected by different rates of animal urine nitrogen returns and application of a nitrification inhibitor—a lysimeter study. Nutrient Cycling in Agroecosystems **79**:281-290.

Di, H. J., and K. C. Cameron. 2012. How does the application of different nitrification inhibitors affect nitrous oxide emissions and nitrate leaching from cow urine in grazed pastures? Soil Use and Management **28**:54-61.

Di, H. J., K. C. Cameron, S. Moore, and N. P. Smith. 1998a. Nitrate leaching and pasture yields following the application of dairy shed effluent or ammonium fertilizer under spray or flood irrigation: Results of a lysimeter study. Soil Use and Management **14**:209-214.

Di, H. J., K. C. Cameron, S. Moore, and N. P. Smith. 1998b. Nitrate leaching from dairy shed effluent and ammonium fertiliser applied to a free-draining pasture soil under spray or flood irrigation. New Zealand Journal of Agricultural Research **41**:263-270.

Di, H. J., K. C. Cameron, S. Moore, and N. P. Smith. 1999. Contributions to nitrogen leaching and pasture uptake by autumn-applied dairy effluent and ammonium fertilizer labeled with ^{15}N isotope. Plant and Soil **210**:189-198.

Di, H. J., K. C. Cameron, J. P. Shen, C. S. Winefield, M. O'Callaghan, S. Bowatte, and J. Z. He. 2010a. Ammonia-oxidizing bacteria and archaea grow under contrasting soil nitrogen conditions. FEMS Microbiology Ecology **72**:386-394.

Di, H. J., K. C. Cameron, J. P. l. Shen, C. S. Winefield, M. O'Callaghan, S. Bowatte, and J. Z. He. 2009. Nitrification driven by bacteria and not archaea in nitrogen-rich grassland soils. Nature Geoscience **2**:621-624.

Di, H. J., K. C. Cameron, R. R. Sherlock, J.-P. Shen, J.-Z. He, and C. S. Winefield. 2010b. Nitrous oxide emissions from grazed grassland as affected by a nitrification inhibitor, dicyandiamide, and relationships with ammonia-oxidizing bacteria and archaea. Journal of Soils and Sediments **10**:943-954.

Diaz, R. J. 2001. Overview of hypoxia around the World. Journal of Environmental Quality **30**:275-281.

Dickinson, N. M., A. J. M. Baker, A. Doronila, S. Laidlaw, and R. D. Reeves. 2009. Phytoremediation of inorganics: Realism and synergies. International Journal of Phytoremediation **11**:97-114.

Dijkstra, F. A., N. E. Bader, D. W. Johnson, and W. Cheng. 2009. Does accelerated soil organic matter decomposition in the presence of plants increase plant N availability? Soil Biology and Biochemistry **41**:1080-1087.

Dijkstra, F. A., Y. Carrillo, E. Pendall, and J. A. Morgan. 2013. Rhizosphere priming: A nutrient perspective. Frontiers in Microbiology **4**:1-8.

Dosskey, M. G., P. Vidon, N. P. Gurwick, C. J. Allan, T. P. Duval, and R. Lowrance. 2010. The role of riparian vegetation in protecting and improving chemical water quality in streams. Journal of the American Water Resources Association **46**:261-277.

Douglas, G. B., M. B. Dodd, and I. L. Power. 2007. Potential of direct seeding for establishing native plants into pastoral land in New Zealand. New Zealand Journal of Ecology **31**:143-153.

Drake, J. E., A. Gallet-Budynek, K. S. Hofmockel, E. S. Bernhardt, S. A. Billings, R. B. Jackson, K. S. Johnsen, J. Lichter, H. R. McCarthy, and M. L. McCormack. 2011. Increases in the flux of carbon belowground stimulate nitrogen uptake and sustain the long-term enhancement of forest productivity under elevated CO_2. Ecology Letters **14**:349-357.

Dunbabin, V., A. Diggle, and Z. Rengel. 2003. Is there an optimal root architecture for nitrate capture in leaching environments? Plant, Cell and Environment **26**:835-844.

Edgar, E., and H. E. Connor. 2000. Flora of New Zealand-Vol. V: Gramineae (Grasses). Manaaki Whenua Press, Lincoln, New Zealand.

Environment Canterbury. 2011. Riparian zones: A guide to the protection of Canterbury's rivers, streams and wetlands. Environment Canterbury, Christchurch, New Zealand.

Eory, V., C. F. E. Topp, and D. Moran. 2013. Multiple-pollutant cost-effectiveness of greenhouse gas mitigation measures in the UK agriculture. Environmental Science and Policy **27**:55-67.

Esperschütz, J., F. Buegger, J. B. Winkler, J. C. Munch, M. Schloter, and A. Gattinger. 2009. Microbial response to exudates in the rhizosphere of young beech trees (*Fagus sylvatica*) after dormancy. Soil Biology and Biochemistry **41**:1976-1985.

Fewtrell, L. 2004. Drinking-water nitrate, methemoglobinemia, and global burden of disease: A discussion. Environmental Health Perspectives **112**:1371-1374.

Fillery, I. R. P. 2007. Plant-based manipulation of nitrification in soil: A new approach to managing N loss? Plant and Soil **294**:1-4.

Finlay, J. C., J. M. Hood, M. Limm, M. E. Power, J. D. Schade, and J. R. Welter. 2011. Light-mediated thresholds in stream water nutrient composition in a river network. Ecology **92**:140-150.

Finzi, A. C., N. Van Breemen, and C. D. Canham. 1998. Canopy tree–soil interactions within temperate forests: Species effects on soil carbon and nitrogen. Ecological Applications **8**:440-446.

Fontaine, S., A. Mariotti, and L. Abbadie. 2003. The priming effect of organic matter: a question of microbial competition? Soil Biology and Biochemistry **35**:837-843.

Fukuda, Y., H. Moller, and B. Burns. 2011. Effects of organic farming, fencing and vegetation origin on spiders and beetles within shelterbelts on dairy farms. New Zealand Journal of Agricultural Research **54**:155-176.

Garnier, E. 1991. Resource capture, biomass allocation and growth in herbaceous plants. Trends in Ecology and Evolution **6**:126-131.

Gielen, G., V. Dameron, G. Oliver, I. Nicholas, S. Pearce, and C. J. 1999. Growth and nutrient uptake of effluent irrigated *Eucalypus botryoides*. New Zealand Land Treatment Collective, Technical Session No. 19, Christchurch, New Zealand.

Gift, D. M., P. M. Groffman, S. S. Kaushal, and P. M. Mayer. 2010. Denitrification potential, root biomass, and organic matter in degraded and restored urban riparian zones. Restoration Ecology **18**:113-120.

Gilliam, J. W., J. E. Parsons, and R. L. Mikkelsen. 1997. Nitrogen dynamics and buffer zones. Pages 54-61 *in* Buffer zones: Their Processes and Potential in Water Protection. Proceedings of the International Conference on Buffer Zones, September 1996. Harpenden, UK, Quest Environmental.

Givnish, T. J. 2002. Ecological constraints on the evolution of plasticity in plants. Evolutionary Ecology **16**:213-242.

Golden, M., and C. Leifert. 1999. Potential risks and benefits of dietary nitrate. Pages 269-280 *in* W. S. Wilson, A. S. Ball, and R. H. Hinton, editors. Managing risks of nitrates to humans and the environment. The Royal Society of Chemistry, Cambridge, UK.

Gopalakrishnan, S., T. Watanabe, S. J. Pearse, O. Ito, Z. Hossain, and G. V. Subbarao. 2009. Biological nitrification inhibition by *Brachiaria humidicola* roots varies with soil type and inhibits nitrifying bacteria, but not other major soil microorganisms. Soil Science and Plant Nutrition **55**:725-733.

Greenway, D. R. 1987. Vegetation and slope stability. Pages 187-230 *in* M. G. Anderson and K. S. Richards, editors. Slope stability: Geotechnical engineering and geomorphology. John Wiley and Sons, New York, USA.

Gregory, S. V., F. J. Swanson, W. A. McKee, and K. W. Cummins. 1991. An ecosystem perspective of riparian zones. BioScience **41**:540-551.

Grizzetti, B., i. F. Bouraou, G. Billen, H. van Grinsven, A. C. Cardoso, V. Thieu, J. Garnier, C. Curtis, R. Howarth, and P. Johnes. 2011. Nitrogen as a threat to European water quality. Pages 379-404 *in* M. A. Sutton, C. M. Howard, J. W. Erisman, G. Billen, A. Bleeker, P. Grennfelt, H. van Grinsven, and B. Grizzetti, editors. The European nitrogen assessment: Sources, effects and policy perspectives. Cambridge University Press, Cambridge, UK.

Groffman, P. M., A. J. Gold, and K. Addy. 2000. Nitrous oxide production in riparian zones and its importance to national emission inventories. Chemosphere - Global Change Science **2**:291-299.

Groffman, P. M. A., E. A. Lemunyon, J. L. Sullivan, and W. Michael. 1991. Denitrification in grass and forest vegetated filter strips. Journal of Environmental Quality **20**:671-674.

Guest, R. 1985. Establishment of nursery-raised indigenous seedlings in selectively logged forest in Pureora Forest Park. New Zealand Journal of Forestry **30**:159-166.

Guo, L. B., R. E. H. Sims, and D. J. Horne. 2002. Biomass production and nutrient cycling in *Eucalyptus* short rotation energy forests in New Zealand: Biomass and nutrient accumulation. Bioresource Technology **85**:273-283.

Hahner, J. L., B. H. Robinson, Z. Hong-Tao, and N. M. Dickinson. 2014. The phytoremediation potential of native plants on New Zealand dairy farms. International Journal of Phytoremediation **16**:719-734.

Haile, W., T. Mala, Y. Osotsapar, and V. Verasan. 2006. Screening of Ethiopian traditional medicinal herbs for their nitrification inhibiting ability. Kasetsart Journal (Natural Sciences) **40**:74-82.

Haines, L., and J. Margetts. 2006. How can urban riparian planting chime with residential garden design? New Zealand Garden Journal **9**:2-5.

Hamill, K. D., and G. B. McBride. 2003. River water quality trends and increased dairying in Southland, New Zealand. New Zealand Journal of Marine and Freshwater Research **37**:323-332.

Harmsworth, G. R., and S. Awatere. 2013. Indigenous māori knowledge and perspectives of ecosystems. Pages 274-287 *in* J. R. Dymond, editor. Ecosystem services in New Zealand. Manaaki Whenua Press, Lincoln, New Zealand.

Hartmann, A., M. Rothballer, and M. Schmid. 2008. Lorenz Hiltner, a pioneer in rhizosphere microbial ecology and soil bacteriology research. Plant and Soil **312**:7-14.

Hawkins, B. J., and G. B. Sweet. 1989. The growth of three podocarp species under different nutrient regimes. New Zealand Journal of Botany **27**:305-310.

Haynes, R. J., K. C. Cameron, K. M. Goh, and R. R. Sherlock. 1986. Mineral nitrogen in the plant-soil system. Academic Press, Orlando, USA.

Haynes, R. J., and P. H. Williams. 1993. Nutrient cycling and soil fertility in the grazed pasture ecosystem. Advances in Agronomy **49**:119-199.

Hedley, C. B., S. M. Lambie, and J. L. Dando. 2013. Edaphic and environmental controls of soil respiration and related soil processes under two contrasting manuka and kanuka shrubland stands in North Island, New Zealand. Soil Research **51**:390-405.

Hefting, M., B. Roland, and H. de Caluwe. 2003. Nitrous oxide emission and denitrification in chronically nitrate-loaded riparian buffer zones. Journal of Environmental Quality **32**:1194.

Hefting, M. M., J.-C. Clement, P. Bienkowski, D. Dowrick, C. Guenat, A. Butturini, S. Topa, G. Pinay, and J. T. A. Verhoeven. 2005. The role of vegetation and litter in the nitrogen dynamics of riparian buffer zones in Europe. Ecological Engineering **24**:465-482.

Hendricks, J. J., K. J. Nadelhoffer, and J. D. Aber. 1993. Assessing the role of fine roots in carbon and nutrient cycling. Trends in Ecology and Evolution **8**:174-178.

Hendrickson, O. Q., L. Chatarpaul, and D. Burgess. 1989. Nutrient cycling following whole-tree and conventional harvest in northern mixed forest. Canadian Journal of Forest Research **19**:725-735.

Herlihy, A. T., J. L. Stoddard, and C. B. Johnson. 1998. The relationship between stream chemistry and watershed land cover data in the mid-Atlantic region, US. Water, Air, and Soil Pollution **105**:377-386.

Hesse, P. R. 1971. A textbook of soil chemical analysis. John Murray Publisher, London, UK.

Hewitt, A. E. 1998. New Zealand soil classification. 2nd edition. Manaaki Whenua Press, Lincoln, New Zealand.

Hill, A. R. 1996. Nitrate removal in stream riparian zones. Journal of Environmental Quality **25**:743-755.

Hill Laboratories. 2014. Soil tests and interpretation. Hill Laboratories, Hamilton, New Zealand.

Hill, R. B., and G. P. Sparling. 2009. Soil quality monitoring. Pages 27-51 *in* L. M. Forum, editor. Land and soil monitoring: A guide for SoE and regional council reporting. Land Monitoring Forum, Wellington, New Zealand.

Hinsinger, P., A. G. Bengough, D. Vetterlein, and I. M. Young. 2009. Rhizosphere: Biophysics, biogeochemistry and ecological relevance. Plant and Soil **321**:117-152.

Hobbie, S. E. 1992. Effects of plant species on nutrient cycling. Trends in Ecology and Evolution **7**:336-339.

Houghton, R. A. 2005. Tropical deforestation as a source of greenhouse gas emissions. Pages 13-23 *in* P. Moutinho and S. Schwartzman, editors. Tropical deforestation and climate change. Environmental Defense, Washington, USA.

Houlbrooke, D. J., D. J. Horne, M. J. Hedley, J. A. Hanly, and V. O. Snow. 2004. A review of literature on the land treatment of farm-dairy effluent in New Zealand and its impact on water quality. New Zealand Journal of Agricultural Research **47**:499-511.

Hubbard, C. E. 1954. Grasses. A guide to their structure, identification, uses, and distribution in the British Isles. Harmondsworth, Middlesex, UK.

Hubble, T. C. T., B. B. Docker, and I. D. Rutherfurd. 2010. The role of riparian trees in maintaining riverbank stability: A review of Australian experience and practice. Ecological Engineering **36**:292-304.

Hudson, H. R., and J. S. Harding. 2004. Drainage management in New Zealand. A review of existing activities and alternative management practices. Department of Conservation, Wellington, New Zealand.

Hutchinson, G. L., and A. R. Mosier. 1981. Improved soil cover method for field measurement of nitrous oxide fluxes. Soil Science Society of America Journal **45**:311-316.

IPCC. 2007. Climate Change 2007: Synthesis Report. Contribution of Working Groups I, II and III to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change. Intergovernmental panel on climate change, Geneva, Switzerland.

Jacinthe, P.-A., P. M. Groffman, A. J. Gold, and A. Mosier. 1998. Patchiness in microbial nitrogen transformations in groundwater in a riparian forest. Journal of Environmental Quality **27**:156-164.

Jacinthe, P. A., J. S. Bills, L. P. Tedesco, and R. C. Barr. 2012. Nitrous oxide emission from riparian buffers in relation to vegetation and flood frequency. Journal of Environmental Quality **41**:95-105.

Jacobs, T. C., and J. W. Gilliam. 1985. Riparian losses of nitrate from agricultural drainage waters. Journal of Environmental Quality **14**:472-478.

Jarvis, N. J. 2007. A review of non-equilibrium water flow and solute transport in soil macropores: Principles, controlling factors and consequences for water quality. European Journal of Soil Science **58**:523-546.

Jobbágy, E. G., and R. B. Jackson. 2004. The uplift of soil nutrients by plants: Biogeochemical consequences across scales. Ecology **85**:2380-2389.

Keating, T., and P. O'Kiely. 2000. Comparison of old permanent grassland, *Lolium perenne* and *Lolium multiflorum* swards grown for silage: 3. Effects of varying fertiliser nitrogen application rate. Irish Journal of Agricultural and Food Research **39**:35-53.

Keeney, D. R., and J. M. Bremner. 1966. Comparison and evaluation of laboratory methods of obtaining an index of soil nitrogen availability. Agronomy Journal **58**:498-503.

Kemp, P. D., C. Matthew, and R. J. Lucas. 2004. Pasture species and cultivars. Pages 89-90 *in* J. White and J. Hodgson, editors. New Zealand pasture and crop science. Oxford University Press, Melbourne, Australia.

Kielland, K. 2001. Short-circuiting the nitrogen cycle: Ecophysiological strategies of nitrogen uptake in plants from marginal environments. Pages 376-398 *in* A. Noriharu, J. Arihara, K. Okada, and A. Srinivasan, editors. Plant nutrient acquisition. Springer, Tokyo, Japan.

Kim, C. H., and T. C. Hollocher. 1983. N-15 tracer studies on the reduction of nitrite by the purified dissimilatory nitrite reductase of *Pseudomonas aeruginosa* - evidence for direct production of N_2O without free NO as an intermediate. Journal of Biological Chemistry **258**:4861-4863.

Kirschbaum, M. U. F., S. Saggar, K. R. Tate, D. L. Giltrap, A.-G. E. Ausseil, S. Greenhalgh, and D. Whitehead. 2012. Comprehensive evaluation of the climate-change implications of shifting land use between forest and grassland: New Zealand as a case study. Agriculture, Ecosystems and Environment **150**:123-138.

Knobeloch, L., B. Salna, A. Hogan, J. Postle, and H. Anderson. 2000. Blue babies and nitrate-contaminated well water. Environmental Health Perspectives **108**:675-678.

Knowles, O. A., B. H. Robinson, A. Contangelo, and L. Clucas. 2011. Biochar for the mitigation of nitrate leaching from soil amended with biosolids. Science of the Total Environment **409**:3206-3210.

Kremen, C., and R. Chaplin-Kramer. 2007. Insects as providers of ecosystem services: Crop pollination and pest control. Pages 49–382 *in* A. Stewart, T. New, and O. Lewis, editors. Insect conservation biology. CABI Publishing, Wallingford, UK.

Kroeze, C., A. Mosier, and L. Bouwman. 1999. Closing the global N_2O budget: a retrospective analysis 1500–1994. Global Biogeochemical Cycles **13**:1-8.

Kronzucker, H. J., M. Y. Siddiqi, and A. D. Glass. 1997. Conifer root discrimination against soil nitrate and the ecology of forest succession. Nature **385**:59-61.

Kutera, J., and M. Soroko. 1994. The use and treatment of wastewater in willow and poplar plantations. Pages 37-49 *in* P. Aronsson and K. Perttu, editors. Willow vegetation filters for municipal wastewaters and sludges. Department of Ecology and Environmental Research, Swedish University of Agricultural Sciences, Uppsala, Sweeden.

Kuzyakov, Y. 2010. Priming effects: Interactions between living and dead organic matter. Soil Biology and Biochemistry **42**:1363-1371.

Lambers, H., F. S. I. Chapin, and T. L. Pons, editors. 2008. Plant Physiological Ecology. Springer, New York, USA.

Lambert, M. G., G. A. Jung, and D. A. Costall. 1989a. Forage shrubs in North Island hill country 1. Forage production. New Zealand Journal of Agricultural Research **32**:477-483.

Lambert, M. G., G. A. Jung, R. H. Fletcher, P. J. Budding, and D. A. Costall. 1989b. Forage shrubs in North Island hill country 2. Sheep and goat preferences. New Zealand Journal of Agricultural Research **32**:485-490.

Lambert, M. G., G. A. Jung, H. W. Harpster, and J. Lee. 1989c. Forage shrubs in North Island hill country 4. Chemical composition and conclusions. New Zealand Journal of Agricultural Research **32**:499-506.

Landcorp. 2014. Manuka honey sector gets boost with trial expansion. Landcprop. Retrieved on 5 September 2014. http://www.landcorp.co.nz/assets/NEW---News--Events/28-08-14-manuka-honey-media-release.pdf.

Langer, E. R., M. R. Davis, and C. W. Ross. 1999. Rehabilitation of lowland indigenous forest after mining in Westland. Department of Conservation, Wellington, New Zealand.

Larned, S. T., M. R. Scarsbrook, T. H. Snelder, N. J. Norton, and B. J. F. Biggs. 2004. Water quality in low-elevation streams and rivers of New Zealand: recent state and trends in contrasting land-cover classes. New Zealand Journal of Marine and Freshwater Research **38**:347-366.

Lata, J.-C., J. Durand, R. Lensi, and L. Abbadie. 1999. Stable coexistence of contrasted nitrification statuses in a wet tropical savanna ecosystem. Functional Ecology **13**:762-768.

Lata, J. C., V. Degrange, X. Raynaud, P. A. Maron, R. Lensi, and L. Abbadie. 2004. Grass populations control nitrification in savanna soils. Functional Ecology **18**:605-611.

Laughlin, D. C. 2011. Nitrification is linked to dominant leaf traits rather than functional diversity. Journal of Ecology **99**:1091-1099.

Leathwick, J. L., G. Wilson, D. Rutledge, P. Wardle, F. Morgan, K. Johnston, M. Mcleod, and R. Kirkpatrick. 2003. Land environments of New Zealand, Nga Taiao o Aotearoa. David Bateman Ltd., Auckland, New Zealand.

Ledgard, N., and M. Davis. 2004. Restoration of mountain beech (*Nothofagus solandri* var. *cliffortioides*) forest after fire. New Zealand Journal of Ecology **28**:125-136.

Ledgard, S. F., J. W. Penno, and M. S. Sprosen. 1999. Nitrogen inputs and losses from clover/grass pastures grazed by dairy cows, as affected by nitrogen fertilizer application. The Journal of Agricultural Science **132**:215-225.

Ledgard, S. F., M. S. Sprosen, J. W. Penno, and G. S. Rajendram. 2001. Nitrogen fixation by white clover in pastures grazed by dairy cows: Temporal variation and effects of nitrogen fertilization. Plant and Soil **229**:117-187.

Lee, K. E. 1959. The earthworm fauna of New Zealand. Government Printer, Wellington, New Zealand.

Lee, P., C. Smyth, and S. Boutin. 2004. Quantitative review of riparian buffer width guidelines from Canada and the United States. Journal of Environmental Management **70**:165-180.

Lilburne, L., T. Webb, R. Ford, and V. J. Bidwell. 2010. Estimating nitrate-nitrogen leaching rates under rural land uses in Canterbury. Environment Canterbury, Christchurch, New Zealand.

Linder, H. P., M. Baeza, N. P. Barker, C. Galley, A. M. Humphreys, K. M. Lloyd, D. A. Orlovich, M. D. Pirie, B. K. Simon, and N. Walsh. 2010. A Generic Classification of the Danthonioideae (Poaceae) 1. Annals of the Missouri Botanical Garden **97**:306-364.

Lis-Balchin, M., S. Deans, and S. Hart. 1995. Bioactivity of New Zealand medicinal plant essential oils. Pages 13-30 *in* L. E. Craker, L. Nolan, and K. Shetty, editors. Proceedings of the International Symposium of Medicinal and Aromatic Plants, August 1995. Acta Horticulturae, Amherst, USA.

Liu, L., and T. L. Greaver. 2009. A review of nitrogen enrichment effects on three biogenic GHGs: the CO_2 sink may be largely offset by stimulated N_2O and CH_4 emission. Ecology Letters **12**:1103-1117.

Liu, W., G. Liu, and Q. Zhang. 2011. Influence of vegetation characteristics on soil denitrification in shoreline wetlands of the Danjiangkou reservoir in China. Clean – Soil, Air, Water **39**:109-115.

Longhurst, R. D., A. H. C. Roberts, and M. B. O'Connor. 2000. Farm dairy effluent: A review of published data on chemical and physical characteristics in New Zealand. New Zealand Journal of Agricultural Research **43**:7-14.

Lovett, G. M., K. C. Weathers, M. A. Arthur, and J. C. Schultz. 2004. Nitrogen cycling in a northern hardwood forest: Do species matter? Biogeochemistry **67**:289-308.

Lowrance, R. R., R. Todd, J. Fail Jr, O. Hendrickson Jr, R. Leonard, and L. Asmussen. 1984. Riparian forests as nutrient filters in agricultural watersheds. BioScience **34**:374-377.

Lu, J., D. A. Carter, L. Turnbull, D. Rosendale, D. Hedderley, J. Stephens, S. Gannabathula, G. Steinhorn, R. C. Schlothauer, C. B. Whitchurch, and E. J. Harry. 2013. The effect of new zealand kanuka, manuka and clover honeys on bacterial growth dynamics and cellular morphology varies according to the species. PLoS ONE **8**:e55898.

Lu, J., L. Turnbull, C. M. Burke, M. Liu, D. A. Carter, R. C. Schlothauer, C. B. Whitchurch, and E. J. Harry. 2014. Manuka-type honeys can eradicate biofilms produced by *Staphylococcus aureus* strains with different biofilm-forming abilities. PeerJ **1**:e326.

Luo, J., C. A. M. de Klein, S. F. Ledgard, and S. Saggar. 2010. Management options to reduce nitrous oxide emissions from intensively grazed pastures: A review. Agriculture, Ecosystems and Environment **136**:282-291.

Luo, J., S. Saggar, R. Bhandral, N. Bolan, S. Ledgard, S. Lindsey, and W. Sun. 2008. Effects of irrigating dairy-grazed grassland with farm dairy effluent on nitrous oxide emissions. Plant and Soil **309**:119-130.

Luo, J., R. W. Tillman, and P. R. Ball. 2000a. Nitrogen loss through denitrification in a soil under pasture in New Zealand. Soil Biology and Biochemistry **32**:497-509.

Luo, Y. M., P. Christie, and A. J. M. Baker. 2000b. Soil solution Zn and pH dynamics in non-rhizosphere soil and in the rhizosphere of *Thlaspi caerulescens* grown in a Zn/Cd-contaminated soil. Chemosphere **41**:161-164.

Maddocks-Jennings, W., J. M. Wilkinson, D. Shillington, and H. Cavanagh. 2005. A fresh look at manuka and kanuka essential oils from New Zealand. International Journal of Aromatherapy **15**:141-146.

Magesan, G. N., H. Wang, and P. W. Clinton. 2012. Nitrogen cycling in gorse-dominated ecosystems in New Zealand. New Zealand Journal of Ecology **36**:21-28.

Malcolm, B. J. 2013. The effect of pasture species composition and a nitrification inhibitor on nitrate leaching losses. Lincoln University, Lincoln, New Zealand.

Malcolm, B. J., K. C. Cameron, H. J. Di, G. R. Edwards, and J. L. Moir. 2014. The effect of four different pasture species compositions on nitrate leaching losses under high N loading. Soil Use and Management **30**:58-68.

Mander, Ü., Y. Hayakawa, and V. Kuusemets. 2005. Purification processes, ecological functions, planning and design of riparian buffer zones in agricultural watersheds. Ecological Engineering **24**:421-432.

Mander, Ü., V. Kuusemets, K. Lõhmus, and T. Mauring. 1997. Efficiency and dimensioning of riparian buffer zones in agricultural catchments. Ecological Engineering **8**:299-324.

Marden, M., and C. Phillips. 2009. Native Plant Trial. Conservation Quorum, Issue 54. Gisborne District Council, Gisborne, New Zealand.

Marden, M., D. Rowan, and C. Phillips. 2005. Stabilising characteristics of New Zealand indigenous riparian colonising plants. Plant and Soil **278**:95-105.

Marden, M., D. Rowan, and C. Phillips. 2008a. Karamu: *Coprosma robusta*. Manaaki Whenua Landcare Research. http://icm.landcareresearch.co.nz/research/freshwater/documents/karamu.pdf.

Marden, M., D. Rowan, and C. Phillips. 2008b. Kohuhu: *Pittosporum tenuifolium*. Manaaki Whenua Landcare Research. http://icm.landcareresearch.co.nz/research/freshwater/documents/kohuhu.pdf.

Marden, M., D. Rowan, and C. Phillips. 2008c. Kowhai: *Sophora microphylla*. Manaaki Whenua Landcare Research. http://icm.landcareresearch.co.nz/research/freshwater/documents/kowhai.pdf.

Marden, M., D. Rowan, and C. Phillips. 2008d. Ribbonwood: *Plagianthus regius*. Manaaki Whenua Landcare Research. http://icm.landcareresearch.co.nz/research/freshwater/documents/Ribbonwood.pdf.

Marschner, H. 1995. Mineral nutrition of higher plants. Academic Press, San Diego, USA.

Martin, T. L., N. K. Kaushik, J. T. Trevors, and H. R. Whiteley. 1999. Review: denitrification in temperate climate riparian zones. Water, Air, and Soil Pollution **111**:171-186.

Mason, N. W. H., P. N. Beets, I. Payton, L. Burrows, R. J. Holdaway, and F. E. Carswell. 2014. Individual-based allometric equations accurately measure carbon storage and sequestration in shrublands. Forests **5**:309-324.

Mayer, P. M. R., S. K. McCutchen, M. D. Canfield, and J. Timothy. 2007. Meta-analysis of nitrogen removal in riparian buffers. Journal of Environmental Quality **36**:1172.

McClain, M. E., E. W. Boyer, C. L. Dent, S. E. Gergel, N. B. Grimm, P. M. Groffman, S. C. Hart, J. W. Harvey, C. A. Johnston, and E. Mayorga. 2003. Biogeochemical hot spots and hot moments at the interface of terrestrial and aquatic ecosystems. Ecosystems **6**:301-312.

McClaugherty, C. A., J. Pastor, J. D. Aber, and J. M. Melillo. 1985. Forest litter decomposition in relation to soil nitrogen dynamics and litter quality. Ecology **66**:266-275.

McGlone, M. S. 1989. The Polynesian settlement of New Zealand in relation to environmental and biotic changes. New Zealand Journal of Ecology **12**:115-129.

McGlone, M. S., R. J. Dungan, G. M. J. Hall, and R. B. Allen. 2004. Winter leaf loss in the New Zealand woody flora. New Zealand Journal of Botany **42**:1-19.

McGlone, M. S., and J. M. Wilmshurst. 1999. Dating initial Maori environmental impact in New Zealand. Quaternary International **59**:5-16.

McGruddy, E. 2006. Integrating New Zealand flax into land management systems. Sustainable Farming Fund, Wellington, New Zealand.

McLaren, R. G., and K. C. Cameron. 1996. Soil science: Sustainable production and environmental protection. Oxford University Press, Auckland, New Zealand.

McLenaghen, R. D., K. C. Cameron, N. H. Lampkin, M. L. Daly, and B. Deo. 1996. Nitrate leaching from ploughed pasture and the effectiveness of winter catch crops in reducing leaching losses. New Zealand Journal of Agricultural Research **39**:413-420.

Menyailo, O. V., and B. Huwe. 1999. Activity of denitrification and dynamics of N_2O release in soils under six tree species and grassland in central Siberia. Journal of Plant Nutrition and Soil Science **162**:533-538.

Metcalf, L. 2009. Know your New Zealand native plants. New Holland, Auckland, New Zealand.

Meurk, C. D. 2008. Vegetation of the Canterbury Plains and downlands. Pages 195-250 *in* M. Winterbourn, G. Knox, C. Burrows, and I. Marsden, editors. The natural history of Canterbury. University of Canterbury Press, Christchurch, New Zealand.

Meurk, C. D., and G. M. J. Hall. 2006. Options for enhancing forest biodiversity across New Zealand's managed landscapes based on ecosystem modelling and spatial design. New Zealand Journal of Ecology **30**:131-146.

Meurk, C. D., and S. R. Swaffield. 2000. A landscape ecological framework for indigenous regeneration in rural New Zealand-Aotearoa. Landscape and Urban Planning **50**:129-144.

Millard, P. 1988. The accumulation and storage of nitrogen by herbaceous plants. Plant, Cell and Environment **11**:1-8.

Miller, R. M., C. I. Smith, J. D. Jastrow, and J. D. Bever. 1999. Mycorrhizal status of the genus *Carex* (Cyperaceae). American Journal of Botany **86**:547.

Milne, J. D. G., C. B., P. L. Singleton, and A. D. Wilson. 1995. Soil Description Handbook. Manaaki Whenua Press, Lincoln, New Zealand.

Ministry for the Environment. 2007. Environment New Zealand 2007. Ministry for the Environment, Wellington, New Zealand.

Ministry for the Environment. 2009. Our water: Groundwater quality. Ministry for the Environment, Wellington, New Zealand.

Ministry for the Environment. 2011. Climate change information New Zealand: Agriculture in the emissions trading scheme. Ministry for the Environment, Wellington, New Zealand.

Ministry for the Environment. 2012. New Zealand's Greenhouse Gas Inventory 1990-2010. Ministry for the Environment, Wellington, New Zealand.

Ministry of Health. 2008. New Zealand Drinking Water Standards. Ministry of Health, Wellington, New Zealand.

Mittermeier, R. A., N. Myers, C. G. Mittermeier, and P. Robles Gil. 1999. Hotspots: Earth's biologically richest and most endangered terrestrial ecoregions. Nature **406**:237-238.

Moffat, A. J., A. T. Armstrong, and J. Ockleston. 2001. The optimization of sewage sludge and effluent disposal on energy crops of short rotation hybrid poplar. Biomass and Bioenergy **20**:161-169.

Moir, J. L., K. C. Cameron, H. J. Di, A. H. C. Roberts, and W. Kuperus. 2003. The effects of urea and ammonium sulphate nitrate (ASN) on the production and quality of irrigated dairy pastures in Canterbury, New Zealand. Pages 139-145 *in* Tools for nutrient and pollutant management: Applications to agriculture and environmental quality: Proceedings of the 17th Annual

Workshop, December 2003. Fertiliser and Lime Research Centre, Palmerston North, New Zealand.

Moir, J. L., G. R. Edwards, and L. N. Berry. 2013. Nitrogen uptake and leaching loss of thirteen temperate grass species under high N loading. Grass and Forage Science **68**:313-325.

Molloy, L. 1998. Soils in the New Zealand landscape -The living mantle. Second edition. New Zealand Society of Soil Science, Lincoln, New Zealand.

Monaghan, R. M., R. J. Paton, L. C. Smith, J. J. Drewry, and R. P. Littlejohn. 2005. The impacts of nitrogen fertilisation and increased stocking rate on pasture yield, soil physical condition and nutrient losses in drainage from a cattle-grazed pasture. New Zealand Journal of Agricultural Research **48**:227-240.

Monaghan, R. M., R. J. Wilcock, L. C. Smith, B. Tikkisetty, B. S. Thorrold, and D. Costall. 2007. Linkages between land management activities and water quality in an intensively farmed catchment in southern New Zealand. Agriculture, Ecosystems and Environment **118**:211-222.

Moore, L. B., and E. Edgar. 1970. Flora of New Zealand. Vol. II. Government Printer, Wellington, New Zealand.

Morad, M., and G. M. M. Jay. 2000. Kaitiakitanga: protecting New Zealand's native biodiversity. Biologist **47**:197-201.

Muirhead, R. W., and R. M. Monaghan. 2011. Impacts of ditect faecal inputs and effluent management practices on microbial water quality. AgResearch, Invermay, New Zealand. www.massey.ac.nz/~flrc/workshops/11/Manuscripts/Muirhead_2011.pdf.

Muller, C. 1995. Nitrous oxide emission from intensive grassland in Canterbury, New Zealand. Lincoln University, Christchurch, New Zealand.

Myers, N. 1996. Environmental services of biodiversity. Proceedings of the National Academy of Sciences **93**:2764-2769.

Naiman, R. J., and H. Décamps. 1997. The ecology of interfaces: Riparian zones. Annual Review of Ecology and Systematics **28**:621-658.

Nevison, C. D., R. F. Weiss, and D. J. Erickson. 1995. Global oceanic emissions of nitrous oxide. Journal of Geophysical Research **100**:15809-15820.

Nicholas, I. 2003. Nitrogen uptake in New Zealand short rotation crops. Pages 27-35 *in* Short Rotation Crops for Bioenergy: New Zealand 2003. Forest Reserach, Rotorua, New Zealand.

Norton, D. A., and C. J. Miller. 2000. Some issues and options for the conservation of native biodiversity in rural New Zealand. Ecological Management and Restoration **1**:26-34.

Ogle, M. 1996. Factors affecting the early growth and survival of indigenous seedlings planted for the purpose of ecological restoration. University of Canterbury, Christchurch, New Zealand.

Oksanen, J., R. Kindt, P. Legendre, B. O'Hara, G. L. Simpson, P. Solymos, M. H. H. Stevens, and H. Wagner. 2009. Vegan: community ecology package. R package version 1.15-1.

Old, N. 2013. The medicine of the manuka an investigation of the usages and methods for utilization of honey derived from the pollen of *Leptospermum Scoparium* in holistic nursing practice. Journal of Holistic Nursing **31**:200-203.

Ordoñez, J. C., P. M. Van Bodegom, J. P. M. Witte, I. J. Wright, P. B. Reich, and R. Aerts. 2009. A global study of relationships between leaf traits, climate and soil measures of nutrient fertility. Global Ecology and Biogeography **18**:137-149.

Orwin, K. H., S. M. Buckland, D. Johnson, B. L. Turner, S. Smart, S. Oakley, and R. D. Bardgett. 2010. Linkages of plant traits to soil properties and the functioning of temperate grassland. Journal of Ecology **98**:1074-1083.

Paavolainen, L., V. Kitunen, and A. Smolander. 1998. Inhibition of nitrification in forest soil by monoterpenes. Plant and Soil **205**:147-154.

Pallardy, S. G. 2010. Physiology of woody plants. Elviser, Amsterdam, Netherlands.

Pandey, A., M. Singh, R. Srivastava, and P. Vasudevan. 2011. Pollutant removal potential, growth and nutritional characteristics of short rotation woody crops in grey water vegetation filter system. Journal of Scientific and Industrial Research **70**:610-615.

Parfitt, R. L., and D. J. Ross. 2011. Long-term effects of afforestation with *Pinus radiata* on soil carbon, nitrogen, and pH: A case study. Soil Research **49**:494-503.

Parfitt, R. L., G. J. Salt, and L. F. Hill. 2002. Clear-cutting reduces nitrate leaching in a pine plantation of high natural N status. Forest Ecology and Management **170**:43-53.

Parfitt, R. L., L. A. Schipper, W. T. Baisden, and A. H. Elliott. 2006. Nitrogen inputs and outputs for New Zealand in 2001 at national and regional scales. Biogeochemistry **80**:71-88.

Parkyn, S. M., R. J. Davies-Colley, N. J. Halliday, K. J. Costley, and G. F. Croker. 2003. Planted riparian buffer zones in New Zealand: Do they live up to expectations? Restoration Ecology **11**:436-447.

Patterson, M. G., and A. O. Cole. 1999. Assessing the value of New Zealand's biodiversity. School of Resource and Environmental Planning, Massey University, Palmerston North, New Zealand.

Peterjohn, W. T., and D. L. Correll. 1984. Nutrient dynamics in an agricultural watershed: observations on the role of a riparian forest. Ecology **65**:1466-1475.

Peters, M., and B. Clarkson, editors. 2010. Wetland restoration : A handbook for New Zealand freshwater systems. Manaaki Whenua Press, Lincoln, New Zealand.

Phillips, C. 2005. Erosion and sediment control using New Zealand native plants–What do we know. Erosion Control Seminar, September 2005. Manaaki Whenua Landcare Research.

Phillips, C., M. Marden, J. Ekanayake, and A. Watson. 2008. A peek down under: Structural root architecture of some New Zealand native plants. Manaaki Whenua Landcare Research. Retrieved 23 August 2011. http://icm.landcareresearch.co.nz/knowledgebase/publications/public/Roots_October08.pdf

Phillips, C. J., J. C. Ekanayake, and M. Marden. 2011a. Root site occupancy modelling of young New Zealand native plants: Implications for soil reinforcement. Plant and Soil **346**:201–214.

Phillips, C. J., J. C. Ekanayake, M. Marden, and A. Watson. 2000. Stabilising - Parameters of vegetation: A critical look down-under. Pages 1-13 *in* Proceedings of Landscape 2000, Leura, NSW, Australia.

Phillips, R. P., A. C. Finzi, and E. S. Bernhardt. 2011b. Enhanced root exudation induces microbial feedbacks to N cycling in a pine forest under long-term CO_2 fumigation. Ecology Letters **14**:187-194.

Pinay, G., L. Roques, and A. Fabre. 1993. Spatial and temporal patterns of denitrification in a riparian forest. Journal of Applied Ecology **30**:581-591.

Pinton, R., Z. Varanini, and P. Nannipieri. 2007. The rhizosphere: biochemistry and organic substances at the soil-plant interface. Taylor & Francis Group, New York, USA.

Pollen-Bankhead, N., and A. Simon. 2010. Hydrologic and hydraulic effects of riparian root networks on streambank stability: Is mechanical root-reinforcement the whole story? Geomorphology **116**:353-362.

Polyakov, V., A. Fares, and M. H. Ryder. 2005. Precision riparian buffers for the control of nonpoint source pollutant loading into surface water: A review. Environmental Reviews **13**:129-144.

Porteous, T. 1993. Native forest restoration: A practical guide for landowners. Queen Elizabeth the Second National Trust, Wellington, New Zealand.

Porter, N. G., and A. L. Wilkins. 1999. Chemical, physical and antimicrobial properties of essential oils of *Leptospermum scoparium* and *Kunzea ericoides*. Phytochemistry **50**:407-415.

Pratt, C. 1999. Factors affecting the establishment, growth and survival of native woody plant communities on the Canterbury Plain, New Zealand. Lincoln University, Lincoln, New Zealand.

Price, S., D. Whitehead, R. Sherlock, T. McSeveny, and G. Rogers. 2010. Net exchange of greenhouse gases from soils in an unimproved pasture and regenerating indigenous *Kunzea ericoides* shrubland in New Zealand. Soil Research **48**:385-394.

Price, S. J., R. R. Sherlock, F. M. Kelliher, T. M. McSeveny, K. R. Tate, and L. M. Condron. 2004. Pristine New Zealand forest soil is a strong methane sink. Global Change Biology **10**:16-26.

Prosser, J., C. Anderson, J. Horswell, and T. Speir. 2014. Can manuka (*Leptospermum scoparium*) antimicrobial properties be utilised in the remediation of pathogen contaminated land? Soil Biology and Biochemistry **75**:167-174.

Quinn, J. M., G. P. Burrell, and S. M. Parkyn. 2000. Influences of leaf toughness and nitrogen content on in-stream processing and nutrient uptake by litter in a Waikato, New Zealand, pasture stream and streamside channels. New Zealand Journal of Marine and Freshwater Research **34**:253-271.

Raese, J. T. 1997. Cold tolerance, yield, and fruit quality of 'd'Anjou'pears influenced by nitrogen fertilizer rates and time of application. Journal of Plant Nutrition **20**:1007-1025.

Randhawa, P. S. 2003. Influence of green manuring and phosphate rock inputs on soil phosphorus cycling and availability. Lincoln University, Lincoln, New Zealand.

Reay, S. D., and D. A. Norton. 1999. Assessing the success of restoration plantings in a temperate New Zealand forest. Restoration Ecology **7**:298-308.

Reeves, P., K. Collier, and A. Suren. 2004. Aquatic and riparian vegetation of rivers and streams. Pages 14.11-14.16 *in* J. Harding, P. Mosley, C. Pearson, and B. Sorrell, editors. Freshwaters of New Zealandy. Caxton Press, Christchurch, New Zealand. .

Reich, P. B., M. B. Walters, and D. S. Ellsworth. 1997. From tropics to tundra: Global convergence in plant functioning. Proceedings of the National Academy of Sciences **94**:13730-13734.

Richards, I. R., and K. M. Wolton. 1975. A note on urine scorch caused by gracing animals. Grass and Forage Science **30**:187-188.

Richardson, A. E., J.-M. Barea, A. M. McNeill, and C. Prigent-Combaret. 2009. Acquisition of phosphorus and nitrogen in the rhizosphere and plant growth promotion by microorganisms. Plant and Soil **321**:305-339.

Robinson, B. H., G. Banuelos, H. M. Conesa, M. W. H. Evangelou, and R. Schulin. 2009. The phytomanagement of trace elements in soil. Critical Reviews in Plant Sciences **28**:240-266.

Ross, D. J., N. A. Scott, S. M. Lambie, C. M. Trotter, N. J. Rodda, and J. A. Townsend. 2009. Nitrogen and carbon cycling in a New Zealand pumice soil under a manuka (*Leptospermum scoparium*) and kanuka (*Kunzea ericoide*s) shrubland. Soil Research **47**:725-736.

Rowe, L., M. Marden, and D. Rowan. 1999. Interception and throughfall in a regenerating stand of kanuka (*Kunzea ericoides* var. *ericoides*), East Coast region, North Island, New Zealand, and implications for soil conservation. Journal of Hydrology New Zealand **38**:29-48.

Roygard, J. K. F., B. E. Clothier, S. R. Green, and N. S. Bolan. 2001. Tree species for recovering nitrogen from dairy-farm effluent in New Zealand. Journal of Environmental Quality **30**:1064-1070.

Saarijärvi, K., and P. Virkajärvi. 2009. Nitrogen dynamics of cattle dung and urine patches on intensively managed boreal pasture. The Journal of Agricultural Science **147**:479-491.

Saetre, P., and E. Bååth. 2000. Spatial variation and patterns of soil microbial community structure in a mixed spruce–birch stand. Soil Biology and Biochemistry **32**:909-917.

Saggar, S., J. Luo, D. Giltrap, and M. Maddena. 2009. Nitrous oxide emissions from temperate grasslands: processes, measurements, modelling and mitigation. Pages 1-66 *in* Nitrous oxide emissions research progress. Nova Science Publishers, New York, USA.

Saggar, S., K. R. Tate, D. L. Giltrap, and J. Singh. 2008. Soil-atmosphere exchange of nitrous oxide and methane in New Zealand terrestrial ecosystems and their mitigation options: A review. Plant and Soil **309**:25-42.

Salisbury, F. B., and C. W. Ross. 1992. Plant Physiology. Fourth edition. Wadsworth, Belmont, USA.

Salmon, J. T. 1991. Native New Zealand flowering plants. Reed Books, Auckland, New Zealand.

Sandhu, H. S., S. D. Wratten, R. Cullen, and B. Case. 2008. The future of farming: The value of ecosystem services in conventional and organic arable land. An experimental approach. Ecological Economics **64**:835-848.

Scarsbrook, M. R., and J. Halliday. 1999. Transition from pasture to native forest land-use along stream continua: Effects on stream ecosystems and implications for restoration. New Zealand Journal of Marine and Freshwater Research **33**:293-310.

Schipper, L. A., W. T. Baisden, R. L. Parfitt, C. Ross, J. J. Claydon, and G. Arnold. 2007. Large losses of soil C and N from soil profiles under pasture in New Zealand during the past 20 years. Global Change Biology **13**:1138-1144.

Schipper, L. A., J. C. Williamson, H. A. Kettles, and T. W. Speir. 1996. Impact of land-applied tertiary-treated effluent on soil biochemical properties. Journal of Environmental Quality **25**:1073-1077.

Schultz, R. C., J. P. Collettil, T. M. Isenhart, W. W. Simpkins, C. W. Mize, and M. L. Thompson. 1995. Design and placement of a multi-species riparian buffer strip system. Agroforestry Systems **29**:201-226.

Schwendenmann, L., and N. D. Mitchell. 2014. Carbon accumulation by native trees and soils in an urban park, Auckland. New Zealand Journal of Ecology **38**:213-220.

Scott, N. A., J. D. White, J. A. Townsend, D. Whitehead, J. R. Leathwick, G. M. J. Hall, M. Marden, G. N. D. Rogers, A. J. Watson, and P. T. Whaley. 2000. Carbon and nitrogen distribution and accumulation in a New Zealand scrubland ecosystem. Canadian Journal of Forest Research **30**:1246-1255.

Sherlock, R. R. 1992. Nitrous oxide sources and sinks: A New Zealand perspective. Pages 48-60 *in* Proceedings of Global change: Impacts on agriculture and forestry, August, 1991. The Royal Society of New Zealand, Wellington, New Zealand.

Silva, R. G., K. C. Cameron, H. J. Di, and T. Hendry. 1999. A lysimeter study of the impact of cow urine, dairy shed effluent, and nitrogen fertilizer on drainage water quality. Australian Journal of Soil Research **37**:357-370.

Silva, R. G., K. C. Cameron, H. J. Di, N. P. Smith, and G. D. Buchan. 2000. Effect of macropore flow on the transport of surface-applied cow urine through a soil profile. Soil Research **38**:13-24.

Simpson, P. 2000. Dancing leaves: The story of New Zealand's cabbage tree, ti kouka. Canterbury University Press, Christchurch, New Zealand.

Sims, R. E. H., and D. Riddell-Black. 1998. Sustainable production of short rotation forest biomass crops using aqueous waste management systems. Biomass and Bioenergy **15**:75-81.

Singleton, G. 2007. Ellesmere: The JEWL In the Canterbury crown. Selwyn District Council, Leeston, New Zealand.

Sizmur, T., and M. E. Hodson. 2009. Do earthworms impact metal mobility and availability in soil? - A review. Environmental Pollution **157**:1981-1989.

Smith, C. M., R. J. Wilcock, W. N. Vant, D. G. Smith, and A. B. Cooper. 1993. Towards sustainable agriculture: freshwater quality in New Zealand and the influence of agriculture. National Institute of Water and Atmospheric Research, Wellington, New Zealand.

Smith, V. H. 2003. Eutrophication of freshwater and coastal marine ecosystems a global problem. Environmental Science and Pollution Research **10**:126-139.

Smith, V. H., and D. W. Schindler. 2009. Eutrophication science: where do we go from here? Trends in Ecology and Evolution **24**:201-207.

Soil Survey Staff. 2014. Keys to Soil Taxonomy. USDA-Natural Resources Conservation Service, Washington, USA.

Stephens, J. M., R. C. Schlothauer, B. D. Morris, D. Yang, L. Fearnley, D. R. Greenwood, and K. M. Loomes. 2010. Phenolic compounds and methylglyoxal in some New Zealand manuka and kanuka honeys. Food Chemistry **120**:78-86.

Stephens, J. M. C., P. C. Molan, and B. D. Clarkson. 2005. A review of *Leptospermum scoparium* (Myrtaceae) in New Zealand. New Zealand Journal of Botany **43**:431-449.

Stevenson, B. A., and M. C. Smale. 2005. Seed bed treatment effects on vegetation and seedling establishment in a New Zealand pasture one year after seeding with native woody species. Ecological Management and Restoration **6**:124-131.

Storey, R. G., and D. R. Cowley. 1997. Recovery of three New Zealand rural streams as they pass through native forest remnants. Hydrobiologia **353**:63-76.

Stouthamer, A. H. 1988. Dissimilatory reduction of oxidized nitrogen compounds. Pages 245-303 *in* A. J. B. Zehnder, editor. Biology of anaerobic microorganisms. John Wiley and Sons, New York, USA.

Subbarao, G. V., O. Ito, K. L. Sahrawat, W. L. Berry, K. Nakahara, T. Ishikawa, T. Watanabe, K. Suenaga, M. Rondon, and I. M. Rao. 2006. Scope and strategies for regulation of nitrification in agricultural systems—challenges and opportunities. Critical Reviews in Plant Sciences **25**:303-335.

Subbarao, G. V., K. L. Sahrawat, K. Nakahara, T. Ishikawa, M. Kishii, I. M. Rao, C. T. Hash, T. S. George, P. Srinivasa Rao, and P. Nardi. 2012a. Biological nitrification inhibition—A novel strategy to regulate nitrification in agricultural systems. Advances in Agronomy **114**:-302249.

Subbarao, G. V., K. L. Sahrawat, K. Nakahara, I. M. Rao, M. Ishitani, C. T. Hash, M. Kishii, D. G. Bonnett, W. L. Berry, and J. C. Lata. 2012b. A paradigm shift towards low-nitrifying production systems: the role of biological nitrification inhibition (BNI). Annals of botany **112**:297-316.

Sullivan, J. J., C. Meurk, K. J. Whaley, and R. Simcock. 2009. Restoring native ecosystems in urban Auckland: Urban soils, isolation, and weeds as impediments to forest establishment. New Zealand Journal of Ecology **33**:60-71.

Sun, X., N. Luo, B. Longhurst, and J. Luo. 2008. Fertiliser nitrogen and factors affecting pasture responses. The Open Agricultural Journal **2**:35-42.

Sundqvist, M. K., D. A. Wardle, E. Olofsson, R. Giesler, and M. J. Gundale. 2012. Chemical properties of plant litter in response to elevation: Subarctic vegetation challenges phenolic allocation theories. Functional Ecology **26**:1090-1099.

Sutton, A. J. 2006. Evaluation of agricultural nutrient reductions in restored riparian buffers. University of Maryland Maryland, USA.

Sweeney, B. W., T. L. Bott, J. K. Jackson, L. A. Kaplan, J. D. Newbold, L. J. Standley, W. C. Hession, and R. J. Horwitz. 2004. Riparian deforestation, stream narrowing, and loss of stream ecosystem services. Proceedings of the National Academy of Sciences of the United States of America **101**:14132-14137.

Sylvester-Bradley, R., D. Mosquera, and J. Mendez. 1988. Inhibition of nitrate accumulation in tropical grassland soils: Effect of nitrogen fertilization and soil disturbance. Journal of Soil Science **39**:407-416.

Taghizadeh-Toosi, A., T. J. Clough, L. M. Condron, R. R. Sherlock, C. R. Anderson, and R. A. Craigie. 2011. Biochar incorporation into pasture soil suppresses in situ nitrous oxide emissions from ruminant urine patches. Journal of Environmental Quality **40**:468-476.

Tanner, C. C. 1996. Plants for constructed wetland treatment system - a comparison of the growth and nutrient uptake of eight emergent species. Ecological Engineering **7**:59-83.

Taylor, B. R., D. Parkinson, and W. F. J. Parsons. 1989. Nitrogen and lignin content as predictors of litter decay rates: A microcosm test. Ecology **70**:97-104.

Thompson, S., I. Grüner, and N. Gapare. 2003. New Zealand land cover database version 2. Ministry for the Environment, Wellington, New Zealand.

Tomer, M. D., T. H. Charleson, C. T. Smith, L. Barton, A. J. Thorn, and G. J. Gielen. 2000. Evaluation of treatment performance and processes after five years of wastewater application at Whakarewarewa Forest, New Zealand. Pages 155-162 *in* C. Henry, R. Harrison, and B. Bastian, editors. The forest alternative: Principles and practices of residual use. College of Forest Recourse, University of Washington, Seattle, USA.

Tomlinson, P. B., and J. B. Fisher. 1971. Morphological studies in *Cordyline* (Agavaceae): I. Introduction and general morphology. Journal of the Arnold Arboretum **52**:459-478.

Tripler, C., C. Canham, R. Inouye, and J. Schnurr. 2002. Soil nitrogen availability, plant luxury consumption, and herbivory by white-tailed deer. Oecologia **133**:517-524.

Trotter, C., K. Tate, N. Scott, J. Townsend, H. Wilde, S. Lambie, M. Marden, and T. Pinkney. 2005. Afforestation/reforestation of New Zealand marginal pasture lands by indigenous shrublands: The potential for Kyoto forest sinks. Annals of Forest Science **62**:865-871.

Tsitsilas, A., S. Stuckey, A. Hoffmann, A. Weeks, and L. Thomson. 2006. Shelterbelts in agricultural landscapes suppress invertebrate pests. Animal Production Science **46**:1379-1388.

Tufekcioglu, A., J. W. Raich, T. M. Isenhart, and R. C. Schultz. 1998. Fine root dynamics, coarse root biomass, root distribution, and soil respiration in a multispecies riparian buffer in Central Iowa, USA. Agroforestry Systems **44**:163-174.

Tufekcioglu, A., J. W. Raich, T. M. Isenhart, and R. C. Schultz. 2003. Biomass, carbon and nitrogen dynamics of multi-species riparian buffers within an agricultural watershed in Iowa, USA. Agroforestry Systems **57**:187-198.

Tzanakakis, V. A., N. V. Paranychianakis, and A. N. Angelakis. 2009. Nutrient removal and biomass production in land treatment systems receiving domestic effluent. Ecological Engineering **35**:1485-1492.

Uchida, Y. 2010. The effects of substrate, temperature and soil fertility on respiration and N_2O production in pastoral soils. Lincoln University, Lincoln, New Zealand.

Van Appledorn, M. 2009. Watershed-scale analysis of riparian buffer function. Utah State University, Logan, USA.

van der Krift, T. A. J., and F. Berendse. 2001. The effect of plant species on soil nitrogen mineralization. Journal of Ecology **89**:555-561.

Van der Weerden, T. J., R. R. Sherlock, P. H. Williams, and K. C. Cameron. 1999. Nitrous oxide emissions and methane oxidation by soil following cultivation of two different leguminous pastures. Biology and Fertility of Soils **30**:52-60.

van Epenhuijsen, K. C. W., R. C. Henderson, A. Carpenter, and G. K. Burge. 2000. The rise and fall of manuka blight scale: A review of the distribution of *Eriococcus orariensis* (Hemiptera: Eriococcidae) in New Zealand. New Zealand Entomologist **23**:67-70.

van Haren, J. L. M., R. C. de Oliveira, N. Restrepo-Coupe, L. Hutyra, P. B. de Camargo, M. Keller, and S. R. Saleska. 2010. Do plant species influence soil CO_2 and N_2O fluxes in a diverse tropical forest? Journal of Geophysical Research **115**:G03010.

Van Veen, J. A., R. Merckx, and S. C. Van de Geijn. 1989. Plant-and soil related controls of the flow of carbon from roots through the soil microbial biomass. Plant and Soil **115**:179-188.

van Zwieten, L., B. Singh, S. Joseph, S. Kimber, A. L. Cowie, and K. Y. Chan. 2009. Biochar and emissions of non-CO_2 greenhouse gases from soil. Pages 227-249 *in* J. Lehmann and S. Joseph, editors. Biochar for environmental management: Science and technology. Earthscan, London, UK.

Vangronsveld, J., R. Herzig, N. Weyens, J. Boulet, K. Adriaensen, A. Ruttens, T. Thewys, A. Vassilev, E. Meers, and E. Nehnevajova. 2009. Phytoremediation of contaminated soils and groundwater: Lessons from the field. Environmental Science and Pollution Research **16**:765-794.

Vant, W. N. 2001. New challenges for the management of plant nutrients and pathogens in the Waikato River, New Zealand. Water science and technology **43**:137-144.

Vitousek, P. M., J. D. Aber, R. W. Howarth, G. E. Likens, P. A. Matson, D. W. Schindler, W. H. Schlesinger, and D. G. Tilman. 1997. Human alteration of the global nitrogen cycle: Sources and consequences. Ecological Applications **7**:737-750.

Vitousek, P. M., and R. W. Howarth. 1991. Nitrogen limitation on land and in the sea: How can it occur? Biogeochemistry **13**:87-115.

Vitousek, P. M., and P. A. Matson. 1985. Disturbance, nitrogen availability, and nitrogen losses in an intensively managed loblolly pine plantation. Ecology **66**:1360-1376.

Walker, S., R. Price, D. Rutledge, R. T. T. Stephens, and W. G. Lee. 2006. Recent loss of indigenous cover in New Zealand. New Zealand Journal of Ecology **30**:169-177.

Ward, B. B., K. J. Courtney, and J. H. Langenheim. 1997. Inhibition of *Nitrosomonas europaea* by monoterpenes from coastal redwood (*Sequoia sempervirens*) in whole-cell studies. Journal of Chemical Ecology **23**:2583-2598.

Wardle, D. A., R. D. Bardgett, J. N. Klironomos, H. Setälä, W. H. van der Putten, and D. H. Wall. 2004. Ecological linkages between aboveground and belowground biota. Science **304**:1629-1633.

Wardle, D. A., R. D. Bardgett, L. R. Walker, and K. I. Bonner. 2009. Among-and within-species variation in plant litter decomposition in contrasting long-term chronosequences. Functional Ecology **23**:442-453.

Wardle, D. A., and L. G. Greenfield. 1991. Release of mineral nitrogen from plant root nodules. Soil Biology and Biochemistry **23**:827-832.

Wardle, J. 2011. Wardle's Native Trees of New Zealand and their story. New Zealand Farm Forestry Association, Wellington, New Zealand.

Wardle, P. 1985. Environmental influences on the vegetation of New Zealand. New Zealand Journal of Botany **23**:773-788.

Wardle, P. 2002. Vegetation of New Zealand. The Blackburn Press, Cadwell, USA.

Watanabe, F. S., and S. R. Olsen. 1965. Test of an ascorbic acid method for determining phosphorus in water and $NaHCO_3$ extracts from soil. Soil Science Society of America Journal **29**:677-678.

Watkinson, J. H., and K. W. Perrott. 1990. A new soil test for sulphate and mineralisable organic sulphur. Pages 189-198 *in* Proceedings of the New Zealand Feriliser Manufacturers' Research Association Conference, Auckland, New Zealand.

Watson, A., M. Marden, and D. Rowan. 1995. Tree species performance and slope stability. Pages 161-171 *in* D. H. Barker, editor. Vegetation and slopes. Thomas Telford, London, UK.

Watson, A., and C. O'Loughlin. 1985. Morphology, strength, and biomass of manuka roots and their influence on slope stability. New Zealand Journal of Forestry Science **15**:337-348.

Watson, A. J., and M. Marden. 2004. Live root-wood tensile strengths of some common New Zealand indigenous and plantation tree species. New Zealand Journal of Forestry Science **34**:344-353.

Webb, J., R. Sylvester-Bradley, and F. Seeney. 1997. The effects of site and season on the fate of nitrogen residues from root crops grown on sandy soils. The Journal of Agricultural Science **128**:445-460.

Webb, T. H. 2008. Soils. Pages 89-118 *in* M. Winterbourn, G. Knox, C. Burrows, and I. Marsden, editors. The natural history of Canterbury Canterbury University Press, Christchurch, New Zealand.

Wehi, P. M., and B. D. Clarkson. 2007. Biological flora of New Zealand 10. *Phormium tenax*, harakeke, New Zealand flax. New Zealand Journal of Botany **45**:521-544.

Weller, D. E., D. Correll, and T. E. Jordan. 1994. Denitrification in riparian forests receiving agricultural discharges. Pages 117-132 *in* W. J. Mitsch, editor. Global Wetlands; old world and new. Elviser, Amsterdam, Netherlands.

Weston, R. J., L. K. Brocklebank, and Y. R. Lu. 2000. Identification and quantitative levels of antibacterial components of some New Zealand honeys. Food Chemistry **70**:427-435.

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

Williams, K. 2005. Native plant communities of the Canterbury Plains. Department of Conservation, Christchurch, New Zealand.

Williams, N. D., and E. L. Petticrew. 2009. Aggregate stability in organically and conventionally farmed soils. Soil Use and Management **25**:284-292.

World Health Organisation. 2007. Nitrate and nitrite in drinking-water, back-ground document for development of WHO Guidelines for drinking water quality. World Health Organization, Geneva, Switzerland.

Wright, I. J., P. B. Reich, M. Westoby, D. D. Ackerly, Z. Baruch, F. Bongers, J. Cavender-Bares, T. Chapin, J. H. Cornelissen, and M. Diemer. 2004. The worldwide leaf economics spectrum. Nature **428**:821-827.

Zakir, H. A. K. M., G. V. Subbarao, S. J. Pearse, S. Gopalakrishnan, O. Ito, T. Ishikawa, N. Kawano, K. Nakahara, T. Yoshihashi, H. Ono, and M. Yoshida. 2008. Detection, isolation and characterization of a root-exuded compound, methyl 3-(4-hydroxyphenyl) propionate, responsible for biological nitrification inhibition by sorghum (*Sorghum bicolor*). New Phytologist **180**:442-451.

Zaman, M., S. Saggar, J. D. Blennerhassett, and J. Singh. 2009. Effect of urease and nitrification inhibitors on N transformation, gaseous emissions of ammonia and nitrous oxide, pasture yield and N uptake in grazed pasture system. Soil Biology and Biochemistry **41**:1270-1280.

Zaman, M. D. H. J., H. J. Di, K. C. Cameron, and C. M. Frampton. 1999. Gross nitrogen mineralization and nitrification rates and their relationships to enzyme activities and the soil microbial biomass in soils treated with dairy shed effluent and ammonium fertilizer at different water potentials. Biology and Fertility of Soils **29**:178-186.

Zatylny, A. M., and R. G. St-Pierre. 2006. Nitrogen uptake, leaf nitrogen concentration, and growth of saskatoons in response to soil nitrogen fertility. Journal of Plant Nutrition **29**:209-218.

Zhang, S., and H. L. Allen. 1996. Foliar nutrient dynamics of 11-year-old loblolly pine (*Pinus taeda*) following nitrogen fertilization. Canadian Journal of Forest Research **26**:1426-1439.