Investigations of soil extractable aluminium and toxicity in New Zealand soils

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by

Amy Elizabeth Whitley

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Te Whare Wānaka o Aoraki

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Chapter 4 - a manuscript from this section has been published as follows:

Whitley, A.E., Moir, J.L., Almond, P.C., Moot, D.J., Giona Bucci, M., Nelson, J. (2018). A field survey of soil pH and extractable aluminium in the Ashburton Lakes Catchment, Canterbury, New Zealand. *Journal of New Zealand Grasslands.* 80. (pg *149-154*).

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- Whitley. A.E., Moir, J. L., Almond, P. C., & Moot, D. J. (2016). Soil pH and exchangeable aluminium in contrasting New Zealand high and hill country soils. In: Hill country symposium. April 12th - 13th, 2016, Rotorua, New Zealand: (*Grassland Research and Practise series 16: 169-172*).

The Hill country symposium paper presented data from Chapter 4 and Chapter 5. Three of the 21 soil profiles from the Ashburton Lakes catchment were included in this paper and the profile pit samples from the 10 sites where the soils for the glasshouse experiment were collected (actual data not presented in Chapter 4). Data included pH_{H2O} and 0.02 M $CaCl_2$ extractable Al for each sample (10 cm vertical increments).

Chapter 4 - a manuscript from this section has been published as follows:

- Whitley, A.E., Moir, J.L., Moot, D.J. (2015). A field survey of aluminium toxicity in New Zealand upland soils varying in parent material and climate. *In:* Proceedings of the 9th International Symposium on Plant-Soil Interactions at Low pH. October 18th – 23rd, 2015, Dubrovnik, Croatia: (pg *128-129*).

The two page manuscript for the Proceedings of the 9th International Symposium on Plant-Soil Interactions at Low pH involved data from Chapter 4. The samples included bulk soil (0-15 cm), cores (0-7.5 cm and 7.5-15 cm) and soil profile samples from the 10 sites where the soil was collected for the glasshouse experiment. Data included pH_{H2O} and 0.02 M $CaCl_2$ extractable Al for each sample.

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

Investigations of soil extractable aluminium and toxicity in New Zealand soils

by

Amy Elizabeth Whitley

Soil acidity and associated soil aluminium (Al) toxicity severely restrict the establishment, yield and persistence of legumes in New Zealand high and hill country pastures. There is an urgent need to identify soils which are most susceptible to Al toxicity and to determine what factors are involved in regulating soil extractable Al concentrations. This study investigated the relationship between soil chemical, physical and environmental variables and soil extractable Al for New Zealand soils in national, catchment and rhizosphere contexts.

The relationship between soil chemical, physical and environment variables and soil extractable Al_{KCl} were investigated using the National Soils Database (NSD). Base saturation (BS), soil pH_{H2O}, cation exchange capacity (CEC), total nitrogen, total carbon and soil order were strongly associated with Al_{KCl} concentrations and relationships differed among the depth zones (0-20 cm, 20-50 cm and 50-120 cm). Soil acidity and high CEC contributed to high Al_{KCl} concentrations, whereas a high BS and total C had a negative effect. Total N decreased with increasing Al_{KCl} in the top 20 cm, likely as a response to Al toxicity induced limitations on biological N fixation by pasture legumes. An Al_{KCl} concentration > 1.0 $cmol_c/kg$, which can be toxic to sensitive plants, occurred across a pH_{H2O} range of 3.8-6.4. Brown Soils and Podzols had the highest mean Al_{KCl} concentrations across all depths and are likely to be more susceptible to Al toxicity.

A soil survey in the Ashburton Lakes catchment was conducted to determine which soils are most susceptible to Al toxicity and to identify which factors drive soil pH_{H2O} and extractable Al_{CaCl2} at a landscape scale. Depth in the soil profile was the strongest explanatory variable for pH_{H2O} and Al_{CaCl2}. Soil pH_{H2O} increased with depth and Al_{CaCl2} declined. Rainfall and age were significant factors for Al_{CaCl2}, however, there were no systematic patterns of an increase in Al_{CaCl2} with increasing rainfall and soil age. Differences in pH_{H2O} among landform types were found, in contrast to no difference in Al_{CaCl2} concentrations. The soil pH_{H2O} ranged from 4.7-6.0 and Al_{CaCl2} concentrations from 1.2 mg kg^{-1} to 39.1 mg kg^{-1}, with a mean of 7.8 mg kg^{-1}. Maps of soil pH_{H2O} and Al_{CaCl2} in the 20 cm depth zone were

constructed using the rules established by decision trees. Distinct areas in the landscape were identified which had higher concentrations of Al_{CaCl2}. Higher Al_{CaCl2} concentrations were found at the wettest sites in the catchment (≥1266 mm), areas that seem to mirror those that were identified as most acidic in the soil pH_{H2O} map.

The growth response and nutrient uptake of legumes (*Medicago sativa L.* and *Trifolium ambiguum L.*) as bioindicators of Al toxicity were assessed in a range of acidic soils in a glasshouse experiment. Soil extractable Al_{CaCl2} concentration was strongly associated with lucerne shoot yields. Lucerne shoot yield increased one to six fold with lime application, particularly between the L0P0 and 2 t lime ha^{-1} treatments. Yield increases were strongly associated with declines in the soil extractable Al_{CaCl2} concentration to below toxic levels (≤2.5 mg kg^{-1}) and clearly demonstrated the severe plant growth restriction of Al toxicity in these high country soils. On most soils lucerne shoot yields responded more to lime than P applied. In contrast, Caucasian clover (CC) shoot yields were not affected by soil extractable Al concentration, with more consistent yields across the range of pH_{H2O} (5.0-7.5) and the P rates applied than lucerne. This study clearly highlights the potential importance of CC use in the high country, where the growth of more sensitive species such as lucerne is restricted by Al toxicity.

The plant effect on Al mobilisation and immobilisation at the root-soil interface and the effects of pH_{H2O} were investigated in a rhizobox experiment with legumes (*Lupinus polyphyllus* L. and *Medicago sativa* L.) in an acidic high country soil. The pH_{H2O} was more acidic (0.1- 0.3 pH units lower) and Al_{Cacl2} concentrations were higher (0.5 mg kg^{-1} and 5.4 mg kg^{-1}) in the rhizosphere of lupin plants compared to the bulk soil. Lucerne plants had a similar soil pH_{H2O} between the bulk and rhizosphere. Hot water extractable organic carbon levels appeared to be consistently higher in the rhizosphere and seemed to increase at the highest lime application. DGT data showed increased mobilisation of Al at the root tip of lupin and depletion along the root axis, indicative of previous removal of Al by the plant from the soil. This is the first study that has shown, in high resolution using DGT and LA-ICP-MS analysis, distinct patterns of soil Al mobilisation induced by the roots of important pasture species.

A laboratory investigation was conducted to determine if changing the molarity and extraction time of the standard $CaCl_2$ and KCl soil Al tests altered the Al concentrations extracted. Overall, the Al concentration extracted by the KCl standard test was 16 times higher than the $CaCl_2$ across all soils. The effect of molarity and extraction time on the Al concentrations extracted differed among the five soils tested for the two extraction methods. For the $CaCl_2$ test, the extractable Al concentration increased (P<0.001) with an increase in the molarity (by 8.7-17.7 mg kg^{-1}) of $CaCl_2$ for most soils. Only

the Allophanic Soil measured a difference (P<0.001) in extractable Al_{CaCl2} with an increase in extraction time (decrease in Al_{CaCl2}). The interaction of molarity and extraction time only extracted different (P<0.05) concentrations of Al on two soils for the $CaCl_2$ extraction. For the KCl extraction, on most soils the Al extracted increased (P<0.001) with an increase in molarity to 1 M (by 0.5-1.0 $cmol_c$/kg), with no increase (P>0.05) with a further increase in molarity. For two of the soils, the Al extracted was significantly affected by the extraction time, however, the results were contrasting. On most soils the interaction of molarity and extraction time extracted significantly different concentrations of Al_{KCl}. These findings suggest that the Al concentrations measured by the two extraction methods are affected by specific soil properties in the topsoil related to soil order.

This research has identified key variables driving soil extractable Al concentrations in a suite of New Zealand soils, and at different scales. Legume species responded differently to soil extractable Al_{CaCl2} and influenced pH and the amount of soil Al in the rhizosphere. Soil properties of different soil orders were also found to affect the amount of Al extracted from the soil by the two $CaCl_2$ and KCl extraction methods. The knowledge generated from this thesis has identified specific sets of conditions (environmental, soil chemical and soil order) that have higher concentrations of extractable Al and therefore areas most likely to be susceptible to Al toxicity in New Zealand.

Key words: acidic soil, National Soils Database, Ashburton Lakes, lucerne, Caucasian clover, Russell lupin, *Medicago sativa L.*, *Trifolium ambiguum L.*, *Lupinus polyphyllus L.*, yield response, lime, phosphorus, soil fertility, Diffusive gradient in thin films (DGT), LA-ICP-MS, rhizobox, rhizosphere, KCl soil test and $CaCl_2$ soil test.

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List of Acronyms and Abbreviations

AAS	Atomic Absorption Spectroscopy
AIC	Akaike Information Co-efficient value
ANOVA	Analysis of variance
Al	Aluminium
Al_{CaCl2}	Soil aluminium extracted by $CaCl_2$
Al_{KCl}	Soil aluminium extracted by KCl
Al_o	Soil aluminium extracted by oxalate
BS	Base Saturation
C	Carbon
CC	Caucasian clover
CEC	Cation exchange capacity
$CaCl_2$	Calcium chloride
DEM	Digital elevation model
DGT	Diffusive gradient in thin-films
DM	Dry matter
DOC	Dissolved organic carbon
DoC	Department of Conservation
HWEC	Hot water extractable carbon
ICP-OES	Inductively coupled plasma optical emission spectrometry
K	Potassium
KCl	Potassium chloride
kyr	1000 years
LGM	Last glacial maximum
LA-ICP-MS	Laser ablation inductively coupled plasma mass spectrometry
LSD	Least significant difference
MAR	Median annual rainfall
m.a.s.l	Metres above sea level
N	Nitrogen
NIWA	National Institute of Water and Atmospheric Research
NSD	National Soils Database
NZ	New Zealand
NZSC	New Zealand soil classification
OM	Organic matter
P	Phosphorus
S	Sulphur
SEM	Standard error of the mean
SSP	Single super phosphate
USDA	United States Department of Agriculture

1 Introduction

1.1 Background

The high and hill country of New Zealand is a challenging environment to farm because of substantial limitations and variability in climate, soils, topography, and low soil fertility. Farmers have had to utilise hill and lower altitude high country areas for sheep and beef as a result of recent conversions of lowland areas from sheep and beef finishing to dairying and dairy grazing (Moot *et al.*, 2009). This has been compounded by land use intensification resulting from a loss of land due to the tenure review process (Brower, 2016; Ministry of Agriculture and Forestry, 2018). These higher altitude areas are often dryland (seasonal moisture deficits, temperature extremes and a short growing season), low in fertility (N, P, S) and acidic (Langer, 1990; Moir & Moot, 2010; Scott *et al.*, 1985). On an estimated 500, 000 ha of farmed high country, soils are acidic, increasing the potential for Al toxicity (Moir & Moot, 2010). Soil acidity, coupled with high extractable soil Al and low availability of essential nutrients, can limit the establishment and persistence of pasture legumes in this environment (Haynes & Williams, 1993; Moir *et al.*, 2000). This is important because legumes play a critical role in providing N via biological N fixation, which drives productivity, and are a high quality forage for grazing stock (Caradus *et al.*, 1996; Haynes & Williams, 1993; Hofmann *et al.*, 2007). Legumes vary in their sensitivity to acidity and Al (Moir *et al.*, 2016). However, soil Al toxicity reduces legume yields through the restriction of root growth and nutrient uptake, which affects the farming system as a whole (Barcelo & Poschenrieder, 2002; Rout *et al.*, 2001; Scott *et al.*, 2008). To maximize economic returns, farmers need to increase productivity on a reduced land area, intensify marginal (less productive) land and manage the variation. However, traditional pasture species, such as white clover (*Trifolium repens* L.) and lucerne (*Medicago sativa* L.*)*, are sensitive to acid soils (Scott *et al.*, 2008; Su & Evans, 1996) and research has been conducted to look at alternate legume species, particularly at Lincoln University (Keenan, 2014; Moir *et al.*, 2016; Schwass, 2013; Whitley, 2013). Lime is a common amendment applied to mitigate acidity and decrease soil extractable Al concentrations. In many cases, however, the lime application is uneconomic in the high and hill country because of the high cost of aerial topdressing over large areas. Where liming is not practicable, soils can become more acidic and the productivity declines (Edmeades *et al.*, 1983).

Large areas of the high and hill country have acidic soils, which can increase the extractable Al concentrations and therefore exacerbate the potential for Al toxicity. However, the extent of the issue in New Zealand is unclear. In order to mitigate Al toxicity, there is a need to understand which New Zealand soils and areas may be more susceptible to high concentrations of extractable Al that could

be toxic to plants. In addition, it is important to determine what factors, other than soil pH, are involved in the spatial variability of soil extractable Al in New Zealand soils, both at a national and catchment scale. If the depth at which high Al concentrations occur in the soil profile can be isolated, land previously unable to grow lucerne because of Al may be remediated. The extractable Al may occur in layers, rather than through the whole profile, therefore this could enable solutions to be developed across large areas.

Legumes are critical for the productivity of high and hill country and it is important to determine if there are measurable growth responses to reducing extractable Al concentrations. Further, to determine if such responses vary among soil orders and the reason for this. Plants also influence their immediate environment, the rhizosphere. It would be useful to examine the influence that different plant species have on soil Al concentrations in proximity to the legume roots in a New Zealand soil, as this is the primary site for Al toxicity. The diagnostic test used by farmers to determine if there is a potential soil Al issue on their farms is the standard soil 0.02 M $CaCl_2$ extraction, conducted by commercial laboratories in New Zealand. The 1 M KCl test has been used in the past. It is important to determine if the Al extracted from different New Zealand soil orders is affected by changes in the test methodology and whether this differs due to soil properties specific to those soils. The findings of this research programme will also provide new knowledge on soil extractable Al and potential for toxicity in New Zealand soils, which will be valuble to both scientists and farmers.

1.2 Aims, Objectives and thesis structure:

To address gaps in the knowledge five objectives were developed with the overarching aim to improve the understanding of soil extractable Al and to investigate and determine the key factors that drive the variation in soil extractable Al in New Zealand high and hill country soils. Soil extractable Al was studied in a number of experiments at different scales (national, catchment, glasshouse, laboratory and rhizosphere) to achieve the research aim. This thesis is structured into nine Chapters and a flow diagram of the thesis structure is shown in Figure 1.1. Following the general introduction chapter, a comprehensive literature review is presented in Chapter 2. The subsequent research was divided into five experimental chapters, each with specific objectives that will be addressed separately in Chapters 3 (Objective 1), 4 (Objective 2), 5 (Objective 3), 6 (Objective 4) and 7 (Objective 5). Finally, the key results of this thesis and implications are discussed in Chapter 8, with the conclusions and suggested future research presented in Chapter 9.

The objectives for this thesis are:

Objective 1:

To determine, using a large database of New Zealand soils: i) if the same variables identified in the literature, or others, are important in influencing exchangeable Al; and ii) if soil orders of the New Zealand Soil Classification are effective in partitioning variability in exchangeable Al.

Objective 2:

To determine the key factors driving variation in soil extractable Al_{CaCl2} concentrations on different landforms of similar parent material in a landform context.

Objective 3:

To compare and contrast key New Zealand soils with known acidity and soil extractable Al_{CaCl2} issues using plants (legumes) as bioindicators.

Objective 4:

To examine the effect of pH_{H2O} on the extractable Al concentrations at the rhizosphere scale.

Objective 5:

To determine if changing the molarity and extraction time of the standard $CaCl_2$ and KCl soil Al tests alters the Al concentrations extracted.

Figure 1.1 Flow diagram of thesis structure.

2 Literature review

2.1 The high and hill country of New Zealand

The high country covers around 25% of New Zealand's land mass and an estimated 40% of the high country is utilised for pastoral farming (Beer *et al.*, 2006; Wright, 2009). The South Island high country covers around 6 million hectares which extends from the main divide, east to the coastal plains and almost to the coasts near Marlborough, South Canterbury and Otago (Wright, 2009). There are an estimated 3.4 million hectares of developed pastures in the South Island High Country of New Zealand (Scott *et al.*, 1995). Around 10 million ha of New Zealand's land area (37%) is classified as hill country and 63% is located in the North Island. Hill country is defined as land with slopes greater than 15° and below 1000 m a.s.l. (Kerr, 2016). An estimated 5 million hectares (18%) has been designated as pastoral hill country farm land, 80% of the 6000 farms are located in the North Island and they are generally much smaller compared with South Island hill country farms (Kerr, 2016). The expansion of dairy farming and urbanisation has led to the intensification of high and hill country areas and their utilisation as both a breeding platform and for finishing stock for the red meat sector (Kerr, 2016; Moot, 2012; Moot *et al.*, 2010). This is important as the red meat sector provides nearly $8 billion per year in export earnings for New Zealand (Kerr, 2016). Sheep and beef farming systems are often on less productive high and hill country land and soils of lower fertility status. To remain a viable business farmers must be able to produce economic returns on a smaller land area and cost effectively intensify less productive land.

2.1.1 Soils

There is a large diversity in soils in New Zealand, which is demonstrated by the 15 soil orders shown in Table 2.1 (Hewitt, 2013). The dominant soil forming factors which influence soil character and development are parent material, time, climate, organisms (vegetation) and topography/relief.

Much of the high and hill country landscape is dominated by sedimentary soils, and in particular Brown Soils in both the North and South Islands, and also on many river terraces in the Southland region (Hewitt, 1998). Brown Soils are the most dominant soils in New Zealand, and cover 43% of the total land area. These soils extend over the mountainous and hilly backbone of New Zealand, and in areas rainfall of >800 mm yr^{-1} (South island) or >1000 mm yr^{-1} (North island) with free drainage (Hewitt, 2013).

Table 2.1 New Zealand soil orders using the New Zealand Soil Classification (NZSC) and their important characteristics and the equivalent soils in United States Department of Agriculture classification (USDA). From Hewitt (2013), with permission.

Age	Other factors	NZSC	USDA
Young soils		Raw Soils Recent Soils Anthropic Soils	Entisols or not soil Entisols, Inceptisols or Andisols Entisols
Mature soils *Soils that have well developed topsoil and subsoil horizons*	**Climate** *Soils formed in quartz rich materials that show the effects of climate*	Semiarid Soils Pallic Soils Brown Soils Podzols	Aridisols Inceptisols or Alfisols Inceptisols Spodosols
	Wetness *Soils with prolonged high water tables*	Gley Soils Organic Soils	A range of soil orders with aquic sub orders Histosols
	Rock *Soil parent materials are formed from rocks that dominate soil character e.g. limestone, basalt, pumice and volcanic ash*	Melanic soils Pumice Soils Allophanic Soils	Mollisols or Vertisols Andisols Andisols
Old soils *On land surfaces with parent materials that have attributes of advanced weathering*		Ultic Soils Granular Soils Oxidic Soils	Ultisols Ultisols or Alfisols Oxisols

Pallic Soils are the second most common soils in New Zealand (12% of total land area)(Hewitt, 2013). These soils are located in moderate rainfall climates such as Manawatu and South Otago, on either poorly drained terraces or rolling landscapes. Alternatively, Pallic Soils can also be found in low rainfall environments in areas of the Hawkes Bay, Wairarapa, Manawatu and Canterbury, on free draining terraces or rolling landscapes (Hewitt, 1998; Roberts & White, 2016).

There are several areas of New Zealand, where the soils are derived from volcanic activity, overlain by sedimentary rock and these are predominantly Allophanic (Hewitt, 1998). Allophanic Soils occupy around 6% of total land area (Hewitt, 2013) and are found in Taranaki, Waikato and parts of Northland. The Central Plateau is dominated by Pumice Soils, which are derived from rhyolitic volcanic material and represent 7% of New Zealand's total land area (Hewitt, 1998 and 2013). There are many other soil

orders which represent a much smaller proportion of soils in New Zealand which include, Podzols (12%), Recent (6%), Gley (3%), Ultic (3%), Semi-arid (1%) and Organic (1%)(Hewitt, 2013). More detailed descriptions of the physical and chemical properties of the New Zealand soil orders are given in Hewitt (1998) and Hewitt (2013). Each area of New Zealand experiences variation in climate, topography and soil type which present both challenges and opportunities for farming (Roberts & White, 2016). Limitations also include the slope and aspect, which can cause variation in moisture and increase nutrient transfer and loss (Roberts & White, 2016).

2.1.2 Importance of legumes

Many farmed areas of the high and hill country have acidic and low fertility soils (N,P,K,S) which present substantial challenges in establishing legume species that will grow and persist (Langer, 1990; Moir & Moot, 2010). Legumes are critical for high and hill country farming systems, as they provide nitrogen via biological N_2 fixation, which is a key driver of overall pasture production (Caradus *et al.*, 1996; Haynes & Williams, 1993). In addition, legumes are high quality feed and so are important for the live-weight gain of grazing animals (Caradus *et al.*, 1996; Hofmann *et al.*, 2007). Legume roots also enhance soil conservation by improving soil structure and reducing the rate of erosion e.g. *Lupinus polyphyllus* in loose textured soils (Rowland *et al.*, 1986). Traditional legume species used for pastures such as lucerne (*Medicago sativa* L.*)* and white clover (*Trifolium repens* L.) are sensitive to acidic soils (Scott *et al.*, 2008; Su & Evans, 1996). Furthermore, soil Al toxicity occurs on many New Zealand high and hill country farms and is a key factor limiting the growth and persistence of legumes for production (Moir & Moot, 2010). Thus, acid and Al tolerant legume species are critical for grassland ecosystem survival and economically viable farm systems (Moir & Moot, 2010). As such, legumes are critical to the economic viability of sheep and beef production in New Zealand.

2.1.3 Challenges of farming

There is substantial variation of site specific factors in the high and hill country, both between and within farms, including soils, climate, slope, aspect, rainfall and fertility. This presents challenges for farmers to maximise the productivity of their system and manage the variation (Kerr, 2016). The high and hill country environment is often characterised by temperature extremes, a short growing season and seasonal moisture deficits (Scott *et al.*, 1985). This can restrict both the establishment and pasture growth, and also increases the time required for fertiliser applications to take effect.

A main limitation on high and hill country farms is the topography of the land, as often areas of rugged steep terrain dominate. The majority of farms tend to be hilly >15° slope, which is expensive to fertilise

and prone to erosion (Kerr, 2016). Steep slopes increase the mechanical weathering of soils via erosion caused by wind and water (Eriksen *et al.*, 1998). Nutrients move down slope with gravity and this results in lower fertility soils on steeper slopes. The application of fertilisers such as single superphosphate (SSP) can cause direct and indirect acidification of the soil through chemical reactions and also the increased yield of pasture legumes and N fixation (Bolan & Hedley, 2003b). Sufficient amounts of lime must be applied to increase the soil to pH_{H2O} 5.8-6.0, to allow acidity and Al sensitive species to be grown (Edmeades *et al.*, 1983). It is often uneconomic to apply the large quantities of lime required (aerially) across extensive farms to reduce soil acidity, due to high application costs. For example, Ag lime for Canterbury White Rock Rangiora, the cost of lime was $30.46 per tonne, plus transport at $1.20 per tonne per km. The cost of application by fixed wing aircraft at an application rate of 1 tonne per hectare is $60 to $90 per tonne, and a helicopter costs $120 to $300 per tonne (Askin & Askin, 2016). Therefore, the legume content of the sward is reduced as are overall yields produced (Craighead, 2005). Significant challenges and variability must be overcome to farm successfully in the high and hill country of New Zealand.

2.2 Soil pH

Soil pH is a common diagnostic measurement for soil fertility and is a measure of the concentration of hydrogen ions present in solution, calculated using:

Equation 2.1 Soil pH

$$pH = -\log_{10}[H^+]$$

Soil pH is a critical factor that affects the chemistry of the soil. Acidic soils have H^+ ions and Al^{3+} which reduce pH and alkaline soils are dominated by basic cations (Ca^{2+}, Mg^{2+}, K^+ and Na^+) that increase the pH (McLaren & Cameron, 1996c). The soil pH at which nutrient availability is optimal for plant growth and biological requirements, differs among nutrients. The optimum pH_{H2O} for grassland soils in New Zealand through field evidence was found to be 5.8-6.0 (Edmeades *et al.*, 1984).

2.2.1 Acidification of soils

Soil acidification is a critical issue which limits crop production worldwide. Soils with a pH<5.5 in the upper horizons are estimated to cover 30% (~ 3950 million ha) of the world's ice-free land area (Von Uexküll & Mutert, 1995). Figure 2.1 illustrates the estimated global distribution of topsoil pH_{H2O} (FAO & ITPS., 2015). Acidic soils are predominantly located in the two geographical regions: the northern belt, with a cold and temperate climate and the southern tropical belt (Von Uexküll & Mutert, 1995).

Of the soils globally affected by soil acidity, around 75% have subsoil acidity which is more difficult to remediate, as it requires the addition of amendments at depth (Von Uexküll & Mutert, 1995).

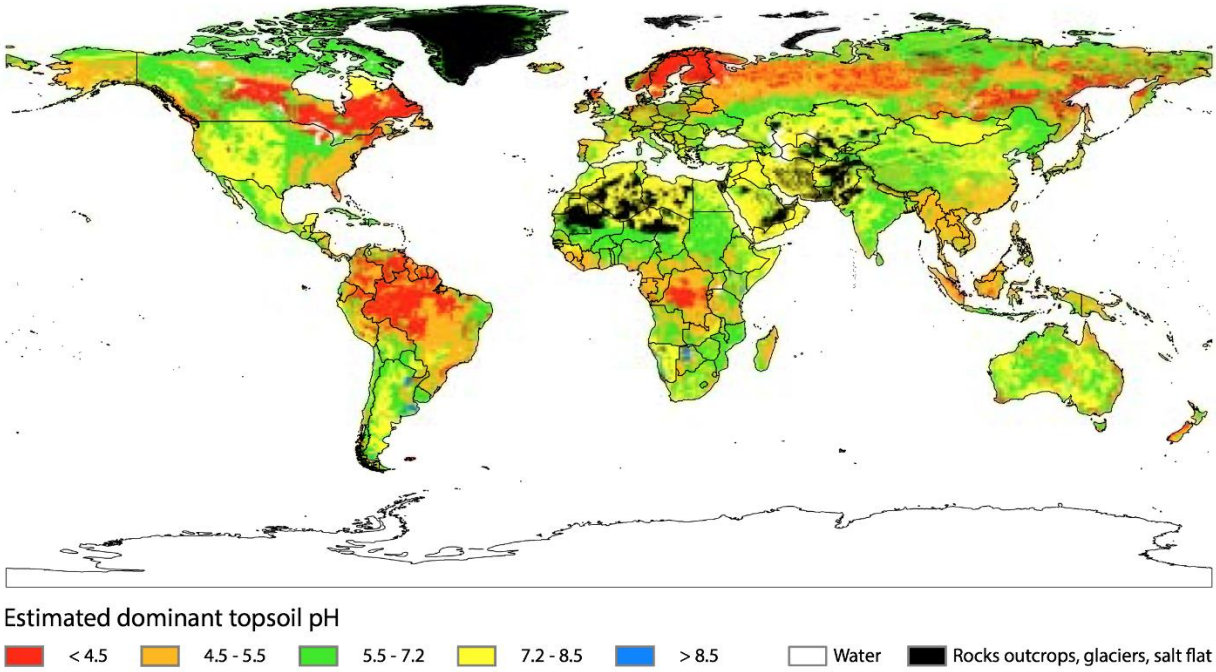

Figure 2.1 A world map of the estimated top soil pH_{H2O} from FAO and ITPS. (2015), with permission.

Soil acidity is a measure of the H^+ ions which can be present as either active acidity, in the soil solution, or bound (exchangeable) onto soil colloids as reserve acidity (McLaren & Cameron, 1996c). Soil pH and Al^{3+} are highly associated and reflect one another. Soil acidification enhances the mobilisation of toxic metals including Al in soils, particularly at a pH<5.5, resulting in increased uptake by plants (Bolan and Hedley, 2003, Haynes and Swift, 1986). The exchangeable Al in the soil solution of acidic soils (Al^{3+}) is widely accepted as the main factor which impacts on plant growth, especially root growth, compared to the H^+ ions (Edmeades & Ridley, 2003; Fox, 1979). Strongly acidic soils, commonly defined as pH<5.5, can be detrimental to plant growth by either facilitating nutrient deficiencies or toxicity (Marschner, 1995).

Soil acidification occurs naturally and is enhanced by anthropogenic processes (Rengel, 2003). The hydrolysis of Al and Fe during the weathering of aluminosilicate minerals leads to acid production (McLaren & Cameron, 1996c; Rengel, 2003). The remaining minerals, rich in Iron and Aluminium Oxides, acidify the soil (Läuchli & Grattan, 2012). The specific reactions that occur during the hydrolysis of Al, the speciation and the thermodynamic hydrolysis constants for each reaction will be presented in Section 2.3. Oxidation and reduction reactions contribute to acid generation including the process of Ferrolysis. Ferrolysis requires cycles of oxidation and reduction in soils that experience alternating

periods of saturation and dry (Schaetzl & Anderson, 2005). Under anaerobic conditions, soil organisms reduce Fe^{3+} to Fe^{2+} as an energy source and OH^- ions are released when the OM is oxidised (increasing the soil pH) (Brinkman, 1977). In dry conditions (aerobic) Fe^{2+} is adsorbed to clay surfaces and oxidised to Fe^{3+}. Some of the Fe^{3+} combines with water and oxygen to form goethite (FeOOH) and/or ferric hydroxide ($Fe(OH)_3$ (Bartelli, 1973; Eaqub & Blume, 1982). During this process bases are lost via leaching and H^+ is released. The H^+ ions release Al^{3+} from the clays into soil solution, which further acidifies the soil (Schaetzl & Anderson, 2005).

The amount of acidity determines the feedback loop. An increase in plant growth means an increase in carbon and an increase in cation uptake, which increases the H^+ and the organic acids exuded into the soil. If enough acidity is generated the Al^{3+} will increase to a point that it reduces the plant yield (carbon). In a closed system, after a period of time the cations will be restored back into the soil. For long term acidification to occur there needs to be the removal of cations from the ecosystem either through grazing or harvest or naturally via leaching.

Of the soil forming factors (mentioned in section 2.1.1), vegetation type, and succession over time and bioactivity drive acid generation in the formation of mineral soils in New Zealand. In areas where the impacts from acid rain or previous inputs of N (NH_4^+, $-NH_2$) and S^0 fertilisers are negligible, soil acidification is mainly caused by the release of protons (H^+) during the transformation and cycling of carbon, nitrogen and sulphur in the soil-plant-animal system (Bolan & Hedley, 2003a; Robson, 1989; Ulrich & Summer, 1991). In particular, the dissolution of CO_2, the synthesis and dissociation of carboxylic acids produced by plants and microorganisms, nitrification and oxidation of elemental sulphur contribute to the generation H^+ in the New Zealand soil forming environment (Bolan & Hedley, 2003a; De Klein *et al.*, 1997). The strength of the acids generated from these processes determines whether there is sufficient H^+ present to create monomeric Al e.g. the oxidation of C to CO_2 generates a weak acid that is unable to generate sufficient acidity (H^+) to create monomeric Al (pKa˜5.0), however, nitrification and S oxidation can (Bolan & Hedley, 2003a; De Klein *et al.*, 1997). The generation of exchangeable Al (extractable in KCl) results from aluminium silicates buffering acid generated by the oxidation of C, N and S.

For many processes, particularly related to N cycling, the form of the element that is taken up and the method of assimilation by the plant affects whether the plant releases H^+, OH^- or HCO_3^- into the soil to maintain a charge balance (Bolan & Hedley, 2003a; Haynes, 1983; Haynes & Goh, 1978). E.g. the uptake

of NH_4^+ and N_2 fixation result in the release of H^+ ions which are replaced by base cations that enter the plant root.

Vegetation type, in addition to vegetation activity (a function of moisture and temperature), is important for acid generation in soils. In forest environments, the rate of acidification is often much greater than in grasslands e.g. the soil under *Pinus radiata* had a lower pH and higher extractable Al than under pasture (Giddens *et al.*, 1997). Legume species acidify the soil when fixing N_2. Temperate legumes often acidify the soil more than tropical species (Israel & Jackson, 1978). The form and amount of amino and organic acids produced by the plant during carbon assimilation determines the amount of H^+ released from the plant during N fixation (Israel & Jackson, 1978).

2.3 Soil aluminium chemistry

Aluminium is the most abundant metal in the earth's crust and is a non-essential nutrient for plant and animal growth (Baes & Mesmer, 1976; McLaren & Cameron, 1996c). The total Al content is determined by the parent material but only the Al that is easily mobile and exchangeable plays a role in soil fertility (Kabata-Pendias, 2001). The largest pool of Al is present in the sediment, soil and rock. Most soil Al^{3+} is bound up in many crystalline, aluminosilicate, oxyhydroxide, hydrous oxide and non-silicate containing minerals (Driscoll & Postek, 1996; McLaren & Cameron, 1996c). A small proportion is present in the soil solution which can be taken up by plants (Bhalerao & Prabhu, 2013; Foy, 1984).

The process of Al dissolution in the soil has two distinct phases, i) the Al removed from soil exchange sites and ii) slow Al released from secondary reactive minerals in the soil (Jones & Kochian, 1996). Minerals undergo chemical and physical weathering and as a result a fraction of Al which was previously immobile is released and becomes more available for biochemical pathways (Driscoll & Postek, 1996). Figure 2.2 is a schematic diagram, which shows the Al chemistry in the environment and interactions between Al pools. Aluminium can be present as soluble Al^{3+}, exchangeable Al^{3+} sorbed to soil colloids and unavailable Al bound up into soil colloids. The exchangeable and solution Al^{3+} make up the bioavailable Al which can be taken up by plants and can cause toxicity effects (Foy, 1984; McLaren & Cameron, 1996c). The soluble Al^{3+} ions in solution are toxic to plants, however, the exchangeable Al is not phytotoxic. Exchangeable Al has the potential under certain conditions to be removed from exchange sites into soil solution and become toxic to plants. Low and high molecular weight organic species bind strongly to Al in the soil e.g. carboxylate, phenolic and alcohol groups

(Vance *et al.*, 1996), and are important for controlling the solubility and speciation of Al (Driscoll *et al.*, 1985; Lundström, 1993).

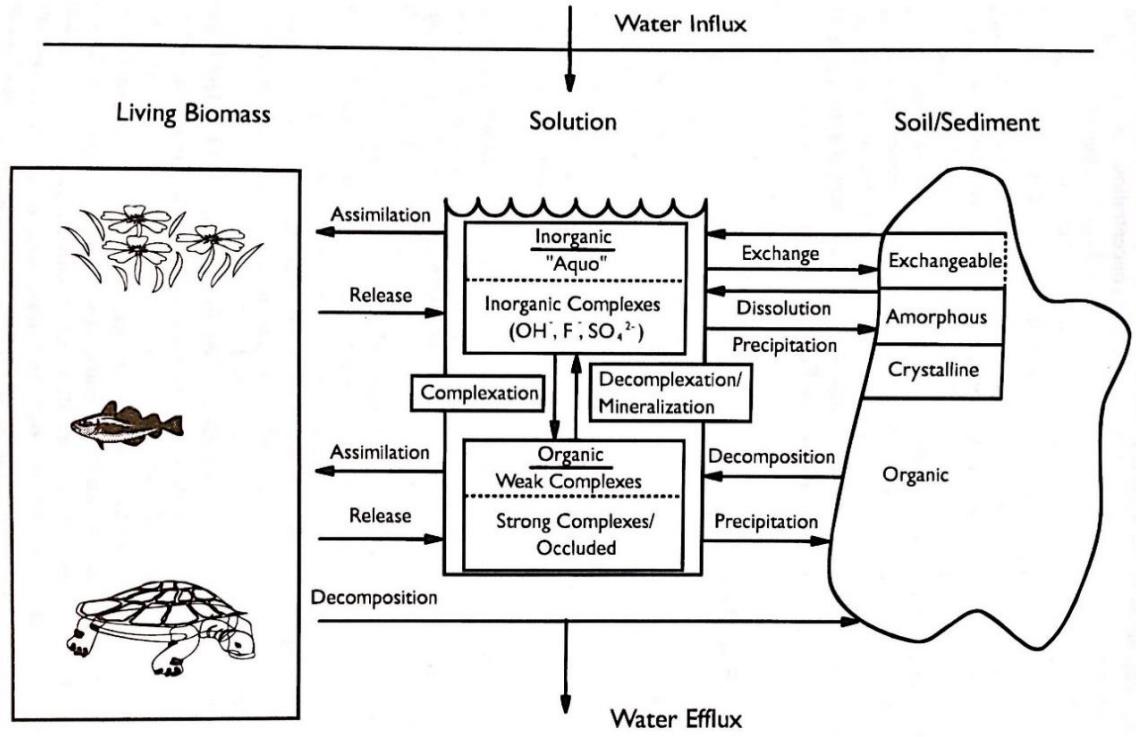

Figure 2.2 A schematic representation of the Al cycle and the links between different pools of Al in the environment. From Driscoll and Postek (1996), with permission.

There are several mechanisms for the solubilisation and transport of Al^{3+} from the solid phase. Organic acid transport of Al through the soil, the hydrological pathways of water through the soil and the specific parent materials of the soil (Adams, 1999). During weathering a large amount of Al is released from the parent materials that contain Al. The weathering process is very slow, therefore it is likely that the Al measured in aqueous systems is Al from more bioavailable sources such as soil exchange sites, dissolution of $Al(OH)_3$ minerals or the dissolution/mineralisation of organic Al species (Driscoll & Postek, 1996).

Aluminium is released into soil solution by the chemical weathering of primary and secondary minerals e.g. Gibbsite and Kaolinite (Gustafsson *et al.*, 2001). The Al which moves into the solution joins with six water molecules that undergo hydrolysis determined by soil pH (Lindsay, 1979). The hydrolysis reactions of monomeric Al (without waters of hydration for simplicity) are presented below (Equations 2.2-2.5), including their thermodynamic hydrolysis constants (log K; Nordstrom & May, 1996).

Equation 2.2 $Al^{3+} + H_2O \rightleftarrows Al(OH)^{2+} + H^+$ log K = -5.00

Equation 2.3 $Al(OH)^{2+} + H_2O \rightleftarrows Al(OH)_2^+ + H^+$ log K = -10.1

Equation 2.4 $Al(OH)_2^+ + H_2O \rightleftarrows Al(OH)_3 + H^+$ log K = -16.8

Equation 2.5 $Al(OH)_3 + H_2O \rightleftarrows Al(OH)_4^- + H^+$ log K= -23.0

The soil pH drives these reactions and determines which Al species are active in the soil (Kinraide, 1991). During hydrolysis H^+ is released from water ($Al^{3+} + 3H_2O \rightarrow Al(OH)_3 + 3H^+$), which acts to further acidify the soil (Sparks, 2003). At a pH of 7.0, gibbsite $Al(OH)_3$ is formed in the soil and at pH 7.4, the aluminate ion is formed (Krstic *et al.*, 2012). With decreasing soil pH below neutral, the concentration of $Al(OH)_3$ drops below its saturation index and gibbsite solubility increases and once released from the solid phase, undergoes hydrolysis reactions (Chadwick & Chorover, 2001). The hydrolysis of Al is most important above pH 4.0, as at pH 4.9 over 80% of the total Al present in the soil is hydrolysed (Menzies, 2003). Below soil pH 5.5 the dissolution of aluminosilicate clays and aluminium hydroxide minerals releases aluminium-hydroxy cations and Al^{3+} are exchanged with other cations. Under these acidic conditions the Al^{3+} ion forms mononuclear Al species including $Al(OH)^{2+}$, $Al(OH)_3$ and $Al(OH)_4$ (Kinraide, 1997). Gibbsite precipitation and dissolution and hydrolysis of dissolved Al are able to both consume and produce significant amounts of soil acidity (Lindsay & Walthall, 1996a; Nordstrom & May, 1996). Aluminium plays a role in the buffering of soil solution acidity (Chadwick & Chorover, 2001).

In acidic soils, Al^{3+} and H^+ occupy a large proportion of the cation exchange sites on negatively charged soil colloid surfaces (McLaren & Cameron, 1996c). This creates a large pool of exchangeable Al that can be rapidly mobilized into soil solution. The loss of the basic cations from the soil has negative implications on the soil buffering capacity and base saturation. At a near-neutral pH, Al combines with soluble silicic acid, $Si(OH)_4$, and forms short range order minerals including allophane, halloysite, kaolinite and imogolite (Lindsay & Walthall, 1996b; McLaren & Cameron, 1996c).

Organic acids are key in the weathering of Al minerals in the soil. The formation of stable, soluble Al organic complexes facilitates the chemical weathering of minerals. A large proportion of the Al that is surface-absorbed is bound to clay and mineral surfaces, or to humic complexes and the rate of exchange is slow. The ability of organic complexes to maintain Al^{3+} in solution contributes to the movement of Al into lower soil horizons (Adams, 1999). There is strong evidence to suggest that the organic phase in acidic soils controls the concentration of Al in the soil solution (Bloom *et al.*, 1979). Many interrelating factors including climate, elevation, slope, temperature, vegetation and the nature

of soil organic matter have been shown to affect Al^{3+} mobilisation and soil development in areas with soil acidity issues (Adams, 1999).

2.3.1 Aluminium toxicity

The speciation of Al in the soil is determined by pH (Figure 2.3), which directly influences the toxicity of Al in the soil, because Al species differ in their relative toxicities to plants (Rengel, 2004). Soluble Al are the most bioavailable forms but are only a small fraction of total Al in the environment (Adams, 1999). The critical issue is that Al is soluble at a low soil pH and can become toxic to plants. The relationship between soil pH and Al has been reported in several studies (Edmeades *et al.*, 1983; Kinraide, 1991; Moir & Moot, 2010). Generally, at pH values below 5, aluminium is present as $Al(H_2O)_6^{3+}$ which is usually written as Al^{3+} (Kinraide, 1991) .

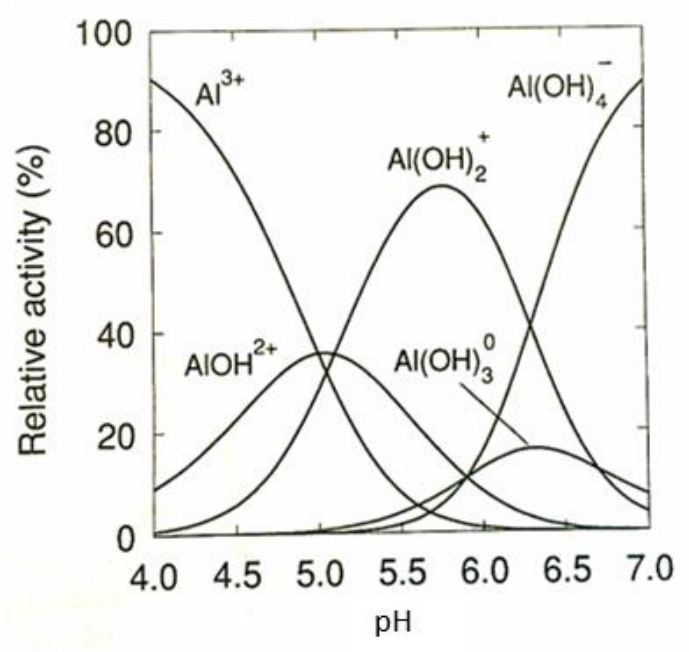

Figure 2.3 A diagram which shows the relative activity of the different aluminium species present in the soil as a response to soil pH. From Kinraide (1991), with permission.

Al^{3+} has been identified as the most rhizotoxic form of Al, and is soluble under acid soil conditions. The assumption has been made that Al^{3+} is the only toxic species, however, polycationic Al (charge >2) are also considered to be toxic e.g. Al_{13} (Kinraide, 1991; Kochian, 1995; Manoharan *et al.*, 1996a; Ryan & Delhaize, 2012). The non-labile Al in soil solution (non-monomeric), inorganic polycation Al_{13} is toxic and is believed to be at least 10 times more toxic than Al^{3+} (Kinraide, 1991). The solution chemistry of Al is complicated, as several species co-exist in the same pH range, which means that it is difficult to investigate Al species involved in rhizotoxicity in isolation (Kinraide, 1991). Kinraide (1997) states that

the exact role that $Al(OH)^{2+}$ plays in the soil is uncertain, however, based on experimental data it appears the $Al(OH)^{2+}$ could be toxic, but less toxic than Al^{3+}. Different Al species have different toxicity effects on plants and are arranged from the most toxic to least toxic: $Al^{3+}>Al(OH)^{2+}>Al(OH)_2^{+}>Al(OH)_4^{-}$ (Alleoni *et al.*, 2010). It is the Al^{3+} in the soil solution which is toxic and to a lesser extent those species which are attached to one or two OH groups**.**

Aluminium poses a significant challenge for plant growth in acid soils and the phytotoxicity and chemistry of Al has been extensively researched over the years. However, the identification and isolation of toxic species in the soil still remains difficult and the mechanism of phytotoxicity is not fully understood (Krstic *et al.*, 2012; Menzies, 2003). The critical level that Al becomes toxic to plants is determined by the plant species, soil organic matter content, P status and electrolyte concentration in the soil (Kabata-Pendias, 2001). But again, 'critical' soil exchangeable Al concentrations for plant toxicity are poorly understood (Moir *et al.*, 2016). This poses a real issue for farmers trying to manage for soil extractable Al and acidity.

2.3.2 Soil pH and nutrient availability

Soil pH affects the availability of essential nutrients to the plant (Figure 2.4), and therefore growth. The movement of soil pH outside the optimum range for plants leads to stress through insufficient nutrient uptake, toxicity and deficiencies (Läuchli & Grattan, 2012). Under acidic conditions (pH <5.5), Molybdenum (Mo), P, Ca and Mg are the most limiting nutrients which can cause deficiencies in plants (Marschner, 1995). Simultaneously soil Al, Mn, Cu, Fe and Zn become more bioavailable and are most likely to become toxic. Lime application (increase in pH) can improve the availability of the limited nutrients, and reduce the toxicity of Al and Mn. Phosphorus is the major limiting nutrient in acidic soils and results from the precipitation of P with Al in the soil (Wright, 1943). Phosphorus availability increases up to a pH_{H2O} of 6.5 and then declines beyond that point. Boron bioavailability declines with an increase in pH_{H2O} (Sherrell, 1983).

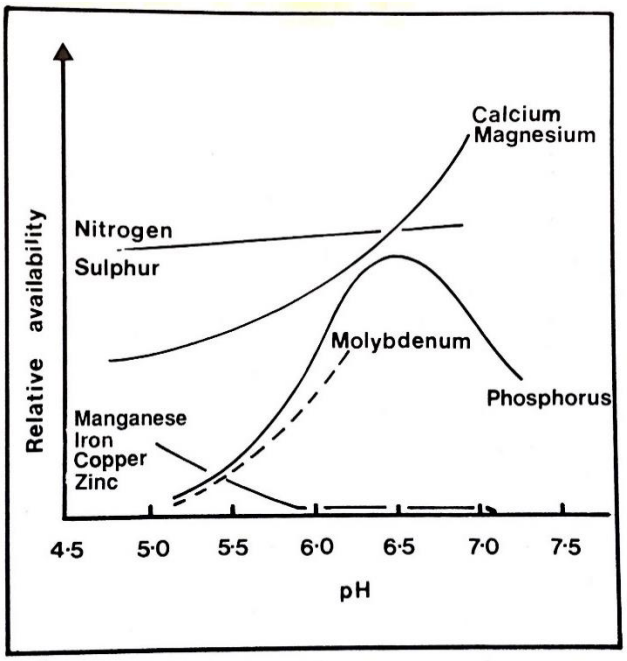

Figure 2.4 The effect of soil pH on the relative availability of nutrients. From McLaren and Cameron (1996c), with permission.

2.4 Factors driving soil Al concentrations in New Zealand soils

To date there have been no Al maps constructed for New Zealand soils, only estimations using the CEC or P retention values based on soil type. The National Soils Database (NSD) contains the data for an extensive number of soils collected and measured for exchangeable Al_{KCl}. From the NSD a total of 3280 samples were measured for Al_{KCl} and ranged from 0.05-35.9 $cmol_c$/kg. The average concentration was 2.4 $cmol_c$/kg (Landcare Research New Zealand Ltd, 1995). For a sensitive species such as white clover, Al toxicity is associated with soil concentrations of ≥1.0-2.0 $cmol_c$/kg (Edmeades *et al.*, 1983). The mean exchangeable Al concentration for the NSD samples was above this reported threshold value. The extent of the Al issue in New Zealand is difficult to define as a result of variability both with depth in the soil profile and spatially, at a paddock scale. New Zealand soil data looking at soil pH and exchangeable Al is scarce, particularly across different soil orders and including data related to plant growth/toxicity. It is of interest to know why at similar soil pH, some soils have Al toxicity and others do not. This may be because of soil and site specific factors. There is speculation as to the factors driving exchangeable Al in soils, pH is a main factor but is not the only driver.

2.4.1 Soil type

2.4.1.1 Soil pH

Al availability is affected by soil pH but the relationship is poorly understood across soil orders. Currently, soil pH is the main indicator used to determine if a soil is likely to have an Al toxicity issue or not and Al soil testing is recommended within a particular pH range in New Zealand. However, there can be toxic concentrations of Al measured at sites which are outside the recommended pH_{H2O} range for Al toxicity (pH_{H2O}>5.5). Two soil series were sampled in the Te Kauwhata area for pH, Al and Mn at depth (Singleton *et al.*, 1987). Ruawaro clay loam is a Typic Yellow Ultic Soil from weathered pumiceous alluvium. Kauwhata clay loam is an Orthic Granular Soil from strongly weathered volcanic ash. The extracted Al concentration tended to increase with depth and differed between sites. In general it is an accepted trend for Al to increase as the pH decreases (Al^{3+} species), however, Singleton *et al.* (1987) noted that there are exceptions to this rule. In their study of two soils, the Al extracted from the top of the soil profile (0-20 cm) was high, even though the pH_{H2O} was 6-7. This finding suggests that soil pH may not always be a reliable factor for predicting the Al status of the soil in relation to the toxicity (which was defined by the extraction methods used). The authors found there were no factors from the sites sampled which could explain the high concentrations of Al in the topsoil at high pH and further work would need to be conducted (Singleton *et al.*, 1987). Nielsen *et al.* (2017) measured a soil pH 0.5-0.8 units lower via in situ measurement of topsoils of organic horizons in heathland and pine forest compared to laboratory standard pH measurements on the same soils. They suggested that sample processing including drying, grinding and rewetting of soil samples may increase pH due to the release of buffering ions from soil biota. Therefore the pH of the previous studies which measured high pH and high Al, may be more acidic in situ than the measurement attained through standard laboratory practises.

The soil pH is influenced by net inputs of either acidic or basic amendments to the soil, the initial soil pH and the buffering capacity (BC) of the soil (Nelson & Su, 2010). Many soil components affect the ability to buffer the addition of acids and bases effectively resisting an overall pH change in the soil. These include calcium carbonate content, organic matter (OM) and oxides of Fe and Al formed within the soil (Bolan & Hedley, 2003a). Soil type plays a critical role in the toxicity of metals and the variability in pH. Bishop and Quin (2013) investigated the variability of pH_{H2O} and potential for metal phytotoxicity on three different soils, a recent Rangitikei silt soil, a highly weathered Allophanic Brown and Gley Soils. Soil cores were taken along a transect (1.5 m) and started at a residual urine patch and radiated outwards and at 5 cm depth increments to 20 cm depth. Of the three soils, the Rangitikei silt soil, which had the lowest CEC and buffering capacity showed the largest amount of micro variation in soil pH

compared with the other soils (Bishop & Quin, 2013). One site on the Rangitikei soil had clusters of soil pH_{H2O} of 4.5–4.7 and 6.1–6.3, which authors attributed to aged and relatively recent urine deposition, respectively. The Allophanic Brown Soil was located on a hill country plateau, a highly weathered soil with a pH_{H2O} of 4.8 and therefore a greater susceptibility to Al toxicity. The Gley Soils had imperfect drainage and showed resistance to acidification with little pH variation. However, waterlogging in the winter could mean the soil became phytotoxic seasonally (Mn and Fe) as a result of the anaerobic conditions (Bishop & Quin, 2013).

It is important to note that the standard mixed cores sent in for commercial testing can produce high results which are not reflective of the true micro-variability in soil pH. Bishop and Quin (2013) measured the pH_{H2O} distribution in the root zone and found around 25% of the root zone soils had pH less than (0.3-0.6 units) the true average. However, this could be attributed to the plant effects on the soil, exuding H^+ ions and increasing the acidity local to the roots. Plants take up nutrients from the soil and plant roots release H^+ ions in conjunction with this into the soil environment (Bolan *et al.*, 1991). As a consequence of these findings, paddocks with a measured pH_{H2O} above 5.6, which are deemed non-toxic, may have pockets of significantly lower pH_{H2O} in the soil where Al toxicity could be reducing yields but is not picked up by the macro scale of the sampling (Bishop & Quin, 2013).

2.4.1.2 Soil parent rock, mineralogy and texture

Soil parent material is the mineral material the soil is formed from e.g. alluvium, loess, till (McLaren & Cameron, 1996c). The parent rock is the rock which the parent material is derived from and both the parent rock and parent material influence the structure and chemistry of the soil. Soils formed from parent rocks containing high levels of Silica (rhyolite and granite), high levels of sand with low buffering capacities and in areas which receive high annual rainfall are more likely to undergo acidification (McCauley *et al.*, 2003). The cation and anion exchange capacity specific to each soil determines the ability to retain and supply nutrients to plants. Soils with high OM and/or clay content have increased ability to retain nutrients and a greater buffering capacity compared with more silty or sandy soils (McCauley *et al.*, 2003).

Soil texture is an important property of soils which could have a significant impact on the variation in Al across different soil groups. A study by Vendrame *et al.* (2013) in Brazil assessed the acidity control of Oxidic (Latosol) soils under long-term pastures. It was determined that the amount of exchangeable Al and the saturation rates of the soil differed as a response to different clay mineral fractions in the soil. Soils with increased mineral gibbsite had much lower exchangeable Al compared with soils with

increased kaolinite, which showed higher exchangeable Al. Vendrame *et al.* (2013) suggested the kaolinite: gibbsite ratio as a valuable indicator of the sensitivity of specific soils subjected to acidity and Al toxicity. As the kaolinite: gibbsite ratio increased, so too did the Al^{3+} in the soil. This parameter is measured by near infra-red reflectance spectrometry and could prove a particularly useful method of gauging the impacts of both acidity and Al toxicity. Different levels of gibbsite and kaolinite across the study area were attributed to the regional variation in parent material (Vendrame *et al.*, 2013). The proton consumption through mineral weathering was mainly due to the dissolution of kaolinite and gibbsite in the soils and resulted in the release of Al. The solubility of the Al containing minerals differs; kaolinite is the least soluble, followed by gibbsite and amorphous Al_3, which above pH_{CaCl2} 3.4-5.7 is the most soluble of these minerals (Percival, 1995; Stumm & Morgan, 1996). However, the solubility of gibbsite compared with kaolinite naturally in soils is debated (Watanabe *et al.*, 2006). This study showed the effect of different clay minerals on the Al concentrations in the soil and the ratio between kaolinite: gibbsite is suggested as a means of determining potential Al toxicity. However, in the context of farming in New Zealand, this method would be expensive for farmers and not easily adopted as a test for potential Al toxicity in the soil.

2.4.1.3 Soil OM and carbon

Bloom and Erich (1995) reported that the Al complexed to organic compounds is considered non-toxic because it becomes unavailable to plants for uptake. Most of the Al in solution occurs at pH 4.4-5.5 and is complexed to fulvic acids (Brown *et al.*, 2008). The relationship between Al and soil pH is strongly altered by the presence of organic matter (van Hees *et al.*, 2000). High levels of OM play a key role in the binding and complexation of Al ions in acidic soils and preventing Al reaching toxic concentrations in soil solution (Brady & Weil, 2002; Ismail *et al.*, 1994). Soluble Al can be complexed to the dissolved organic carbon (DOC,) which decreases bioavailability. This is prevalent in no-till systems (NTS) in Brazil, where the top few cm of the soil profile has increased organic matter/crop residues present, which may reduce Al toxicity compared to soils cultivated under conventional systems (Alleoni *et al.*, 2010). This OM can provide a natural buffer to soil acidity, as well as reduce the bioavailability of Al and therefore reduce toxicity.

Alleoni *et al.* (2010) conducted a lime application field experiment on two soils in Brazil, Rhondonopolis Typic Orthic Oxidic soil (Rhodic Haplustox) and a Ponta Grossa Typic Orthic Oxidic soil (Typic Hapludox). Sites had similar organic matter but differed in the time under NTS, crop rotation, soil acidity, climate and the clay content. The Rhondonopolis site has been under NTS for seven years (more weathered) and experiences tropical weather with a dry winter. The Ponta Grossa site (higher Al) has been under

NTS for longer (15 years) and had mild and humid weather with cool summers and frequent winter frosts. Surface lime was applied at several rates using a formula used in many states in Brazil which incorporates the difference between target Base Saturation (BS) and current soil BS x total CEC (which includes the potential acidity of the soil). Lime rates were calculated to increase the BS in the top 20 cm by 50, 70 and 90% compared to the control soil. The effect of lime on Al^{3+} was more pronounced at the Ponta Grossa site, although, there was no correlation between pH_{CaCl2} and Al. The likely explanation for this is that most of the Al present is complexed to organic acids in soil solution. At the Rhondonopolis site the soil contained more clay and had a shorter time under NTS. However, a negative correlation between soil pH_{CaCl2} and Al^{3+} was found as a response to lime applications.

Previous work conducted at these sites showed that the maximum amount of time for the lime to react in the top 10 cm of the soil profile was 2.5 years after surface lime application. At a soil $pH_{CaCl2} < 5.0$ most of the soil solution Al is complexed with dissolved organic carbon (DOC) which represents 70-80% of the total Al and at a pH_{CaCl2} of greater than 5.0 Al bound was 30-40%. Below a soil pH_{CaCl2} of 5.5 the results closely correlated to the solubility line of amorphous Al. This suggests that organic complexes formed by high molecular weight organic compounds and Al^{3+} may control the solubility of Al at pH_{CaCl2} <5.5 and therefore reduce Al toxicity in no till systems (Alleoni *et al.*, 2010). Their study suggests that Al toxicity to plants can be reduced through the complexation of Al with high molecular weight organic compounds, which are greater under long term NTS. The vegetation grown in the area affects the organic acids which are present in the soil and generally soils with a greater vegetative cover have increased organic acids. This is because the organic acids in the soil are produced by microbial activity which is greater in forest litter compared to cultivated soils. Aluminium complexing acids include oxalic, citric, malic and malonic which are more abundant in forest soils (Hue *et al.*, 1986)

2.4.2 Rainfall, soil age and development

Soils acidify naturally over time. Low pH soils are formed by long term acidification processes including the weathering of parent material and leaching over time which strips the soil of basic cations, reducing the buffering capacity and soil pH (Kidd & Proctor, 2001). Consequently, older soils in either humid climates or formed from acidic parent rock are more acidic than younger soils, soils from dryland environments or those derived from basic parent rocks (Helyar & Porter, 1989; Von Uexküll & Mutert, 1995). Weathering and soil development is influenced by rainfall and location in the landscape. Enhanced acidification through weathering processes could influence the Al concentrations in the soil. Two New Zealand studies were conducted which examined the soil physical, chemical and mineral properties along development sequences. Webb *et al.* (1986) examined the effect of rainfall on the

pedogenesis in a climosequence study of four Allophanic Brown Soils near Lake Pukaki. Sites ranged in rainfall from 640 to 2000 mm y^{-1}, and were selected to compare rainfall effects and reduce variation in other soil forming factors. The leaching of bases, soil acidity, exchangeable Al_{KCl}, soil P retention and clay % increased markedly with rainfall. Exchangeable Al and H^+ contributed to 60-85% of the cations under high leaching conditions (20-70 cm depths). Their findings were attributed to increased weathering and a higher leaching environment. Evidence of podzolised soil morphology was found at the highest rainfall sites of 1800 mm y^{-1} and 2000 mm y^{-1}, despite the 2000 mm y^{-1} site being on the youngest landform. This indicates that the effects of soil age on soil development are overwhelmed by the effects of climate, in this case rainfall and the leaching it promotes. Their work highlights the importance of rainfall and soil development on the pH of the soil and consequently the Al present.

In another study, Harrison *et al.* (1990) examined eight different soils across two chronosequences in different climates in a montane region in Canterbury, New Zealand. A sequence of five terrace soils (T1-T5) which increased in height above the stream (age) and three moraine soils (M1-M3) with increasing age of the surface and in a higher rainfall zone (1447 mm y^{-1} compared with 830 mm y^{-1}). The exchangeable Al_{KCl} concentration, for both sequences of soils, increased with soil age and development (Figure 2.5). The moraine sites had higher rainfall and overall extractable Al_{KCl} concentrations were higher compared to the alluvial terrace soils.

Dixon *et al.* (2016) examined the climate driven thresholds for chemical weathering at 28 profile sites close to the sites sampled by Webb *et al.,* (1986) along the McKenzie Basin climate gradient, with mean annual rainfall ranging from 400 to 4700 mm y^{-1}. A pedogenic threshold was found at ~800 mm y^{-1} rainfall, which coincided with associated cation weathering, acidification, reduced buffering capacity and the release and rapid mobilisation of trivalent metallic ions. Between 400 mm yr^{-1} and 800 mm yr^{-1} there was a trend of decreasing pH and a rapid loss of exchangeable cations (Ca, Mg and K) with increasing rainfall. Consistent changes in precipitation overprinted the differences in age and weathering in their sequence, following a north to south increase in weathering along the rainfall gradient, rather than a north to south decrease with relative age.

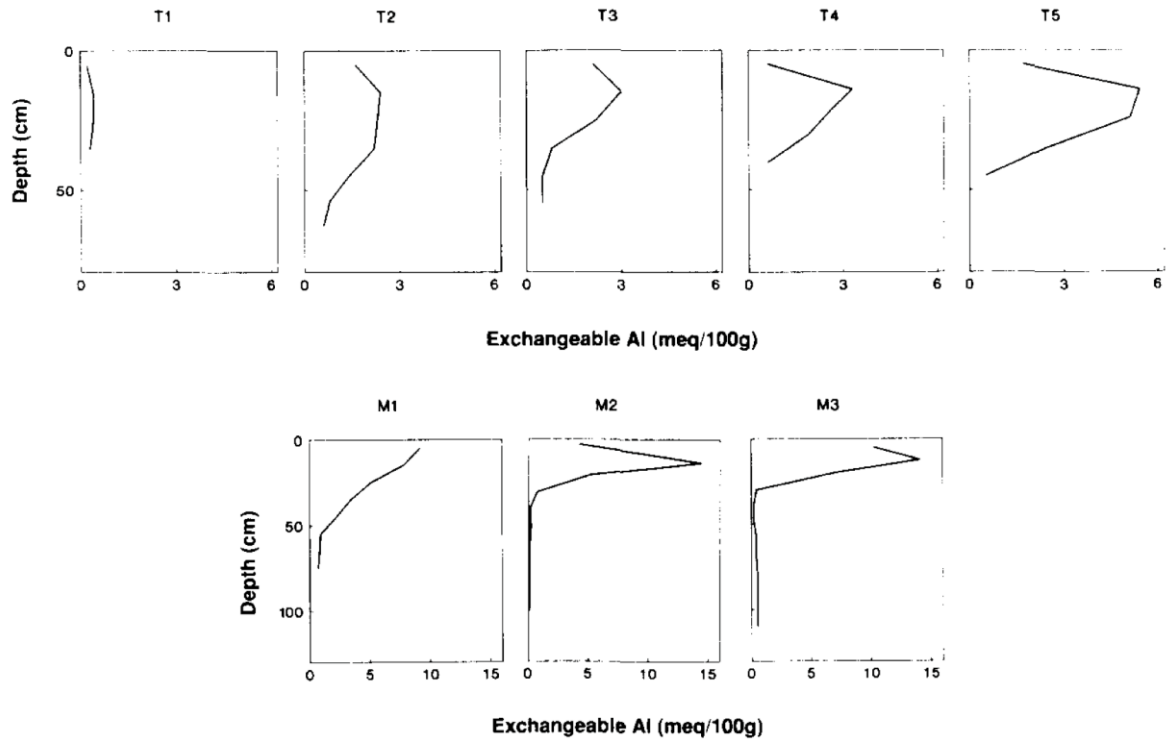

Figure 2.5 Variation of exchangeable Al_{KCl} with depth in the soil profile for soils in the terrace (T) sequence and moraine (M) sequence. From Harrison *et al.* (1990), with permission.

Webb *et al.* (1986) and Harrison *et al.* (1990) measured exchangeable Al_{KCl}, among other forms of extractable Al, which is a soil test used to indicate the potential Al toxicity to plants grown in the soil. However, there were no links in their study to soil Al_{KCl} measured in relation to plant growth, as this was not an aim of their work.

Eger and Hewitt (2008) conducted a study of soils in the South Island high country and the relationship between soil pedogenesis and aspect and vegetation, with high rainfall at all sites (1400-1500 mm y^{-1}). Authors found a strong relationship between Al_o and P retention, the P retention was higher in southern aspects compared with northern (90% and $\leq$70%). This is important as soils with a medium to high P retention (ASC) and significant clay content are more at risk of Al toxicity under acidic conditions (Hill Laboratories, 2014). South facing sites had more OM movement, Al-OM complexes (Al_p) and were more acidic. The south facing shady slopes showed evidence of stronger leaching, enhanced weathering and a more acid soil environment. Greater annual vegetation growth on shady aspects drives more acid generation and a reduction in the base saturation, particularly at sites where the winter drainage is high. Their work highlights the influence that the position in the landscape has on soil properties.

Other studies of New Zealand high country soils which related the Al measurements to potential plant toxicity were conducted by Adams *et al.* (1999) and Adams *et al.* (2001). In the first study, Adams *et al.* (1999) examined the soil Al solution chemistry (free and organically bound Al) and the Al-CC (ability of humic substances in the soil solution to bind Al into non-labile and slowly labile forms) for the top soils of six sites at three altitudes (730 m.a.s.l, 945 m.a.s.l and 1190 m.a.s.l). Their findings included a decrease in Al complexation capacity with a decrease in soil pH, shady aspects were more prone to toxicity due to their reduced Al-CC and sunny aspects had higher organically bound Al. Their main finding was that soils at the lowest elevation had the lowest Al-CC. This could mean that Al toxicity is more likely at these sites, with vegetation inputs and continued acidification. This was an important finding, as the lower altitude sites are those more likely to be utilised for farming, however, their study only looked at the top 7.5 cm of the soil profile. The Al-CC appears to be an important parameter for assessing the potential for Al toxicity at a site, as dissolved soil organic matter complexes Al and reduces toxicity.

Adams *et al.* (2001) compared the exchangeable and solution Al chemistry of grassland and forest areas at three sites. Their findings were that both reactive Al and Al bound in labile organic complexes at all sites was higher in the forest soils compared with grassland. The 1 M KCl and 0.02 M $CaCl_2$ Al concentrations indicated greater (P<0.01) soil Al acidification at two of the forest sites in the top 10 cm of the soil profile, compared with the grassland site. Soil solution 'reactive Al' concentrations were also higher under forest soils, but were only significantly different to the grassland site, which had the lowest pH and base saturation. The Ca:Al ratio, a soil characteristic which is linked to Al toxicity, was lower in the forest soils, which highlights a greater risk of toxicity. The Al-CC was higher in forest soil solutions compared with grassland. The higher Al-CC at the forest sites was consistent with higher amounts of humic and fulvic matter which provide potential binding sites for Al and therefore amelioration of Al toxicity.

Previous sections have outlined processes involved in soil acidification and the issues associated with Al toxicity in a wider context. Soil chemical, physical and environmental variables have been identified that may influence Al toxicity in New Zealand soils. There have been limited studies in New Zealand examining the drivers of soil pH and Al variability. Therefore the next step would be to conduct a study on New Zealand soils to determine which variables are influencing extractable Al on a national scale. At a landscape scale (finer), the studies above (Section 1.4.2) all indicate potential variability of exchangeable Al related to landscape-scale effects yet there has been no systematic study of exchangeable Al variability at this scale. Therefore, in addition to the national scale study, a survey of

a single catchment is required to determine which factors drive soil pH and extractable Al_{CaCl2} at a landscape scale.

2.5 Remediation options

2.5.1 Liming

The use of lime as a soil amendment has three important roles (Alleoni *et al.*, 2010), i) it increases the soil pH, ii) promotes Al precipitation as a response to increased pH and iii) reduces Ca and Mg deficiencies in the soil. The increase in pH can ameliorate soil Al and Mn toxicity. Soil type (chemistry) and environmental factors specific to each site determine the amount of lime required and the time taken for the lime to increase the soil pH by one unit i.e. the pH buffering capacity. In New Zealand liming is recommended for grassland soils with a pH of <5.8-6.0 (Edmeades *et al.*, 1983). The biological lime requirement is determined by the crop and soil type combination and is influenced by the initial soil pH, all of which are site specific (Edmeades & Ridley, 2003). Soil pH values rarely reach below 4.0 because the pH is buffered by aluminosilicate and oxide mineral dissolution within this soil pH range (Ulrich, 1991). Lime can be applied to acidic soils which are currently at their biological optimum, to prevent inevitable acidification over time (Edmeades & Ridley, 2003). The lime application advised should be both site specific and based on individual paddock scale testing to determine the best treatment, rather than large scale assumptions for the area i.e. targeted applications.

Many New Zealand high and hill country farmers are unable to apply lime as there are large application costs, particularly in areas where the farm extends across challenging topography. Acidification of the soils compounds over time and must be maintained by annual applications of limestone of around 100-600 kg ha^{-1}, which is site specific (Edmeades & Ridley, 2003). The application of lime to the topsoil is well practised in New Zealand, however, this does not alleviate subsoil acidity and in some cases toxic Al concentrations. Applied lime may only move a few cm down the profile annually and is influenced by placement, solubility, lime quality, rainfall and texture. There have been clear advantages shown in research conducted by incorporating lime at depth to the soil profile to alleviate subsoil acidity and Al toxicity. However, this may not be economic and the land may be unsuitable for this operation, particularly in the South Island high country (Scott *et al.*, 1997).

Edmeades *et al.* (1985a) conducted an experiment on a Moanatuatua Peat soil which showed that the physical incorporation of lime into the soil increased pasture production (kg DM ha^{-1}) across all lime rates applied, compared with traditional surface applied lime. At the 2.5 t lime ha^{-1} and 5.0 t lime ha^{-1} application rates the pasture production increased by 34% and 47% respectively with 50/50

incorporated and surface applied lime compared to 100% surface application. This shows the influence that physical incorporation has on both the movement of lime through the profile and pasture response, highlighting the importance of the placement of lime to achieve the required response (Edmeades *et al.*, 1985a). Physical incorporation also improves the aeration of the soil, which likely also contributes to the increase in plant growth.

Moir and Moot (2014) assessed the effects of lime rate on the soil pH and exchangeable Al at three long term lime trial sites in the South Island high country. Site 1 was on a stony Brown Soil (North Canterbury), Site 2 was on a Brown Soil (South Canterbury) and Site 3 was on a dense Brown hill soil (Central Otago). Mean annual rainfall across all sites was 600 mm y^{-1}, however, the elevation differed, site 1) 430 m.a.s.l, site 2) 700 m.a.s.l and site 3) 750 m.a.s.l. The lime rates applied differed among the sites and the pH and exchangeable Al were measured at 0-7.5 cm and 7.5-15 cm soil depths. Liming had a strong effect at all three sites, especially in the top 7.5 cm, where an increase in pH and a reduction in Al was observed with increased lime applied. However, at all sites, exchangeable Al was present at high concentrations in the subsoil (7.5-15 cm), even at a lime application rate of 4-5 t lime ha^{-1}. The surface applied lime took 5-8 years to affect soil pH and Al at a depth of 7.5-15 cm (Moir & Moot, 2014). The effectiveness of lime application differed among sites and is likely a result of site specific factors, which are poorly understood (Moir & Moot, 2014). Future research is required to explain the key factors driving exchangeable Al concentrations in New Zealand soils.

2.5.2 Other methods

Lime is the most common amendment to New Zealand soils, however, there are other methods which can remediate soil pH and Al issues in the soil. Iqbal (2014) assessed the ability to ameliorate Al toxicity through the application of phosphorus. The addition of P to the soil via fertilizer application reduces the soluble Al present as a result of the precipitation reactions of Al-P on the roots or in the soil (McCormick & Borden, 1974 ; Pellet *et al.*, 1997) and this precipitate is believed to be non-toxic to plants (Zheng *et al.*, 2005). There are reports of P application causing a reduction in the Al present in toxic form, and an increase in plant dry matter production and root growth (Ismail, 2005). Iqbal (2014) found that a P rate of 80 mg P kg^{-1} (highest rate applied) was most effective at decreasing soil extractable Al concentrations, suggesting a detoxification effect. Iqbal (2014) measured a decrease in soil pH with an increase in P rate applied, which was suggested to be the result of the formation of insoluble Al-phosphate in the soil and the release of protons (H^+) in the process. Manoharan *et al.* (1996a) also found a decrease in toxic Al species (Al^{3+}, $Al(OH)^{2+}$, $Al(OH)_2^+$) in soil solution with applications of North Carolina phosphate rock (NCPR) and Single Superphosphate (SSP). The authors

attributed this to the formation of non-toxic Al-F complexes formed from the Fluorine derived from the fertilisers. Moreover, they suggested that long-term application of reactive phosphate rock may contribute to the amelioration of both acidity and Al toxicity under legume based pastures in New Zealand (Manoharan *et al.*, 1996a). However, this approach (P additions to reduce Al) is unlikely to be economically feasible for high country farmers in New Zealand, although it is cheaper to apply than lime.

2.6 Soil Al test

The soil test for Aluminium is a common test used by farmers to examine the soil for elevated concentrations of Al, which could be toxic to plants. Generally, the tests are recommended for New Zealand farmers if their soils are within a certain pH zone, generally pH_{H2O}<5.5 (Hill Laboratories, 2014). Exchangeable Al is defined as the amount of element extracted from an unbuffered neutral salt solution and the use of different salts often produce different measures of Al in the soil (Coscione *et al.*, 1998). The measured Al concentration represents the availability at the pH of the soil, as the solution is unbuffered (Houba *et al.*, 2000; Rayment & Lyons, 2011a). 1 M KCl has been used in the past in New Zealand for the measure of labile or exchangeable Al in soils and also to estimate toxicity status (Edmeades *et al.*, 1983; Hume *et al.*, 1988; Singleton *et al.*, 1987). The KCl extraction is still used in many parts of the world, including Australia, China, South America, the USA and Canada as a measure of Al toxicity and as an index for lime requirement (Abreu Jr *et al.*, 2003; Amedee & Peech, 1976; Guo *et al.*, 2012; MacLeod & Jackson, 1967; Marques *et al.*, 2002; Schroder *et al.*, 2011; Shuman, 1990; Vendrame *et al.*, 2013; Wang *et al.*, 2016). The 0.01 M and 0.02 M dilute $CaCl_2$ tests have been used as diagnostic criteria of Al toxicity in New Zealand, Australia and Canada in acid soils (Close & Powell, 1989b; Conyers *et al.*, 1991; Hoyt & Nyborg, 1971; Hoyt & Nyborg, 1972; Hoyt & Webber, 1974; Rayment & Lyons, 2011b). Dilute neutral salts, particularly 0.01 M and 0.02 M $CaCl_2$, are close to the ionic strength of most soil solutions and disturb the ionic equilibrium as little as possible (Houba *et al.*, 2000; Soon *et al.*, 2008). There are also measures of the Al in soil solution using different methods. Al in solution was determined by Adams and Lund (1966) through centrifugation of the soil sample and measurement using Atomic Absorption Spectroscopy (AAS). Another method uses the colorimetric chromeazurol S (CAS) reaction between the colorimetric reagent and Al^{3+}(Close & Powell, 1989b; Hawke & Powell, 1994). Subsequent work on the CAS method has led to the analysis of the Al complexation capacity of soil solutions and also the analysis of 'reactive Al' (Powell & Hawke, 1995; Powell *et al.*, 1997). However, although much work has been conducted using these tests and linking the Al solution concentrations to toxicity, these tests are not used in commercial laboratories to identify the potential toxicity of New Zealand soils.

There is an on-going debate in the scientific community around the accuracy of methods for measuring soil exchangeable Al in relation to potential plant toxicity (Menzies, 2003). As a consequence, no single method has been adopted that has gained widespread acceptance internationally (Wright, 1989). It is important to be aware that in the literature there is not consistent terminology used around the soil Al test and what each test measures. In particular, for the 0.02 M $CaCl_2$ test terms used interchangeably include exchangeable, plant available and extractable Al. While for the KCl test, there is general agreement that this measures 'exchangeable' Al from soil exchange sites. In this thesis, to make it clearer the terminology used is extractable Al_{CaCl2} and extractable Al_{KCl}, to describe the amount of Al present in the supernatant using standard soil Al extraction methods.

There have been many studies internationally and a small number in New Zealand which compare the measurement of Al by the different soil tests and assess their reliability for indicating potential plant toxicity. Critical studies were conducted by Hoyt and Nyborg (1971 and 1972) which examined variations in molarity and extraction time for the $CaCl_2$ extractable Al test. Importantly the measurement of Al was linked to plant growth on Canadian soils in glasshouse experiments, barley, rapeseed and lucerne species were used as bioindicators of toxicity. The first studies identified that the 0.01 M $CaCl_2$ tests (16hr shake) showed promise as an extraction method for plant available Mn and Al in acidic soils (Hoyt & Nyborg, 1971). The Al concentration in lucerne and the yield response of barley to lime were closely related to the $CaCl_2$ test. The latter study confirmed that the best method for determining plant available Al and Mn was through the 1:2 ratio 0.02 M $CaCl_2$ method (1 hr shake) (Hoyt & Nyborg, 1972). Their study encompassed 40 different soils from Alberta and British Columbia, which ranged in acidity from pH_{H2O} 4.0-5.6. Higher molarities have been tested such as 0.04 M $CaCl_2$, and gave reasonable correlations with plant yield data (Hoyt & Nyborg, 1972). However, such concentrations of extractant have not been implemented into common practise tests for diagnostics. Hoyt and Webber (1974) modified successful methods for measuring plant available Al, suggested in Hoyt and Nyborg (1971; 1972), to determine if the method could be improved, for 33 Canadian acidic topsoils (0-15 cm) ranging in pH_{CaCl2} from 3.7-5.0. In this study Al concentrations extracted by 0.01 M $CaCl_2$ and a 5 minute extraction time. The authors found that correlations with crop data were similar, if not better, than those used in previous studies. This was proposed as a diagnostic measure for plant available Al (Hoyt & Webber, 1974). Although there are many advantages to using the 0.01 M $CaCl_2$ test, including the measurement of other metals and nutrients in the one extract (e.g. pH, soluble organic C, N, P, S, Cu, Pb and Zn mobility), there have been no critical levels proposed for toxicity to plants (Houba *et al.*, 1996; Houba *et al.*, 2000; Hoyt & Webber, 1974; Pueyo *et al.*, 2004; Soon *et al.*,

2008). The 0.02 M $CaCl_2$ test is the current and preferred method of soil Al testing in New Zealand by the commercial laboratories to determine plant toxicity and has replaced the 1 M KCl test.

The $CaCl_2$ extraction method has been used to measure and assess the potential of Al toxicity in the soil and has proven successful across a range of soils and plant species in Australia (Slattery *et al.*, 1999). This is contradictory to other research using this soil Al test. The parameters which may be a good predictor of Al toxicity in a particular soil may not be plausible across a range of soils and plant species (Menzies, 2003). Not all studies have found these tests ($CaCl_2$ and KCl) reliable for indicating potential Al toxicity in the soil, particularly for several New Zealand soils. Studies of dissolution by Lee (1988) suggest that the 1 M KCl releases the Al cations from the interlayers of the Al rich minerals such as smectite/mica and vermiculite clays. The Al extracted from the soil by 1 M KCl could be from organic Al complexes, precipitated or amorphous $Al(OH)_3$, disordered aluminosilicates and hydroxyl Al interlayers (Amedee & Peech, 1976; Bache & Sharp, 1976).

Percival *et al.* (1996) conducted a study on the 0.02 M $CaCl_2$ and 1 M KCl extracted Al concentrations of 14 New Zealand subsoil samples from the NSD including samples from the Pallic, Brown, Allophanic, Recent and Gley Soil Orders. These concentrations were then related to total Al and monomeric Al in soil solution to determine any relationships. Authors found that for most of the soils measured, Al concentrations extracted by both KCl and $CaCl_2$ exceeded the toxicity thresholds for white clover which were recommended by Edmeades *et al.* (1983). In comparison, the monomeric Al concentrations in soil solution did not exceed minimum plant toxicity thresholds in this study. It was suggested that the lower concentrations of Al in the soil solution were a result of the majority of the Al strongly bound in non-labile forms, possibly strongly bound to organic complexes and some inorganic polycationic species (unlikely Al_{13}) (Percival *et al.*, 1996). There were no significant relationships found between the total and monomeric Al and the 0.02 M $CaCl_2$ and 1 M KCl extracted Al. Consequently, it was concluded by Percival *et al.* (1996) that the current tests used for soil extractable Al, 1 M KCl and 0.02 M $CaCl_2$ produced falsely high values from the soil types tested, giving a misrepresentative toxicity status. The soil concentrations measured in this study suggested Al toxicity, but this was not observed by any other factors including plant growth. The high concentrations of Al measured could be a result of the soil minerals present and the aggressive nature of the extractant (KCl) on the particular soils measured. A similar process could explain high $CaCl_2$ extractable Al. Soil solution Al measurements provided a more reliable measure of potential Al toxicity for these soils under field conditions (Percival *et al.*, 1996). The Al tests currently (KCl and $CaCl_2$) do not distinguish between toxic and non-toxic forms of Al in the soil (Carr *et al.*, 1991). The Al must be in a plant available form to be toxic to plants. Percival *et al.* (1996)

raised the important point that with Al extracted by KCl and $CaCl_2$ being so high in their study, soils may be fragile as the additions of salts from sources including fertilisers may raise the Al concentrations in solution to those toxic to pasture species.

Marques *et al.* (2002) studied a suite of soils in the Western region of the Amazon Basin, including two Fluvial Recent Soils (typic Udifluvents), one Gley Soil (Aeric Endoaquent) and two Mottled Yellow Ultic Soils (typic Hapludults). The crops grown at each site differed and included pasture (*Brachiaria. sp*), peach palm (*Bactris gasipaes H.B.K.*) and cupuazu tree (*Theobroma grandiflorum Willd.ex Sprang*) on the Ultic Soils, cassava (*Manihot esculenta Crantz*) on the Gley Soil and maize (*Zea mays* L.) on the Recent Soils. Soils in this region had KCl extractable Al concentrations of 5 to 10 times higher than expected from highly weathered soils which contain kaolinite and gibbsite minerals. Moreover, even measuring very high soil exchangeable Al, no severe symptoms of Al toxicity were present in crops grown in these soils. The researchers suggested that the reason crops grown in these Amazonian soils did not exhibit toxicity symptoms when the soil exchangeable Al was high was because the 1 M KCl extracted the Al in the interlayers of the hydroxyl-interlayered smectite. This was not correlated with the soil Al activity in the field. It is only the plant available soluble fraction of Al which is associated with Al toxicity (Marques *et al.*, 2002). This is another example of why the KCl extractable Al test is unsuitable for all soil types in determining the Al toxicity of the soil. These Amazonian soils are in a contrasting environment to New Zealand with a tropical climate and different soil groups. It is uncertain if the misrepresentative Al concentrations measured by the KCl test in these Amazonian soils will hold true for soils in New Zealand and in particular those in the high and hill country.

The overall results in the literature of the success of these soil Al tests in measuring and predicting soil Al toxicity is varied and conflicting. This raises the question of the reliability of these tests in determining Al toxicity to plants in soils. This needs to be investigated further, as this soil test is the current measure that farmers have available to inform them of potential soil Al toxicity on their farms and to assist in land-use decisions. Further research is required to determine if changing the molarity and extraction time of the standard $CaCl_2$ and KCl soil Al tests, alters the Al concentrations extracted for different New Zealand Soil Orders. Results should be related to which specific soil properties affect the quantity of Al extracted, as research to date has shown that the diagnostic tests for Al toxicity are not successful across all soil types. In addition, the plant roots are the site most affected by Al toxicity and it would therefore be beneficial to compare rhizosphere measurements of extractable Al to bulk soil samples, as most soil tests for Al toxicity are conducted on bulk soil.

2.7 Plant effects

2.7.1 Effects on plants

For most agriculturally significant plants, Al ions rapidly inhibit root growth at micromolar concentrations (Krstic *et al.*, 2012). The primary site for Al toxicity is the root apex. The root apex (root cap, meristem, and elongation zone) accumulates more Al and undergoes greater physical damage than mature root tissues (Delhaize & Ryan, 1995). A reason for this could be that Al ions translocate very slowly into the upper parts of plants (Ma *et al.*, 1997).

Al^{3+} damages plant roots, reducing cell elongation and division at the root tips. The roots become short and thickened, with reduced branching and can often become brownish black in colour (Schroth *et al.*, 2003). Al causes extensive root injury and stunts growth, which restricts water and nutrient uptake (Barcelo & Poschenrieder, 2002; Rout *et al.*, 2001). This can induce drought stress on the plant, as roots are unable to fully exploit resources in the soil and root stores (Curtin *et al.*, 2008; Edmeades & Ridley, 2003). Damage has also been reported in the root cells of plants as lesions, which has the potential to increase the vulnerability of the plant to pests and disease (Ryan & Delhaize, 2012). Plants often selectively grow away from regions in the soil which contain toxic concentrations of an element. As a result, the first observation of a toxicity presence in the soil is the inhibition of the plant root growth and rooting depth, restricted by an acidic layer in the soil (Matsumoto, 2002). Increased soluble Al can restrict root growth in sensitive plant species to the top of the soil profile. Berenji (2015) found that lucerne had horizontal root growth and abnormal branching in response to high soil Al_{CaCl2} concentrations (>8.9 mg kg^{-1}) in field trials at two high country sites on Acidic Orthic Brown Soils. High concentrations of exchangeable soil Al (Al^{3+}) can reduce pasture growth, quality and overall yield, particularly for sensitive species (Läuchli & Grattan, 2012; Scott *et al.*, 2008).

There are several mechanisms through which Al may cause toxicity in plants (Delhaize & Ryan, 1995; Rosseland *et al.*, 1990). Firstly Al may bind directly with proteins and pectins in cell walls, which can displace other cations from these sites, especially Ca^{2+}. The soluble Al interferes with the uptake and transport of essential nutrients in plants, including Cu, Zn, Ca, Mg, Mn, K, P and Fe, by reducing the membrane potential of the cell wall (Roy *et al.*, 1988). Secondly Al can move into the cytoplasm and bind to important ligands required for specific plant functions e.g. enzymes, proteins. Thirdly Al may also have the ability to inhibit DNA synthesis by binding to DNA phosphate groups in the nucleus. Al also induces the increased production of reactive oxygen species and this is thought to cause root growth inhibition (Rengel, 2004).

Prolonged exposure to Al can lead to the yellowing and browning of roots, wilting, reduced crop yields and reduced Ca and Mg in the plant roots and shoots (Rosseland *et al.*, 1990; Russell, 1988). Aluminium effects phosphorus availability in the soil, through the reduction of root growth and binding P to the root surfaces and cell walls. At high soil extractable Al concentrations, plant symptoms may appear similar to P or Ca deficiency and therefore can be difficult to diagnose (Delhaize & Ryan, 1995; Rowell, 1988). The mechanism of Al toxicity is unclear, but it is suggested that it may involve an interaction with Ca, as the apoplastic Ca^{2+} is displaced by Al^{3+} and inhibits plant uptake (Delhaize & Ryan, 1995). Most plant species show a reduced persistence in acidic soil environments (Haling *et al.*, 2010). However, the concentration at which Al toxicity impacts plant growth is species specific and some species are more tolerant to acidity and associated high concentrations of Al than others e.g. *Subterranean clover* and *Lotus pedunculatus* L. (Bouma *et al.*, 1981; Lowther, 1980).

2.7.2 Legumes

Legumes are critical for farming systems in New Zealand, particularly in the high and hill country. However, legume nodulation and overall N fixation is reduced by soil acidity and associated Al toxicity. Al and Mn are not the only cause of damage for plants living in acidic soils. For example lucerne growth and N fixation, may be restricted by the soil acidity even when toxic amounts of Al and Mn are not present (Hoyt & Nyborg, 1972). Reduced persistence of legumes in low pH and high Al soils can be related to the effects on the plant, and the rhizobia which are important for facilitating symbiosis with the plant and nodulation during N fixation. A critical finding by Berenji *et al.* (2017) on an acidic (pH_{H2O} <6.0) and high Al soil was a reduction rhizobium populations which led to less effective nodulation by lucerne. In addition, the soil Al toxicity supressed lucerne fine root growth which lead to N deficiencies which limited growth. In contrast, Caucasian clover root growth and nodulation were not affected by the presence of Al. Their study suggested a soil Al_{CaCl2} concentration of <1.0 mg kg^{-1} for effective lucerne nodulation and highlighted the effects of environmental stresses on the symbiotic relationship between legume and rhizobia. *Sinorhizobium meliloti* is known to be particularly sensitive, the attachment to lucerne roots, which is required for successful nodulation and N fixation, is limited in low pH soils by acidity and Ca (Soto *et al.*, 2004). Wigley (2017) found that the rhizobia population capable of nodulating lucerne was very low in the no lime and high Al (7.6 mg kg^{-1}) treatment. In contrast the rhizobia population was highest in the 4 t lime ha^{-1} treatment, which raised the pH by 0.1 units (5.3) and reduced the Al concentration to 4.9 mg kg^{-1}.

The exact reference level for Al toxicity is difficult to define because the concentration of Al in the soil, the plant species and environmental factors differ from site to site (Carnan & Grigal, 1995). Plant

species differ in their sensitivity to soil extractable Al. There have been several critical studies in New Zealand which have examined the Al toxicity thresholds of plants using different soil extractable Al tests. Edmeades *et al.* (1983) grew white clover in two New Zealand soils, an Allophanic Brown Soil and an Acid Brown Soil in a glasshouse experiment, with a range of pH and P treatments. Three different measurements of Al were used, including the 1 M KCl, 0.02 M $CaCl_2$ and soil solution Al, and the measures were related to plant yield. The Al toxicity thresholds for white clover on these soils were Al_{Kcl} ≥ 1.0-2.0 $cmol_c/kg$, Al_{CaCl2} ≥ 3.0-5.0 mg kg^{-1} and soil solution Al activity of ≥9-15 µM. They found that the critical values for pH_{H2O} and Al in soil solution were dependent on the soil type and the P treatment applied (Edmeades *et al.*, 1983). Edmeades *et al.* (1983) attributed this to the application of P increasing plant growth, increasing the removal of nutrients from the soil, and decreasing the ionic strength. At higher P rates applied, the Al activities were higher and the critical Al concentrations were lower in soil solution.

In a later study, Edmeades *et al.* (1991) determined the toxicity threshold for 11 temperate legume species in a solution culture experiment. Cultivars of white clover, red clover, subterranean clover, and Lotus (*corniculatus* and *pedunculatus*) were included. The ionic strength of solutions in this experiment were chosen to reflect a range of actual measurements from selected New Zealand topsoils. The results showed that the most tolerant species to Al were *L. pedunculatus L.* and subterranean clover (5-7+ µM Al^{3+} activity leading to a 50% reduction in total dry matter). Red clover and *lotus corniculatus* were much more sensitive species and showed a substantial reduction (50%) in total dry matter yield at Al^{3+} of 2-3 µM in the soil (Edmeades *et al.*, 1991). The results of this important study gave thresholds of micromolar Al which would be toxic to each species. However, this was a solution based study and did not take into account the soil characteristics and conditions which are present in a plant-soil system that influence Al toxicity. Although a valuable measure of Al^{3+}, soil solution Al is not currently used as a diagnostic soil test for the identification of Al toxicity for farmers in New Zealand.

Caradus *et al.* (2001) monitored the growth of 15 legume species over three years on three different Otago upland soils with Al concentrations of 11, 45 and 70 mg kg^{-1} under field conditions. Both *L.pedunculatus* L. and *Lotus corniculatus* L. produced the highest yields over the three years, while *L.pedunculatus* L. produced high yields, even at the intermediate and highest Al sites. The authors reported the ability of Caucasian clover to spread via rhizomes at the high Al site and concluded that optimising the performance of Lotus and Caucasian clover in these soils would be a more productive strategy than breeding tolerant white clover, which showed poor performance in their study. Since this study, a large body of research has been conducted, mainly at Lincoln University, Canterbury, to

investigate legume species that are best adapted for growth and survival in the high and hill country of New Zealand (Berenji, 2015; Jordan, 2011; Keenan, 2014; Maxwell *et al.*, 2012; Moot, 2012; Schwass, 2013; Whitley, 2013).

In a more recent glasshouse experiment, Moir *et al.* (2016) grew 11 legume species in an acidic (pH_{H2O} 5.1) and high Al (14.0 mg kg^{-1}) high country soil with lime applied and the critical concentration thresholds for Al toxicity were determined. They found that tagasaste, lotus, persian and gland clovers and *Medicago falcatta* L. species had higher Al toxicity thresholds at 7-8 mg kg^{-1} compared with the other species grown. Their result highlights the potential for these species to be utilised in low pH and high Al soils in the high and hill country of New Zealand. However, it was noted that further testing in the field would be required to confirm these results. Berenji *et al.* (2017) measured the growth of lucerne and Caucasian clover in response to lime and capital P inputs on an acidic (pH_{H2O} 5.2) high country Orthic Brown Soil. This study quantified the differences in production and persistence of both species in a high Al soil (15.1 mg kg^{-1} 0.02 M $CaCl_2$). Their findings were that N deficiency was the main factor limiting lucerne growth as a result of limited nodulation. Caucasian clover was contrastingly more tolerant of Al, and showed persistence of nodulation and root growth in this harsh environment which supports earlier work by Caradus *et al.* (2001). This is another study which shows the potential for Caucasian clover as a species for low fertility high and hill country sites at which it is uneconomic to apply large amounts of lime.

In recent years at Lincoln University several studies have examined the growth of legume species in acidic high country soils, with different soil extractable Al concentrations. However, these have been limited to soils of the South Island high and hill country. As mentioned in Section 2.1.1, there are many Soil Orders in New Zealand. Therefore it would be beneficial to examine the effects of soil type and pH on soil extractable Al_{CaCl2} in different soil orders, including volcanic soils from the North Island, using legume growth as bioindicators of Al toxicity. In addition to the field and glasshouse experiments in pots, it is useful to study the rhizosphere, as the root-soil interface is an important zone for nutrient acquisition and also Al toxicity. A study that examines the plant effects on Al mobilisation and immobilisation at the plant root scale in acidic New Zealand high country soils would be beneficial.

2.7.3 Plant adaptations to Al

There are several plant species which have adaptations that allow them to grow in acidic and Al toxic environments. Some plants are accumulators of Al and can take up over 10 times more Al without toxicity effects e.g. *Camellia sinensis* L. (Krstic *et al.*, 2012; Matsumoto *et al.*, 1976). Two different

mechanisms for Al resistance in plants are 1) Al exclusion from the roots and 2) detoxification of Al ions in the plant (Heim *et al.*, 1999; Kochian *et al.*, 2005; Roy *et al.*, 1988; Taylor, 1991; Zhou *et al.*, 2007). Methods of exclusion of Al include binding of Al to the cell wall, a plant-induced pH barrier and root exudation of Al binding compounds (Delhaize *et al.*, 1993). The exudation of low molecular weight organic acids, which bind Al, from plant roots is suggested as the primary defence mechanism of Al tolerant plants growing in acidic soils (Delhaize & Ryan, 1995; Miyasaka *et al.*, 1991; Rosseland *et al.*, 1990). These low molecular weight organic acids form soluble complexes with aluminium ions that are non-toxic to plants and microbes (Brady & Weil, 2002). Many studies have reported the release of organic acids, including malic and citric acids, from lupin cluster roots, particularly under P deficiency conditions, in order to solubilise P for plant uptake (Dinkelaker *et al.*, 1989; Egle *et al.*, 2003; Li *et al.*, 1997; Neumann *et al.*, 1999). They may also have an additional effect of detoxifying Al through chelation. Hue *et al.* (1986) investigated the detoxifying effects of short chain carboxylic acids e.g. citric and salic acid on cotton (*Gossypium hirsutum L*.) taproots in solution. The cotton taproots were used as a bioindicator for Al toxicity. The ability to detoxify Al was highly correlated with the positions of OH and COOH groups.

It was found that short chain, carboxylic acids could be divided into three categories based on their role of detoxifying Al i) strong (citric, oxalic, tartaric) ii) moderate (malic, malonic, salicylic) and iii) weak (sucinic, lactic, formic, acetic, phthalic) (Hue *et al.*, 1986). The effectiveness in protecting roots against Al^{3+} toxicity is related to the number and positions of the OH/COOH groups on the main carbon chain. Organic acids that can form stable five or six ring structures with Al^{3+} provide the best protection (Hue *et al.*, 1986). Organic residues incorporated into the soil can cause a short term increase in soil pH as a result of complexing protons with organic acids and the consumption of protons with decarboxylation of organic acids (Haynes & Mokolobate, 2001). Organic acids are often released from the root apex of Al tolerant plants, triggered by an increase in soluble Al (Barcelo & Poschenrieder, 2002). Graminous plant species which exhibit tolerance to Al also release organic acids and the amount and type of organic acids differ among plant species (Ma *et al.*, 2014). Some plant species release organic acids immediately after exposure to Al in the soil, while others are known to have a more delayed response. Malate and citrate both detoxify Al in the soil by chelating with Al in the rhizosphere (Ma *et al.*, 2001). Fan *et al.* (2014) found that for rice bean (*Vigna umbellata*) that citrate secretion and regulation, respiration and protein synthesis were all processes important during the rice bean response to Al stress. They point out that root elongation inhibition is more likely associated with the down regulated genes responsible for transport of water and ions, and cell division.

Kobayashi *et al.* (2013) found that adding a non-alkalinising Ca fertiliser (gypsum) to the soil improved the tolerance of sensitive Arabidopsis (*Arabidopsis thaliana L.*) in moderately acid (pH_{H2O} 5.0-5.5) soil culture. These results fitted their model predictions that root toxicity to Al^{3+} and H^{+} in moderately acid soils show interactions between both ions in relation to Ca alleviation. The Ca: Al ratio is an indicator of the risk of Al toxicity in soils. A lower Ca:Al was found in forest soils compared to grassland soils at three sites high country sites in New Zealand, which highlights a greater risk of toxicity (Adams *et al.*, 2001). A study by Manoharan *et al.* (1996b) looked at the effects that the application of P fertilisers (Ca, F and SO_4) and changes in pH had on Al phytotoxicity at low P and high P pasture sites. Their results indicated that the Al^{3+}: Ca^{2+} activity ratio is the most consistent indicator of Al toxicity in soils irrespective of fertiliser effects.

Aluminium resistance mechanisms and genes have been identified in many plant species over the last 10 years. Work is currently being conducted by many research groups using molecular technologies and bioinformatics to create models of how Al^{3+} disrupts root growth either through damaging interactions at the apoplast or altering cellular mechanisms for DNA repair (Ryan & Delhaize, 2015). There has also been work conducted in which cultivars are chosen through genetic selection, to grow plants with a genetic aluminium resistance/tolerance to grow in acidic and Al toxic environments e.g. Maize (*Z. mays* L.), triticale (x Tritosecale), Arabidopsis (*A. thaliana* L.) and wheat (*Triticum aestivum* L.) (Kobayashi *et al.*, 2015; Krill *et al.*, 2010; Lazarević *et al.*, 2015; Ryan *et al.*, 2015).

2.8 Summary and research opportunities

A review of the literature produced the following findings:

- Significant challenges and variability (soil type, topography, aspect, climate and fertility) must be overcome to farm successfully in the high and hill country of New Zealand.
- Lime application is the most common amendment for remediating soil pH and aluminium issues. However in the high and hill country of New Zealand it is not always economic to apply lime, and it may take a number of years to see measureable effects.
- Site-specific factors drive the variation in soil extractable Al, however, the exact combination of factors and mechanisms involved with Al toxicity is poorly understood across different soils. There have been limited studies in New Zealand examining the drivers of soil pH and Al variability. Therefore the next step would be to conduct a study on New Zealand soils to determine which variables are influencing extractable Al concentrations and therefore potential Al toxicity on a national scale (Chapter 3).
- At a landscape scale, although studies have indicated potential variability of exchangeable Al related to landscape-scale effects, there has been no systematic study of exchangeable Al

variability at this scale. Therefore, in addition to the national scale study, a survey of a single catchment is required to determine which factors drive soil pH and extractable Al_{CaCl2} at a landscape scale (Chapter 4).

- Aluminium toxicity severely restricts the growth of legumes in acidic soils of the high and hill country of New Zealand. Legume species vary in their sensitivity to Al. Several studies have been conducted which have examined the growth of pasture legumes in acidic New Zealand high country soils, with different soil extractable Al concentrations. However, New Zealand has many soil orders and therefore it would be beneficial to examine the effects of soil type and pH on soil extractable Al_{CaCl2} in different soil orders, including volcanic soils from the North Island, using legume growth as bioindicators of Al toxicity. Establishing if there is relationship between legume shoot yield and soil extractable Al_{CaCl2} in different New Zealand soils and whether it differs among soils is valuable information for farmers and researchers for addressing this critical issue (Chapter 5).
- Plants have developed adaptations which allow them to live in toxic environments, through either exclusion or detoxification of Al. The root-soil interface (rhizosphere) is a zone for nutrient acquisition and also Al toxicity, as the roots are the primary site for Al toxicity. A study that examines the effects of plants and pH on Al mobilisation and immobilisation at the plant root scale in acidic New Zealand high country soils would be beneficial. With a particular focus on pasture legumes with differing sensitivity to acidity and Al toxicity (Chapter 6).
- There is an on-going debate in the scientific community around the success of the soil Al tests in measuring soil extractable Al and predicting Al toxicity. These tests do not seem to give reliable results on all soil types. This requires further investigation, as this soil test is the current measure that farmers have available to inform them of potential soil Al toxicity on their farms and to assist in land-use decisions. An experiment, which examines the methodology (molarity and extraction time) of the standard $CaCl_2$ and KCl soil Al tests and whether this alters the Al concentrations extracted for different New Zealand Soil Orders would be beneficial (Chapter 7). Results should be related to specific soil properties that may affect the quantity of Al extracted, as research to date has shown that the diagnostic tests for Al toxicity are not successful across all soil types.

3 National Soils Database Analysis

3.1 Introduction

The literature review chapter (Chapter 2) outlined the processes involved in soil acidification and the issues associated with Al toxicity in a wider context. Acidification and Al toxicity were identified as critical issues in New Zealand, particularly in high and hill country areas. A review of the literature identified possible variables, including soil chemical, physical or environmental that may influence Al toxicity in New Zealand soils. These variables included soil pH_{H2O}, total carbon, cation exchange capacity, base saturation, texture, annual rainfall, temperature, soil order, P retention, total Al and total nitrogen.

The objectives of this chapter were to determine, using a large database of New Zealand soils: i) if the same variables identified in the literature, or others, are important in influencing exchangeable Al; and ii) if soil orders of the New Zealand Soil Classification (NZSC) are effective in partitioning variability in exchangeable Al. Forthwith, the extractable Al determined by the KCl method are referred to as Al_{KCl}. The research question was "what are the key covariates of soil Al_{KCl} in New Zealand soils that enable exchangeable Al to be predicted"? The analysis was intended to provide further insights into the environmental and pedological controls on Al_{KCl}, and hence the areas in New Zealand where Al toxicity may be a problem. Furthermore, controls of Al_{KCl} at the national scale will provide a comparison against those revealed at a finer, (catchment-scale) study in the subsequent chapter.

3.2 Materials and methods

3.2.1 Data retrieval from the National Soils Database

This study used the National Soils Database (NSD) to extract information for soil samples with measured exchangeable Al_{KCl} (Landcare Research New Zealand Ltd, 1995). The NSD is a publicly funded primary collection of point, pedon-based data, which contains detailed information about soil samples collected from the 1950's to the 1990's from around New Zealand (Hewitt, 2013; Landcare Research New Zealand Ltd, 1995). Data collected by T. Webb up until 2005, from theses, dissertations and papers were also included in this dataset. The NSD contains soil descriptions of over 1500 soil profiles and all data were obtained from physical standardised sampling and observations at those sites. Information includes analytical data from standardised analytical procedures on soil chemistry, physical and mineralogical characteristics and environmental factors associated with each site sampled.

The NSD is a relational database that comprises a series of tables including sites, horizons, chemistry and mineralogy data (Figure 3.1). Each site record is related in a one-to-many way with horizon records, which include attributes such as designation, boundary, colour and structure. Each horizon record has a one-to-many relationship with records of the samples table, which describes samples taken from the horizons, and then uniquely identifies them by the field Sample ID. Chemical data recorded in tables CHEM 1 and CHEM 2 can be associated with horizon and site data through the Sample ID, and the combination of ident and horizon number, respectively. A similar mode of linking as CHEM 1 and CHEM 2 applies to the XRF table, which stores total element data.

In order to retrieve data from the NSD, a query was created in ACCESS 2003. The tables with the required information related to the soil samples were selected. The tables included NZSITES, NZHOR, NZSAMPLE, NZCHEM1, NZCHEM2 and NZXRF. NZSITES and NZHOR were linked by Ident, NZHOR and NZSAMPLE were linked by Ident and Horizon number, NZCHEM1 and NZCHEM2 were both linked to NZSAMPLE by Sample ID. It was very important to have the correct links between tables to ensure the data were correctly associated. Once the tables were connected, the next step was to select the variables to be included in the data output. A criterion was created for Al_{KCl} of >0 $cmol_c/kg$, to ensure that all records extracted from the database have an Al_{KCl} measurement of greater than zero. The Al_{KCl} measurement was the original measurement used in New Zealand to identify potential soil Al toxicity. This has since been replaced by the Al_{CaCl2} test at commercial laboratories. However, in the database there was no measure of $Al_{CaCl2,}$ therefore, Al_{KCl} was used in this analysis. In order to create a model for Al_{KCl} concentrations in New Zealand soils, the analysis was aimed at evaluating pre-selected variables from the literature that were possible ("best bet") drivers of Al. There were also variables included that are theoretically possible but untested. Once all the variables were chosen, tabulated data were imported into Microsoft Excel.

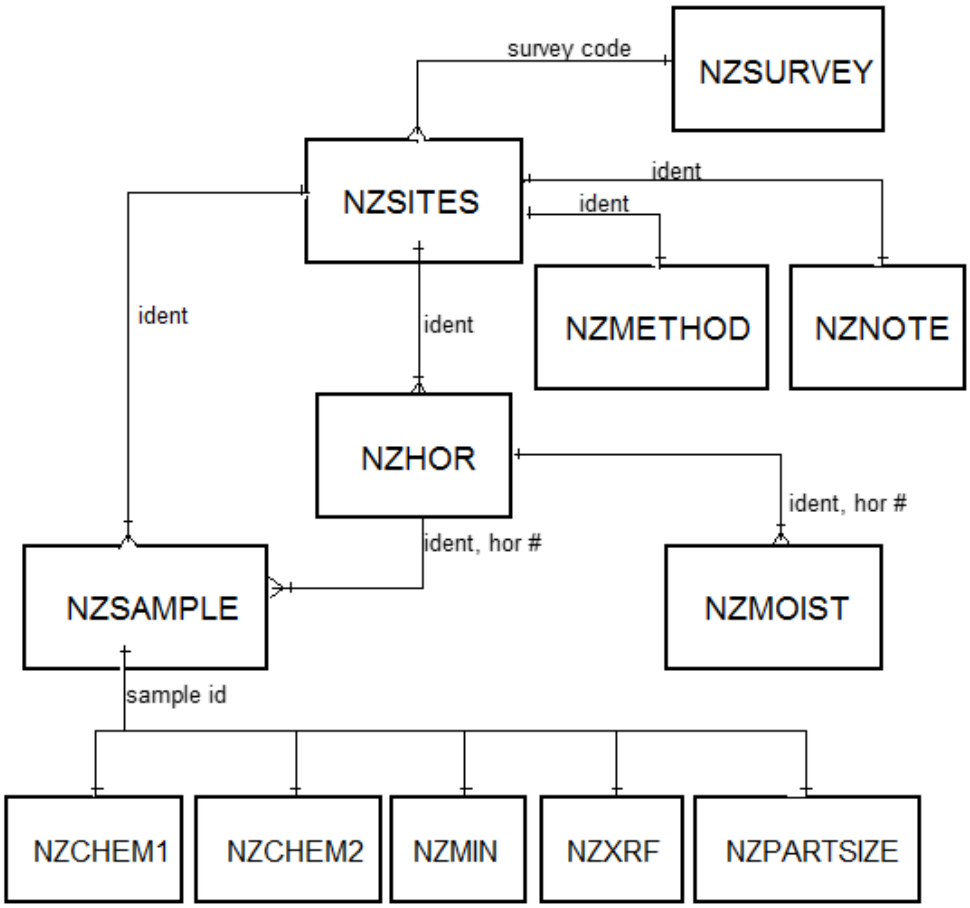

Figure 3.1 The structure of the New Zealand soils database, the tables and links between tables for data extraction. From Willoughby *et al.* (2001), with permission.

3.2.2 Formatting of data

Records with missing data in any field were deleted. Variables extracted for analysis were sample depth, annual rainfall, texture, soil order, pH_{H2O}, total C, total N, P retention, CEC, BS, Al_{KCl}, and total Al. The dataset had 1027 samples in total. The analysis was used to compare the relationships of Al_{KCl} with other soil variables and to determine how much variation is explained by the variables present. Soil order was used in this study to determine soil classification and broad patterns of Al_{KCl} concentration. Lower categories in the system, such as group or subgroup, were not considered. The methods of measurement and explanation of variables included in the analysis are shown in Table 3.1. The data were intersected, using the GPS coordinates, with the NIWA median annual rainfall (50^{th} percentile) to give a more accurate measurement of rainfall at each site than was in the database (National Institute of Water and Atmospheric Research, 2015). Other climate measures included in the analysis were annual temperature (50^{th} percentile = Median annual average temperature) and a measure of precipitation variability (80^{th} percentile annual total rainfall-20^{th} percentile annual total rainfall). The latter variable was included to represent the frequency of wetting and drying events (Dixon *et al.*, 2016; Webb *et al.*, 1986).

Texture was re-formatted to reduce the number of texture classes and improve comparisons. The texture modifiers fine, coarse, stony, deep and moderate at the start of a textural name were ignored. For example, coarse sandy loam became a sandy loam and shallow silt loam became a silt loam. In order to include texture as a numerical value to be included in analysis, it was important to change the qualitative textural names into percentages of sand, silt and clay. The Soil Description Handbook (Milne *et al.*, 1995a) was used as a guide. Each texture was allocated size class percentages based on the centroid of texture classes defined on the texture triangle. Textures included in the dataset that were not defined in the New Zealand soil texture classification, were given percentage values based on the USDA system (Schoeneberger *et al.*, 2012). It is important to note that the percentages were not specifically measured for each soil and were, therefore, an estimate based on their textural class. Clay % was used as a texture variable in the data analysis to determine if it is a key factor driving Al concentrations (Table 3.2).

Table 3.1 Explanation of variables included in the soil analyses, method of measurement and source of data.

Variable	What does it measure?	Method used	Data source
pH_{H2O}	Acidic or basic.	1:2.5 (soil to DI water), (Blakemore *et al.*, 1987)	NSD
BS	% of exchange sites occupied by basic cations (Ca, Mg, K and Na)	(Blakemore *et al.*, 1987)	NSD
CEC	total number of cation sites ($cmol_c/kg$)	(Blakemore *et al.*, 1987)	NSD
Total C	total carbon in the soil (%)	High frequency induction furnace (Blakemore *et al.*, 1987)	NSD
Total N	total N in the soil (%)	Digestion to convert N into $(NH_4)_2SO_4$. NH_4^+ N determined by distillation and titration or colorimetrically (Blakemore *et al.*, 1987)	NSD
% clay	The fraction of clay	Measured in field and classified (Milne *et al.*, 1995a) NZ soil classification	NSD
Depth	Depth of sample (base)	Measured in field	NSD
Soil order	The NZ soil order the sample belongs to.	NZ soil classification	NSD
P retention	Describes the P immobilisation potential of the soil (ASC %).	Standard method (Blakemore *et al.*, 1987)	NSD
Al_{KCl}	Exchangeable aluminium (Al^{3+}) ($cmol_c/kg$)	1 M KCl extraction	NSD
Al total	total Al present in the soil. (%)	It uses nitric acid and hydrogen peroxide to dissolve and measure total metal that could become available (Kovacs *et al.*, 2000)	NSD
Median annual rainfall	Median annual total rainfall ($mm\ y^{-1}$)	50^{th} percentile	NIWA
Median annual temperature	Median annual average temperature (°C)	50^{th} percentile	NIWA
Precipitation variability	Rainfall range ($mm\ y^{-1}$)	80^{th}-20^{th} percentile	NIWA

Table 3.2 The percentage values of sand (0.6-2.0 mm), clay (<0.002 mm) and silt (0.06-0.002 mm) given to each texture class (Milne *et al.*, 1995a; Schoeneberger *et al.*, 2012).

Soil Texture	% Sand	% Clay	% Silt
Clay	15	70	15
Clay loam	45	30	25
Loamy sand	70	5	25
Loamy clay	40	50	10
Loamy silt	30	10	60
Sand	90	5	5
Sandy loam	60	15	25
Silt	5	5	90
Silt loam	20	25	55
Silty clay loam	20	40	40
Silty clay	10	50	40
Sandy clay loam	60	30	10

The final formatting of data were related to the statistical analysis itself. For Multiple Regression Analysis, one of the assumptions is that the data are independent. Soil samples from the NSD that were collected from the same site, at different depths, are correlated. Therefore, it was important to split the data into different depth zones and average data within the depth zones from within a soil profile, to achieve independence. This meant that for each site, there was only one soil sample value (the average) for each depth zone. All samples from shallower than 20 cm depth were grouped into a 20 cm depth zone, samples between >20 and ≤50 were grouped into the 50 cm depth zone and samples between >50 and ≤120 cm were grouped into the 120 cm depth zone. Any samples deeper than 120 cm were not included in the analyses, as they were not considered to have agronomic importance. The 20 cm zone represents the main root zone for many plants but samples up to 120 cm deep are considered because lucerne roots will grow down to this depth to access water and nutrients.

3.2.3 Statistical Analysis

Multiple regression analysis was conducted in GenStat 16.0 to determine which soil and environmental parameters best explained the variation in Al_{KCl} concentrations in the soil. In order to carry out analyses on soil Al_{KCl}, this measure was log transformed to improve the fit of the models and satisfy normal distribution requirements. For the final multiple regression model and single variable regressions against log Al_{KCl}, the fit of the model (R^2) was compared between linear and quadratic polynomial components (squared terms). Adding non-linear components did not improve the model and, therefore, a linear model was used as a best fit model for the data. A series of criteria was created for the removal of variables from the model in order to create the best model for each depth zone, as follows:

1) Removed the single variable with the lowest absolute *t* value from the table of multiple regression coefficients.
2) A variable was only removed from the model if the change in the regression sum of squares was not statistically significant at $P<0.05$, as described below:

The F ratio was calculated for each step and compared to the appropriate 5% critical value for *F*. The 5% critical value for *F* was determined using the degrees of freedom (d.f) of the residual in the full model as the denominator d.f., and the number of terms removed from the model (always 1) as the numerator d.f. If the calculated F ratio was less than the 5% critical value for *F*, the removal of the term resulted in a non-significant change to the model, meaning the term could be removed. If the calculated F ratio was greater than the 5% critical value for *F*, then the removal of that variable caused a significant change in the model and, therefore, that term must remain in the model. The F ratio was calculated by subtracting the Sum of Squares of the current regression model from the previous model S.S and dividing by the change in degrees of freedom (d.f) between the two models. The value calculated was then divided by the residual mean square of the full model.

The variables that were significant for Al_{KCl} in the final model at each depth zone were plotted in a series of graphs to show the relationship between Al_{KCl} concentration and the variable. The $logAl_{KCl}$ concentrations in the graphs were adjusted using the multiple regression coefficients for each variable from the final model and the mean for each variable. Each coefficient can be thought of as the slope of the relationship between log Al_{KCl} and the particular variable, after adjustment for the relationship between log Al_{KCl} and all other terms in the model. The statistical analysis was conducted on log transformed data. The graphs are presented as log Al_{KCl} adjusted for the other variables in the final model, while in the soil order section, means have been back-transformed after analysis on log data and are unadjusted.

The Stepwise Backwards Regression produced a final model for each depth zone that included the most important variables in relation to Al_{KCl}. However, this analysis was unable to determine the relative weighting of each variable in overall importance for contributing to the variance in soil Al_{KCl} concentrations, within the total variance accounted for by the model. This was because each variable was adjusted for the other variables in the model. To give the relative importance of different variables in relation to Al_{KCl}, linear regressions were conducted for each variable singly to give an idea of the variance accounted for by one variable. The regression was conducted with soil order as groups, as was conducted in the main stepwise regression. The % variance accounted for could then be compared

between variables and to the full model. It is also important to recognize that some of the variables are cross correlated (Appendix Tables 1.1-1.3). Therefore, there may be only a small number of variables of importance for soil Al_{KCl} concentrations, however, these may include the effect of other correlated variables.

Soil order was identified as a significant factor associated with Al_{KCl} concentrations in all depth zones in the final model and, as such, soil order was investigated further. In addition, a one-way analysis of variance (ANOVA) was conducted for each of the depth zones between the variate log Al_{KCl} and soil order, to determine if soil order significantly affected soil Al_{KCl}. A table of means and the number of samples for each depth zone was determined. Unlike the multiple regression, the Al_{KCl} concentration was not adjusted for other variables. From this information, the four soil orders with the largest sample sizes were selected (Brown, Pallic, Recent and Podzol) to determine differences in Al_{KCl} concentrations between depths within a soil order and among soil orders for a depth zone. In addition, an analysis of covariance was conducted in which log Al_{KCl} was the variate and depth was the covariate across the soil orders. This analysis produced a table of adjusted means of log Al_{KCl} for each of the soil orders, adjusted for a common x value (x = mean of all depths). Fisher's protected LSD (5%) was used to determine significant differences between the mean Al_{KCl} concentrations of the different soil orders overall.

3.3 Results

3.3.1 Stepwise regression between log Al_{KCl} and soil variables in three depth zones.

Table 3.3 highlights the depth trends of the properties identified as significant in the final models for each of the depth zones. The final reduced models from the stepwise regression for each depth zone are presented in the appendix (Section 1.1). Going from the full model, with all terms included, to the final reduced model for all depth zones, there was no appreciable difference in the variance accounted for in regard to logAl_{KCl} concentrations.

Table 3.3 The multiple regression coefficients for the variables that were significant in the final multiple linear regression model (stepwise backwards regression) for log Al_{KCl}, the significance level for each variable and the overall variance in log Al_{KCl} which is explained by the model in the 20 cm, 50 cm and 120 cm depth zones.

	20 cm	50 cm	120 cm
	Estimate	Estimate	Estimate
Final Model variables			
BS	-0.01728***	-0.01238***	-0.00709***
Total C	ns	-0.1179***	-0.1283***
CEC	0.02087***	0.04079***	0.03849***
Total N	-0.935***	ns	1.105**
pH_{H2O}	-0.2680***	-0.5107***	-0.5103***
% Variance accounted for	78.2	83.9	84.0
SEM	0.02	0.02	0.02
Soil Order	**	***	***

Note: *** *P*<0.001, ** *P*<0.01, * *P*<0.05, ns=non-significant *P*>0.05 in multiple regression model for log $Al_{KCl.}$ Tables of the correlations between soil variables in each depth zone are presented in the appendix (Tables 1.1-1.3). Final outputs for the stepwise regressions are presented for the three depth zones in the appendix (Section 1.1). Overall soil order was significant and the levels are shown in the Table 3.3. For individual soil order coefficients refer to the appendix (Section 1.1).

Base saturation, CEC and pH_{H2O} were all highly significant (*P*<0.001) variables in regard to Al_{KCl} concentrations in the soil at all depth zones. Total C was significant in the 50 cm and 120 cm depth zones. Total N was an important factor associated with Al_{KCl} in the 20 cm and 120 cm depth zones. Other variables that were potential drivers of Al_{KCl} and included in the full model were: median annual rainfall, median annual temperature, precipitation variability, P retention, total Al (%) and clay%. However, none of these variables were significant across all depth zones. This dataset is complex, as some of the soil variables are correlated to the variables that were significant in the final models.

To determine which variables were most important (singly) in relation to Al_{KCl} concentrations, individual regression of variables "with groups" (soil order) were conducted (Table 3.4). The results are shown across all soil orders and not for individual soil orders. The Al_{KCl} concentration trends for particular soil orders are investigated further in Section 3.3.3. The variance accounted for by the individual variable regressions and the overall variance accounted for from the reduced multiple regression model are presented. Note that some of these single regressions may also include the (unknown) indirect effects on log Al_{KCl} of correlated explanatory variables. Soil order was more important in relation to log Al_{KCl} concentrations in the 50 cm and 120 cm depth zones (41.8 and 45.1%) than in the 20 cm depth zone (18.9%). In the 20 cm depth zone, base saturation and pH_{H2O} were most important in relation to log Al_{KCl}. Base saturation decreased in % variance accounted for with increasing depth, while soil pH increased in importance with depth. In the 50 cm depth zone, base saturation,

pH_{H2O} and CEC were most important. In the 120 cm zone, base saturation and pH_{H2O} were important, as were CEC, carbon and nitrogen.

Table 3.4 Total variance accounted for (%) of single variables against Al_{KCl} at different depths, compared with the final variance accounted for (%) by the final multiple regression model.

	20 cm	50 cm	120 cm
Soil order	18.9	41.8	45.1
Variables fitted as straight lines			
BS	71.2	66.8	62.3
Total C	-	42.6	47.0
CEC	18.5	45.5	52.9
Total N	20.1	-	47.0
pH_{H2O}	51.3	67.1	71.6
Total Variance accounted for by Reduced model	78.2	83.9	84.0

Note: Single variables were fitted as linear regressions with groups (soil order) for each depth zone against log Al_{KCl}. (-) is given for variables which were not significant (P>0.05) in the final model for a depth zone.

3.3.2 Relationships between log Al_{KCl} and other soil variables

This section shows the relationship between the variables that were significant in the final multiple regression model and logAl_{KCl} at the different depth zones (Table 3.3). The logAl_{KCl} has been adjusted for all variables using the coefficients from the final multiple regression model and the mean of each variable from each depth zone. Thus, the graphs give a visual representation of the relationship between each factor and logAl_{KCl} in the final model.

3.3.2.1 Total nitrogen

In the 20 cm depth zone, there was a strong relationship (P<0.001) between total nitrogen (N) and Al_{KCl} (Figure 3.2a). The soil N and Al relationship showed a moderate (R^2=0.53) negative relationship across the nitrogen range of 0.0 to 1.8%. Peak log Al_{KCl} concentrations of 0.75 occurred in soils with a lower N content.

In the 120 cm depth zone there was a significant (P<0.01) relationship between nitrogen and the aluminium concentration (Figure 3.2b). The soil nitrogen and Al relationship showed a weak (R^2=0.08) positive relationship across the N range of 0.0 to 0.8%. The trend was opposite to that in the 20 cm zone. In the 120 cm zone, there was high Al and low N, while the reverse was true in the 20 cm zone. The majority of the data were clustered with a N content of <0.25% and there was not a clear trend. There were few samples with a N content >0.3% in this depth zone.

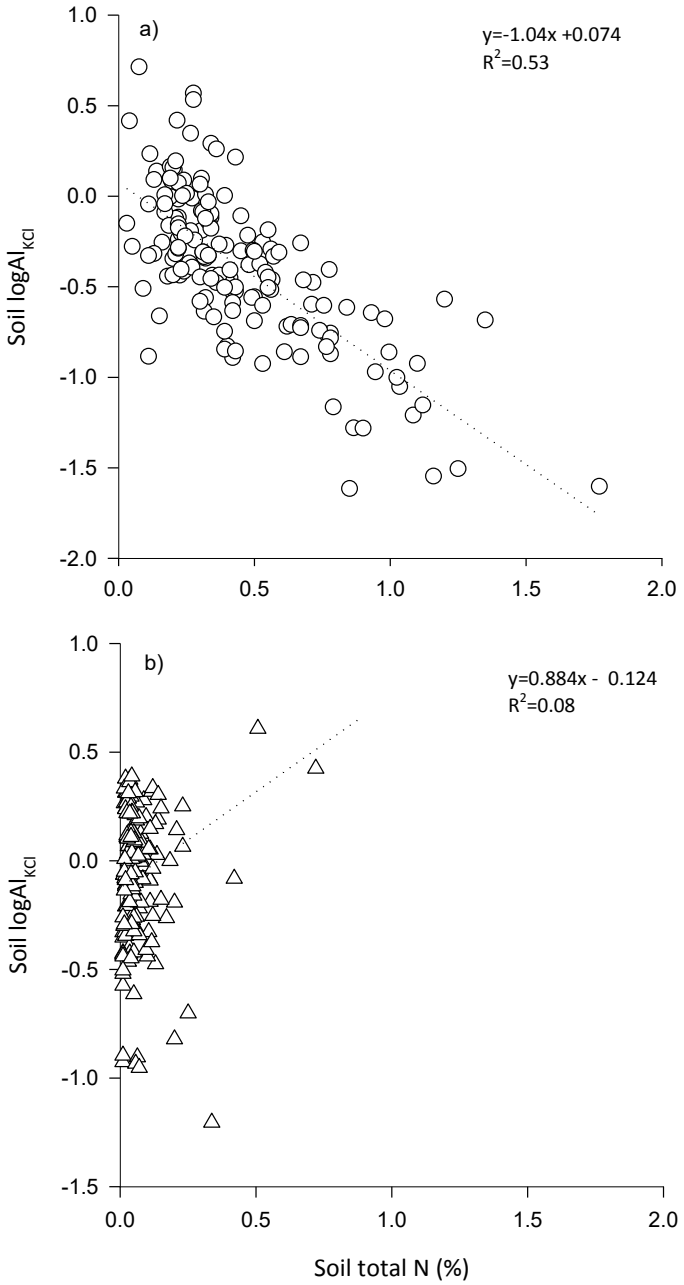

Figure 3.2 The soil relationship between log Al_{KCl} and total nitrogen (%) in the a) 0-20 cm (○) and b) 50-120 cm (△) depth zones. Regressions were adjusted to account for the terms in the final multiple linear regression models for each depth (Table 3.3).

3.3.2.2 Cation exchange capacity (CEC)

A positive (*P*<0.001) relationship existed between CEC and logAl_{KCl} at all depths (Figures 3.3a- 3.3c). The fit of the line increased with depth in the profile for the 20 cm (R^2=0.37), 50 cm (R^2=0.69) and 120 cm (R^2=0.72) depth zones. Furthermore, the slope of the line increased below the 20 cm depth zone: 20 cm-0.02; 50 cm- 0.04; and 120 cm- 0.04).

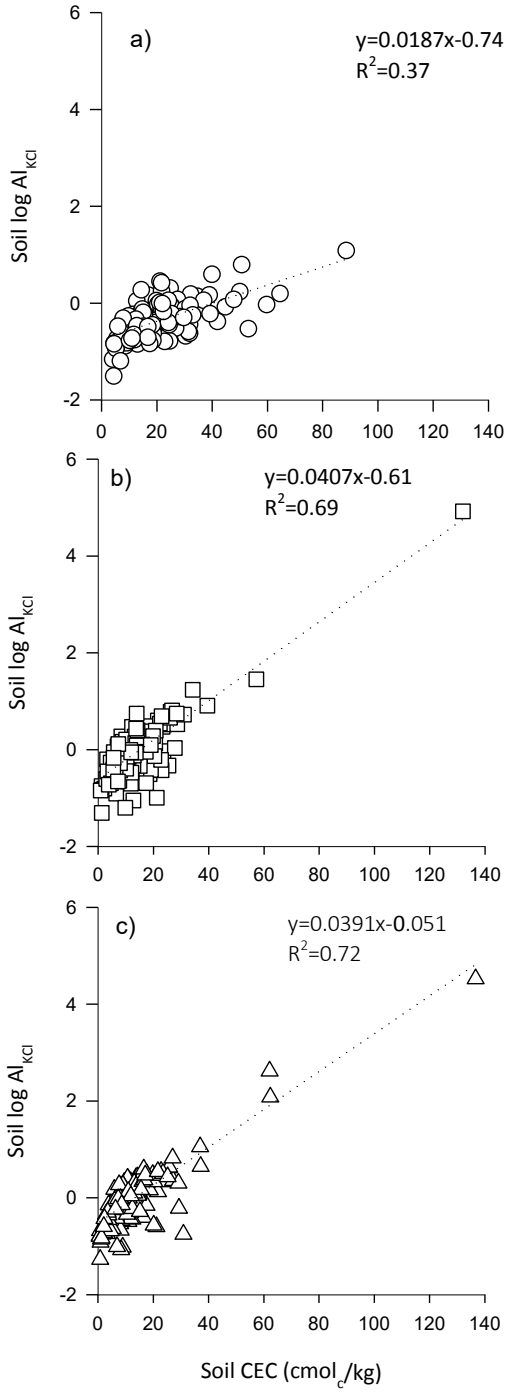

Figure 3.3 The soil relationship between log Al_{KCl} and CEC ($cmol_c$/kg) in the a) 0-20 cm (○), b) 20-50 cm (□) and c) 50-120 cm (△) depth zones. Regressions were adjusted to account for the terms in the final multiple linear regression models for each depth (Table 3.3).

3.3.2.3 Base saturation

A negative (*P*<0.001) relationship existed between base saturation (%) and logAl_{KCl} at all depths (Figures 3.4a-3.4c). The fit of the line declined with an increase in depth and the slope of the line declined below the 20 cm depth zone: 20 cm-0.02; 50 cm- 0.01; and 120 cm- 0.00).

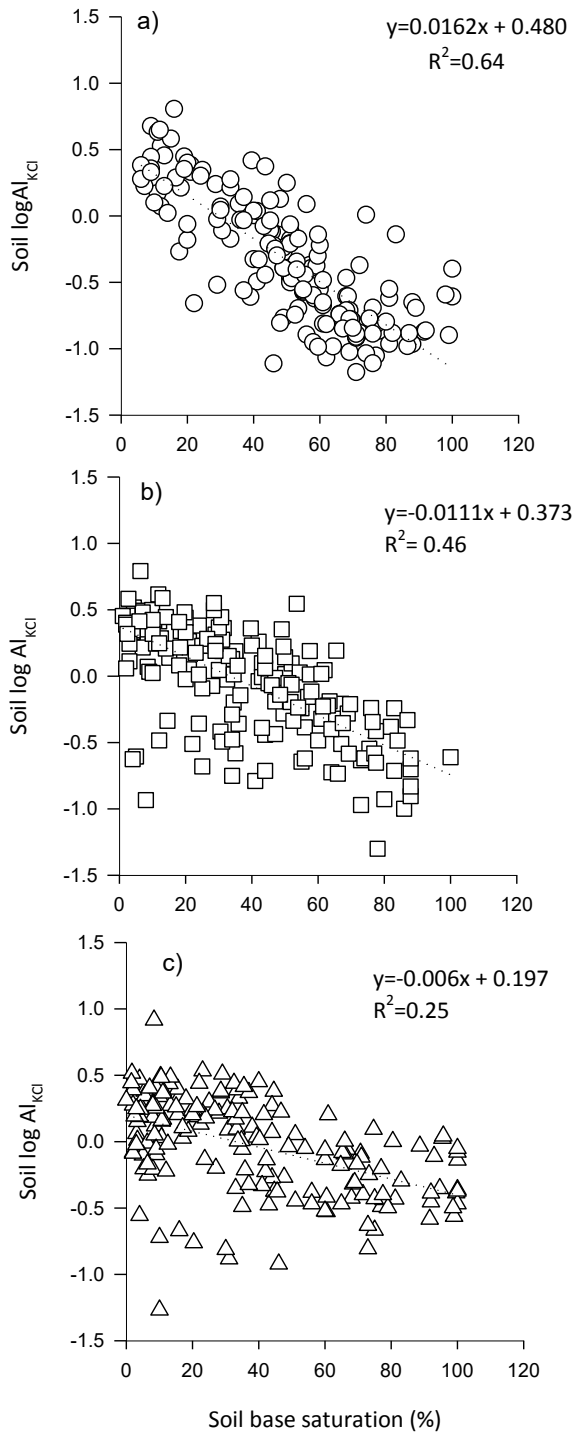

Figure 3.4 The soil relationship between log Al_{KCl} and base saturation (%) in the a) 0-20 cm (○), b) 20-50 cm (□) and c) 50-120 cm (△) depth zones. Regressions were adjusted to account for the terms in the final multiple linear regression models for each depth (Table 3.3).

3.3.2.4 Total carbon

In the 50 cm depth zone, there was a strong negative relationship (P<0.001, R^2=0.68) between total carbon and $logAl_{KCl}$ (Figure 3.5a). However, the majority of data appeared to cluster with carbon levels <10% and few samples had carbon levels >10%.

There was a strong negative relationship (P<0.001, R^2=0.75) between total carbon and $logAl_{KCl}$ concentrations in the 120 cm depth zone (Figure 3.5b). However, few samples had >10% carbon.

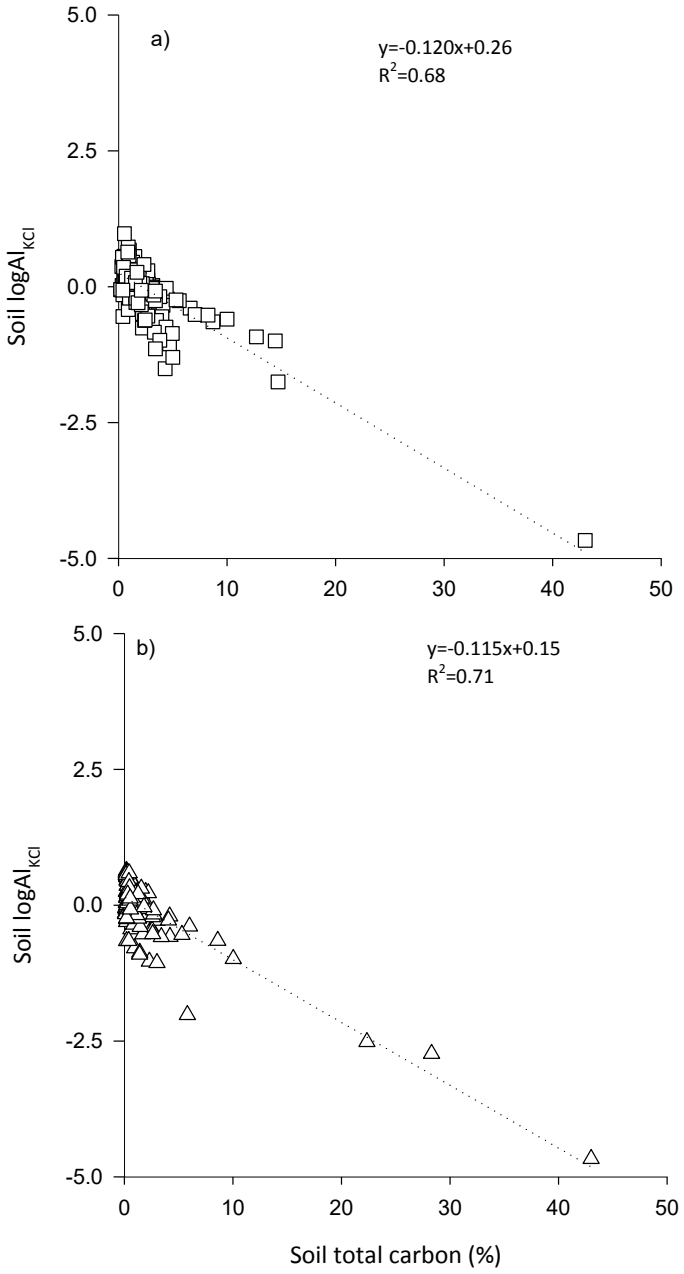

Figure 3.5 The soil relationship between log Al_{KCl} and total carbon (%) in the a) 20-50 cm (□) and b) 50-120 cm depth zones. Regressions were adjusted to account for the terms in the final multiple linear regression models for each depth (Table 3.3).

3.3.2.5 Soil pH

There was a negative relationship (P<0.001) between log Al_{KCl} and soil pH_{H2O} at each of the three depth zones, however, the fit of the line varied (Figures 3.6a-3.6c). The fit of the line increased with depth for the 20 cm (R^2= 0.15), 50 cm (R^2= 0.49) and 120 cm (R^2= 0.59) depth zones. Furthermore, the slope of the line increased below the 20 cm depth zone: 20 cm-0.2; 50 cm- 0.6; and 120 cm- 0.6).

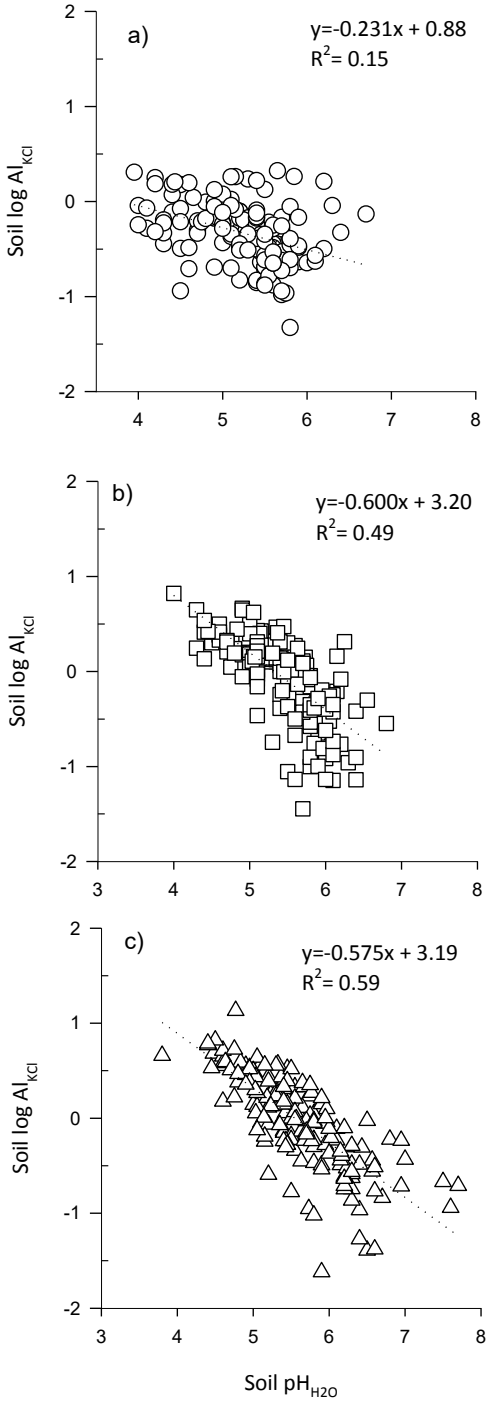

Figure 3.6 The soil relationship between log Al_{KCl} and soil pH_{H2O} in the a) 0-20 cm (○), b) 20-50 cm (□) and c) 50-120 cm (△) depth zones. Regressions were adjusted to account for the terms in the final multiple linear regression models for each depth (Table 3.3).

3.3.3 Soil order and Al_{KCl} concentrations

Soil order had a significant influence on the concentrations of extractable Al_{KCl} in the soil (P<0.001) for all depth zones (Table 3.5). In the 20 cm depth zone, Pumice Soils had the highest mean Al_{KCl} concentration (2.3 $cmol_c$/kg), followed by Podzols and Brown Soils (1.1 and 0.8 $cmol_c$/kg). However, Pumice Soils had one sample as part of the analysis and, therefore, the mean does not reflect a true mean of that soil order. Granular, Oxidic and Melanic Soils had the lowest mean Al_{KCl} concentrations (0.1, 0.1 and 0.2 $cmol_c$/kg). In the 50 cm depth zone, the Organic Soils had the highest mean concentration of Al (5.2 $cmol_c$/kg), followed by Ultic, Podzols and Brown Soils (3.2, 2.6 and 2.0 $cmol_c$/kg). Allophanic Soils had the lowest soil Al_{KCl} concentration in this depth zone (0.2 $cmol_c$/kg). In the 120 cm depth zone, the highest mean Al_{KCl} concentration was for Ultic Soils (10.8 $cmol_c$/kg), then Organic, Podzols and Brown Soils (4.7, 1.7 and 1.6 $cmol_c$/kg). The Pumice and Allophanic Soils had the lowest mean Al_{KCl} concentrations (0.1 and 0.2 $cmol_c$/kg).

Table 3.5 Back-transformed mean Al_{KCl} concentration ($cmol_c$/kg) from an ANOVA between log Al_{KCl} and soil order for soil samples from 20 cm, 50 cm and 120 cm depth zones.

Depth zone		20 cm	50 cm	120 cm
Soil order	symbol	Mean Al	Mean Al	Mean Al
Brown	B	0.8 (45)	2.0 (50)	1.6 (50)
Melanic	E	0.2 (3)	0.3 (6)	0.4 (6)
Gley	G	0.2 (15)	0.7 (18)	0.9(18)
Allophanic	L	0.3 (20)	0.2 (14)	0.2 (14)
Pumice	M	2.3 (1)	0.2 (1)	0.1 (1)
Granular	N	0.1 (1)	1.0 (1)	0.8 (1)
Organic	O	0.3 (5)	5.2 (3)	4.7 (5)
Pallic	P	0.3 (27)	0.7 (34)	0.7 (33)
Recent	R	0.2 (22)	0.3 (23)	0.2 (23)
Ultic	U	0.5 (5)	3.2 (6)	10.8 (7)
Oxidic	X	0.1 (3)	1.0 (1)	2.3 (3)
Podzol	Z	1.1 (17)	2.6 (19)	1.7 (18)
Grand mean		0.4 (163)	0.9 (139)	0.9 (178)
SEM (upper/lower)		(0.46, 0.38)	(0.98, 0.82)	(0.95, 0.81)
P value		<0.001	<0.001	<0.001

Note: The number in parentheses represents the number of sites sampled for each soil order (n) in each depth zone. Samples from the same site were averaged within a depth zone.

Because of low sample numbers of certain soil orders in this dataset, four soil orders were selected for further analysis to determine their relationship between soil order, Al_{KCl} and depth (Table 3.6). The Al_{KCl} concentrations in Brown Soils were significantly different across the three depth zones (P<0.01) and had a mean Al_{KCl} concentration of 1.4 $cmol_c$/kg. The highest Al_{KCl} concentrations were measured in the 50 cm and 120 cm depth zones and there was no significant difference between Al at these two depths. The trend of lower Al_{KCl} in the upper depth zone also occurred in Pallic Soils, however, the mean Al_{KCl}

concentrations were much lower than in the Brown Soils. There was no difference (P>0.05) between the Al_{KCl} concentrations measured within the three depth zones in the Recent Soils and Podzols.

Table 3.6 Back-transformed mean Al_{KCl} concentration ($cmol_c/kg$) of four soil orders across three depth zones (cm) in the soil profile.

Soil Order	Brown	Pallic	Recent	Podzol	Grand mean	SEM (upper/lower)	*P* value
20 cm	0.8_{bA}	0.3_{bB}	0.2_{aB}	1.1_{aA}	0.5	(0.59,0.46)	<0.001
50 cm	2.0_{aA}	0.7_{aB}	0.3_{aC}	2.6_{aA}	1.1	(1.19,0.99)	<0.001
120 cm	1.6_{aA}	0.7_{aB}	0.2_{aC}	1.7_{aA}	0.9	(0.99,0.81)	<0.001
Grand mean	1.4	0.6	0.2	1.7			
SEM (upper/lower)	(1.55,1.27)	(0.79,0.63)	(0.26,0.21)	(1.97,1.45)			
P value	0.003	0.012	0.323	0.062			

Note: Numbers with letter subscripts in common are not different (α= 0.05), based on the Fisher's protected LSD. Capital subscript letters refer to differences along the rows (among soil orders) and lower case subscript letters refer to column differences (within a soil order).

For all depth zones, the Brown Soils and Podzols had the highest mean Al_{KCl} concentrations, and there was no difference (P<0.05) between the Al_{KCl} concentrations of these soil orders. In the 20 cm depth zone, the Pallic and Recent Soils had lower (P<0.05) mean log Al_{KCl} concentrations compared with Brown Soils and Podzols, and there was no difference (P<0.05) between the Pallic and Recent Soils. The mean Al_{KCl} concentrations for the Brown Soils and Podzols were highest in the 50 cm depth zone at 2.0 and 2.6 $cmol_c$/kg, respectively. Pallic Soils were the intermediate soil order with a mean Al_{KCl} concentration of 0.7 $cmol_c$/kg, which was different to the other soil orders (P<0.05). Recent Soils had the lowest Al_{KCl} concentration of 0.3 $cmol_c$/kg. The same trends occurred in the 120 cm depth zone. Pallic Soils were the intermediate soil order with a mean Al concentration of 0.7 $cmol_c$/kg and Recent Soils had lower (P<0.05) log Al_{KCl} concentrations compared with the other soil orders.

In an ANOVA which was adjusted for depth, mean log Al_{KCl} concentrations were highest in Brown Soils and Podzols, 1.4 and 1.7 $cmol_c$/kg respectively, which were significantly higher than Pallic and Recent Soils (Table 3.7). Pallic Soils were the intermediate soil with a mean Al concentration of 0.6 $cmol_c$/kg, which was significantly different from Brown, Podzol and Recent Soils. Recent Soils had a significantly lower mean Al_{KCl} concentration of 0.2 $cmol_c$/kg.

Table 3.7 Back-transformed mean Al_{KCl} concentration ($cmol_c$/kg) of four soil orders from an ANOVA, which was adjusted for depth as a covariate.

Soil order	Mean Al_{KCl}	Sample number
Brown	1.4_{a}	145
Pallic	0.6_{b}	94
Recent	0.2_{c}	68
Podzol	1.7_{a}	54
Grand mean	0.8	361
SEM (upper, lower)	(0.86, 0.76)	
P value (soil order)	<0.001	
P value (soil depth)	0.010	

Note: Numbers with letter subscripts in common are not different (α= 0.05) based on the Fisher's protected LSD.

3.4 Discussion

3.4.1 Al_{KCl} concentrations

The Al_{KCl} concentrations used in this study are those extracted by 1M KCl and are considered to be exchangeable Al removed from cation exchange sites in the soil (Parfitt, 1980). Studies conducted relating to KCl extractable Al are discussed in the literature review (Chapter 2) and the extraction method itself is investigated for several New Zealand soils in Chapter 7 (laboratory investigation). The

Al_{KCl} measurement was the original measurement used in New Zealand to identify potential soil Al toxicity. This has since been replaced by the Al_{CaCl2} test at commercial laboratories. However, in the NSD there was no measure of $Al_{CaCl2,}$ therefore, Al_{KCl} was used in this analysis. The concentrations can be related to thresholds for toxicity for certain plants species in the literature (Edmeades *et al.*, 1983; Wheeler *et al.*, 1992).

3.4.2 Relationships between log Al_{KCl} and other soil variables

The variables found to be significant for soil Al_{KCl} concentrations among the depth zones have been categorised in terms of their relationship with $Al_{KCl.}$ These include supply, capacity, availability and response variables, which are discussed in subsequent sections. Supply variables are those that directly influence the solubility and quantity of Al in the soil, which includes soil pH and base saturation. Capacity variables are those that affect the capacity of the soil to retain Al_{KCl}, which includes CEC. Supply and capacity variables are difficult to separate. Availability variables are those that reflect the capability of the soil to complex and adsorb Al and hence make it non-extractable by KCl including total carbon. Response variables are those variables that, rather than being key drivers of Al_{KCl} concentrations, are likely to respond to Al_{KCl} concentrations in the soil, such as total N.

3.4.2.1 Supply and capacity related variables (pH, BS and CEC)

Soil pH is known to be an important factor associated with Al_{KCl}, which was also evident by its significance in the final model for each depth zone. The relationship between pH_{H2O} and Al_{KCl} differed among the depth zones. The strongest relationships were in the 50 cm and 120 cm depth zones, where as expected, soil pH_{H2O} increased and Al_{KCl} concentration declined (Figure 3.6b and c). The pH affects the solubility of Al in the soil (Krstic *et al.*, 2012), which influences the supply of Al_{KCl}, but also affects the CEC via variable charge and complexation. However, although significant in the final model, there was a poor relationship in the 20 cm zone and there was an increase in the sensitivity of the relationship with an increase in depth in the soil profile, inferred from the slope of the line. Note that this could be a result of the Al_{KCl} being adjusted for other variables in the model and that soil pH_{H2O} correlates with other variables that have a stronger influence on extractable Al_{KCl} in the 20 cm depth zone, such as base saturation, as discussed below.

There appears to be a threshold in the dataset, particularly shown in the 50 cm and 120 cm depth zones, where the variance in logAl_{KCl} concentration appears to increase, above a pH_{H2O} of 5.5, with a larger scatter in the data around the trend line. It is apparent that there is a stronger relationship between pH_{H2O} and log Al_{KCl} concentrations at a pH_{H2O} <5.5 and higher Al_{KCl} concentrations for this

dataset. Other studies have noted similar thresholds when plotting the pH and Al_{KCl} concentrations. Parfitt (1991) found that Al_{KCl} concentrations increased at a soil pH_{H2O} below 5.5, for soil samples from the NSD with Al_{KCl} concentrations > 2.0 $cmol_c$/kg. Moir and Moot (2010) observed a strong relationship between soil pH_{H2O} and Al_{KCl} in the top 15 cm of a high country shallow and stony Brown Soil from North Canterbury. Two critical change points were identified in their study, at pH_{H2O} 5.5 and pH_{H2O} 5.8, at which there was an increase in Al_{KCl} concentrations. Their study supports the existence of a threshold in the Al extracted from the soil by KCl at pH_{H2O} 5.5. The relationship between pH_{H2O} and Al_{KCl} reported here is not as strong as was found by Moir and Moot (2010), even in a similar depth zone. However, this could be attributed to the Al_{KCl} concentration having been adjusted for other parameters in the final model and that many soil orders have been included in the current analysis. Soil pH affects the solubility of Al and therefore is a primary control of the exchangeable Al in the soil. The more acidic the soil is (below pH 5.5), the more acidic cations are present to occupy cation exchange sites and the relative activity of Al^{3+} in the soil increases (Kinraide, 1991).

Overall, the soils were more acidic in the top 20 cm and the pH_{H2O} increased with depth (Figure 3.6). However, the mean Al_{KCl} concentration across all soils was lower in the top 20 cm and increased with profile depth. This is despite there being a relationship of a decline in Al_{KCl} with increasing pH_{H2O}. This can be explained by the spread of the data in the top 20 cm, which was greater at any given pH, and the higher Al_{KCl} concentrations, which were present deeper in the profile, and not in the upper horizon which reduced the overall mean Al_{KCl}. Nevertheless, mean Al_{KCl} was present at concentrations that could be toxic to plants in all depth zones (Edmeades *et al.*, 1983; Moir *et al.*, 2016).

The Al_{KCl} concentration declined as the base saturation increased, with the strongest relationships present in the 20 cm and 50 cm depth zones (Figure 3.4a and b). This was an expected trend and has been reported in other studies (Harrison *et al.*, 1990; Webb *et al.*, 1986). This reflects the increase in the proportion of soil exchange sites occupied by basic cations (Mg^{2+}, Ca^{2+}, K^+ and Na^+) and the decline of Al^{3+} and H^+ (acidic cations) from exchange sites (McLaren & Cameron, 1996c). The difference from 100% represents acidic cations (including Al) on the cation exchange sites. The base saturation interacts with CEC because it affects how many of the cation exchange sites can be occupied by Al. Even if the soils have a high CEC, if the BS is high, there will not be much Al^{3+} stored. There was a declining sensitivity of Al to pH_{H2O} in the 20 cm depth zone but increasing sensitivity to BS (relative to deeper depth zones).

Cation exchange capacity (CEC) was a highly significant factor relating to soil Al_{KCl} concentrations at all depths in the soil profile. There was an increase in Al_{KCl} concentrations with an increase in CEC and the relationship was stronger in the 50 cm and 120 cm depth zones when all other variables within the model are considered (Figure 3.3a and Figure 3.3b). This was an expected result, as the CEC determines the ability of soils to retain/store cations and represents the negatively charged exchange sites that cations can bind to, including those on clay minerals and organic matter. As expected, there were strong (P<0.001) positive correlations between CEC and soil carbon % and clay % in each depth zone (Appendix Tables 1.1-1.3). However, which cations (acidic or basic) are bound to exchange sites is influenced by the soil pH and other soil conditions. The more sites of negative charge in the soil, the more likely that there will be more Al^{3+} present on the exchange sites (Rowell, 1988). The upper horizons (top 20 cm) were more acidic, and had more carbon present. However, the sensitivity of the relationship between carbon and Al was lower at this depth, with carbon not included in the final model for Al_{KCl}. This may be related to Al-OM complexes, which will be discussed in more detail in the following section. The result in this chapter supports findings by Harrison *et al.* (1990) who measured both an increase in CEC and Al_{KCl} in Brown Soils and Podzols in Craigieburn, New Zealand.

3.4.2.2 Availability variables (total carbon)

An increase in soil organic carbon resulted in a decrease (P<0.001) in the soil Al_{KCl} concentration in the 50 cm and 120 cm depth zones (Figure 3.5a and b), although it had no effect in the 20 cm depth zone. Total carbon is generally an indication of concentrations of soil organic matter, especially in New Zealand soils, which generally lack inorganic carbon in the form of calcite.

The trend in the 50 cm and 120 cm depth zones is explainable as per the literature and suggests the complexation effect of carbon on soil $Al_{KCl.}$ Aluminium extracted by 1 M KCl was found to be lower as the organic matter content increased at any given pH (Hargrove & Thomas, 1981; Thomas, 1975). There are different functional groups on soil organic matter including ionisable carboxyl and phenolic hydroxyl groups, which make up humic substances, and are a major component of organic matter (Hargrove & Thomas, 1984; McLaren & Cameron, 1996c). An increase in soil organic carbon, means an increase in soil organic matter, which can provide more exchange sites (CEC) for Al^{3+} and increases the buffering capacity (Bot & Benites, 2005; McLaren & Cameron, 1996c). There may be some kind of complexation with organic matter in the soil that makes Al non-extractable by KCl (non-exchangeable) (Bhumbla & Mclean, 1965; Schnitzer & Gupta, 1965). Aluminium has been found to be strongly complexed by organic substances in Podzols (Dahlgren & Ugolini, 1989; Lundström, 1993). In soil solution there are both high molecular weight organic acids such as fulvic and humic

acids, and low molecular weight organic acids (van Hees *et al.*, 2000). Several of the low molecular weight organic acids can form strong complexes with Al (Hue *et al.*, 1986; McColl & Pohlman, 1986). However, whether there are high Al_{KCl} concentrations depends on the pH of the soil, and the presence of organic matter strongly alters the relationship between pH and Al (van Hees *et al.*, 2000).

The relationship between carbon and Al_{KCl} apparently breaks down in the 20 cm depth zone, and was not a significant factor. The total N is highly correlated to total C and may be essentially a proxy for total C, but see below.

3.4.2.3 Response variables (total nitrogen).

Response variables are those variables that, rather than being key drivers of Al_{KCl} concentrations, are likely to respond to Al_{KCl} concentrations in the soil. Soil total nitrogen was identified as an important variable associated with the log Al_{KCl} concentrations, particularly in the 20 cm depth zone (Table 3.3 and Figure 3.2a respectively). There was a strong linear relationship, with a decline in soil Al_{KCl} concentrations with increasing soil total nitrogen. An explanation for the relationship is that the Al_{KCl} concentrations reflect levels of exchangeable Al that are driving the total nitrogen content in the topsoil via exchangeable Al's influence on legumes and biological N fixation. High concentrations of Al and acidic conditions affect the growth and N fixation by legumes, particularly lucerne (Helyar & Anderson, 1971; Moir & Moot, 2010; Scott *et al.*, 2008). Legume shoot growth, nodulation and N fixation are restricted and less effective in acidic and high Al soils (Berenji *et al.*, 2017; Wigley, 2017), leading to less N in the soil. A concentration of Al_{KCl} of >1.0 $cmol_c/kg$ can be toxic to sensitive legumes such as white clover (Edmeades *et al.*, 1983). This is the equivalent of log Al_{KCl} concentrations of >0. A reduction in soil Al concentrations would improve the conditions in the soil for legumes and rhizobia to undergo nodulation and N fixation (Moir *et al.*, 1997). The amount of N fixed is directly related to the legume yield (Peoples & Baldock, 2001). Therefore, a reduction in the soil Al_{KCl} concentration could increase the N fixation and growth of legumes, increasing the soil total N content. It is also possible that the strong relationship between total N and Al_{KCl} in the 20 cm depth zone is a function of long term vegetation growth and acidification (both current and historic), in which total N is correlated to pH_{H2O} and Al_{KCl}. Unfortunately, there is no information from the database that can be used to confirm the vegetation growth or type (e.g. legumes) or the fixation of nitrogen at these sites. Literature relating the total N content of the soil to exchangeable Al is scarce.

The application of nitrogen fertilisers such as urea, could have been another source of the high total nitrogen content in this depth zone. However, the soils from the database were sampled from the 1950's to 1990's, during a time when N fertiliser applications were low in New Zealand (Ministry for

Primary Industries, 2012). As a consequence, it is unlikely that the trend of total nitrogen and soil Al_{KCl} is related to N fertiliser application. However, information on the current land use at the time of sampling is limited and whether a site was sampled on a dairy farm or sheep and beef station could not be determined. As such, it is difficult to draw solid conclusions based on this.

Although a significant factor in the model, soil total N had a very poor relationship with log Al_{KCl} in the 120 cm depth zone (Figure 3.2b), which suggests that variables other than Al_{KCl} concentrations are driving total N in the 120 cm zone. Total nitrogen was not correlated with soil Al_{KCl} in the 50 cm depth zone, which implies that processes restricted to the topsoil are likely to be influencing the relationship between total N concentrations and Al_{KCl} concentrations.

3.4.2.4 Variables that were not significant in the final model for any depth zone

Median annual rainfall, median annual temperature, precipitation variability, total Al, P retention and clay% were not significant variables associated with Al_{KCl} concentrations in any depth zone. However, although several of these variables were not significant in the final model, they were correlated with variables in the soil that were important for Al_{KCl} concentrations in the final model. For example, texture was a factor hypothesised to be a key driver of Al_{KCl} concentrations in the soil, however, it was not in the final model for Al_{KCl} at any depth zone. However, clay % and P retention were strongly correlated (positively) with the CEC, which in turn showed an increase with increasing Al_{KCl} concentrations. It is important to note that the texture variable (clay%) used in this analysis was averaged for samples at the same site within the same depth zone. Parfitt (1991) also found no relationship between $Al_{KCl,}$ concentration and clay%, despite using a more accurate measure of clay content.

Rainfall and precipitation variability were both correlated with variables in the final model for Al (Appendix Tables 1.1-1.3). Rainfall was negatively correlated with base saturation and pH_{H2O}, which showed a decrease with an increase in soil Al_{KCl}. Although rainfall was also directly correlated to Al_{KCl} concentrations, it was not a significant factor in the final model. Annual rainfall was hypothesized to be one of the factors driving Al_{KCl} concentrations in New Zealand soils, however, other variables explained more variation in Al_{KCl} concentrations. Variables, such as BS and pH_{H2O}, which have a direct (quantity) control on Al_{KCl} remained in the final model and therefore it is unsurprising that rainfall was omitted for variables that captured more of the variation in Al_{KCl} i.e. the rainfall data are a proxy for acidification (pH) and leaching (BS).

The precipitation variability variable, which is the annual rainfall range at the site (80^{th}-20^{th} percentile), had a strong negative correlation with soil pH_{H2O} and a positive correlation with log Al_{KCl} in all depth zones. The inference here is that rainfall variation reflects wetting and drying cycles, which promote weathering and leaching (Dixon *et al.*, 2016; Webb *et al.*, 1986), which in turn result in more acidic soils and higher Al concentrations. However, more direct representations of these effects via the variables soil pH_{H2O} and BS probably eliminated this variable from the final multiple regression model.

Total Al in the soil was not an important factor for Al_{KCl} concentrations in the final models, or in the correlations at any depth zone. The total Al content is determined by the parent material, but only the Al that is easily mobile and exchangeable, plays a role in soil fertility (Kabata-Pendias, 2001). Many variables, both those included in the final model and those which were in the full model, are correlated.

3.4.3 Soil order and Al_{Kcl} concentrations

The data indicated that high Al_{KCl} concentrations followed the sequence Podzols=Brown>Pallic>Recent Soils. This analysis confirmed that Podzols and Brown Soils had the highest mean Al_{KCl} concentrations of the New Zealand soil orders across all depth zones, while Recent Soils had consistently the lowest Al_{KCl} concentrations. This is an important piece of information for farmers, as farmers with Brown Soils or Podzols on their farms are likely to have higher soil Al_{KCl} concentrations compared to the other soil orders. The results of this analysis were somewhat expected, as the trends in Al_{KCl} concentrations follow a general sequence of soil development in relation to the classification of New Zealand soils (Hewitt, 2010). Brown Soils and Podzols form under higher rainfall and are more developed. The natural weathering process leads to increased leaching of basic cations, more acidic soil and a soil with increased Al concentrations (Hewitt, 2013). Whereas, the lowest Al soils (Recent Soils) are younger soils with reduced development and a weak B horizon. These soils are less likely to have high Al concentrations present and are less common, covering only 6% of land area in NZ. While Podzols only cover an estimated 13% of land area, Brown Soils are the most extensive soils, which cover 43% of New Zealand (Hewitt, 2013). This implies that soils with high Al concentrations are widespread and will likely increase in the future, particularly in high and hill country areas where it is expensive to apply lime.

Aluminium toxicity is reflected in the plants grown in the soil and is species specific. This information was not available in the database and therefore could not be analysed. However, Brown Soils and Podzols had Al_{KCl} concentrations that would be considered toxic for many legumes (Edmeades *et al.*, 1983; Moir *et al.*, 2016).

Soil Al_{KCl} concentrations differed among the three depth zones for a single soil order. The depth trends found for soil orders were consistent with the analysed data for the full dataset; an increase in Al_{KCl} concentration with an increase in depth. The exceptions were the Recent Soils and Podzols, which had no overall change in Al_{KCl} in the profile, however, the Al concentrations were much lower in the Recent Soils compared to the Podzols (Table 3.6).

The effect of categories below soil order (group and subgroup) were not considered. The main focus was on the soil chemical variables which may affect Al_{KCl} concentrations and soil order was included to determine broader patterns in Al_{KCl} concentrations for New Zealand soils. Although it would be useful to examine relationships at the group and subgroup level, this would greatly reduce the numbers of samples able to be used in the analysis. This detail was beyond the scope of this study.

This study confirmed that soil order was an important factor affecting Al_{KCl} concentrations in New Zealand soils, which supported part of the objective for this study. Many New Zealand soil orders were not well represented with fewer sites sampled, including organic and volcanic soils such as Pumice and Allophanic Soils. As a result, the analysis was unable to elucidate the trends in Al_{KCl} concentrations and whether there are potential toxicity issues in these soils.

3.5 Conclusions

- From a set of variables preselected on the basis of a literature review, base saturation, soil pH_{H2O}, cation exchange capacity, total nitrogen, total carbon and soil order were identified as the most important variables for Al_{KCl} concentrations in three depth zones for soils from the New Zealand Soils database.
- The importance of these variables differed among the three depth zones, 0-20 cm, 20-50 cm and 50-120 cm, which reflected in part interactions among them.
- Soil acidity and high CEC were antagonistic effects (contributing to high Al_{KCl}) whereas as high BS and total C had negative effects.
- There were important differences in the response of extractable Al_{KCl} to different soil variables in the top 20 cm and lower depth zones. The 20 cm zone was less sensitive to CEC, more sensitive to BS, non-sensitive to carbon, less sensitive to pH_{H2O} and more sensitive to N. The major difference in carbon points to the importance of carbon as a modulator of Al.

- Total C had a dual effect. In the deeper depth zones with lower C, as the C increased the Al_{KCl} decreased, which is probably due to complexation. By contrast, in the upper 20 cm, there was a higher carbon content and a lack of a relationship with Al_{KCl}.
- Total N decreased with increasing Al_{KCl} probably as a response to Al toxicity induced limitations on biological N fixation.
- Rainfall did not appear as an important variable in a multiple regression model. This was probably because the effects it is often used as a proxy for (weathering and leaching intensity) were more effectively represented by pH_{H2O}, CEC, and BS.
- Higher Al_{KCl} concentrations in the two deeper depth zones highlights the issues of subsoil acidity and Al toxicity issues, which can be difficult to mitigate.
- Soil order has a predictive ability which allows a first order identification of areas prone to toxic levels of Al. Some soil orders are poorly represented. Variability within soil orders taxonomically was not considered. The next chapter considers spatial variability within a landscape dominated by Brown Soils.
- Brown Soils and Podzols had the highest mean Al_{KCl} concentrations across all depths and, therefore, are likely to be more susceptible to Al toxicity. An Al_{KCl} concentration > 1.0 $cmol_c/kg$ can be toxic to plants.

4 A field survey of soil pH and extractable Al in the Ashburton Lakes catchment, Canterbury, New Zealand

4.1 Introduction

There have been a limited number of studies in New Zealand examining the drivers of soil pH and Al variability. The main studies undertaken in New Zealand, which focused on differences in soil chemistry among different landforms and aged surfaces, were conducted along rainfall or age gradients. Climosequence and chronosequence studies by Webb *et al.* (1986) and Harrison *et al.* (1990) measured different forms of soil Al as part of a full suite of chemical analyses, including the 1 M KCl Al extraction. Webb *et al.* (1986) found that weathering and soil development increased with an increase in rainfall (640 mm yr^{-1} to 2000 mm y^{-1}) at the four sites in the McKenzie basin and Podzols formed at the two highest rainfall sites, and the other two soils were classified as a Pallic Firm Brown and Acidic Allophanic Brown Soils. Harrison *et al.* (1990) examined chronosequences of soils in the Puffer stream terrace sequence (830 mm y^{-1}) and on moraines on the Craigieburn Range (1447 mm yr^{-1}) in the Waimakariri basin. Four of the five terrace soils were classified as Allophanic Brown Soils and the site closest to the floor of Puffer Stream was a Recent Soil. In contrast, the moraine soils differed in elevation, the youngest site was an Allophanic Brown Soil, and the two older sites were classified as Podzols, with morphology that reflected time under forest vegetation. Both increasing rainfall and age increased soil extractable Al_{KCl} concentrations. Dixon *et al.* (2016) examined the climate driven thresholds for chemical weathering at 28 profile sites close to the sites sampled by Webb *et al.,* (1986) along the McKenzie Basin climate gradient, with mean annual rainfall ranging from 400 to 4700 mm y^{-1}. Among the many chemical parameters included in their study, oxalate extractable Al (Al_o) was also measured. Dixon *et al.* (2016) did not measure exchangeable Al but noted threshold behaviour related to mean annual rainfall (MAR) with other variables.

Adams *et al.* (1999) examined aluminium speciation and Al complexation capacity of high country top soils from six sites varying in altitude. The results from their study were linked to threshold values of toxicity and implications based on the location of the site in the study area. Another study compared the effects of afforestation on Al concentrations in soil and soil solution, in comparison to grassland soils at three sites (Adams *et al.*, 2001). Similar to the previous study, the Al complexation capacity of the soil was assessed and compared. The exchangeable 1 M KCl Al and the 0.02 M $CaCl_2$ extractable Al concentrations were higher in the top 0-10 cm of the forest soil compared to the grassland soil at two of the sites.

Eger and Hewitt (2008) examined the relationship between soils and aspect and vegetation history at eight sites in the South Island high country. The chemical properties focussed on in their study were the Al, Si, and Fe fractions extracted by oxalate and pyrophosphate, and allophane and ferrihydrite concentrations. Although they did not report exchangeable Al, they found distinct aspect differences in soil development that may be reflected in concentrations of exchangeable Al. Powell *et al.* (1997) measured the reactive Al, total Al, pH, natural organic matter and phosphate in soil solutions from seven sites under *Hieracium pilosella* L. from a high country field trial. The aim of their research was to determine if the drop in soil pH and increase in organic carbon reported under *H. pilosella* L. in other studies by Scott (1975) and McIntosh and Allen (1993), corresponded with an increase in Al (free Al in soil solution) and therefore possibly toxicity.

The studies above all indicate potential variability of exchangeable Al related to landscape-scale effects yet there has been no systematic study of exchangeable Al variability at this scale. This chapter involves a catchment field study, which represents a high and hill country environment with commonly farmed landforms. The scope of this study was soil extractable Al variability within a single catchment, and it is focussed on determining which factors drive soil pH_{H2O} and extractable Al_{CaCl2} at a landscape scale. The implications of this work may potentially give some insight into soil extractable Al patterns in other catchments. The Ashburton Lakes catchment, in the South Island of New Zealand, was selected as a study area based on several criteria: i) the catchment contains valley-floor landforms commonly farmed in the high country; ii) there is a distinct rainfall gradient and iii) the age of the land surfaces have been well defined in previous work in this area (Barrell *et al.*, 2011). The Ashburton Lakes catchment has been well studied in the past, by authors who examined the glacial chronology (Mabin, 1984; Rother *et al.*, 2014), mapped the geomorphology (Barrell *et al.*, 2013), studied the soils (Harvey, 1974) and assessed drainage patterns (Wadworth-Watts, 2013). The soils of this area have recently been mapped by Landcare Research (Lynn *et al.*, 2015). Furthermore, it is an area of known soil acidity and potential Al toxicity problems. Black *et al.* (2014) conducted two field trials and a glasshouse experiment on Lake Heron Station, examining the effects of acidic, and potentially Al toxic soils, on growth of Caucasian clover *(Trifolium ambiguum L.*). However, the link between the variation in soil acidity and Al toxicity at a landscape scale remains unexplored.

The present study involved a field soil survey in the Ashburton Lakes catchment between 2014 and 2016. Soil samples were collected from 21 sites on different landforms and sites differed in landform age, elevation, rainfall and land-use. The first field campaign was conducted in December 2014 and

January 2015, and 12 sites were sampled. During the second field campaign, a further 9 sites were sampled in December 2015 and March 2016.

The aim of this chapter was to study soil extractable Al_{CaCl2} and determine the extent of Al variation in the Ashburton Lakes catchment. In particular, this chapter evaluated the variation in extractable Al concentration among different landforms and aged surfaces in the catchment, assessed whether the location of a site (elevation and rainfall) affected the extractable Al_{CaCl2} concentrations in the soil and determined which areas were likely to be more susceptible to higher extractable Al concentrations (Al toxicity). Soil extractable Al in this study was measured using the standard Al test used in New Zealand (0.02 M $CaCl_2$). Determining the location and depth of high Al concentrations (within the soil profile) is important for remediation and management, and therefore this study also examines Al depth profiles. This field study provided new information about the drivers of extractable Al in a catchment context and the ability to predict areas most susceptible to elevated Al concentrations, which will greatly assist farmers to make the most effective land use decisions and to prioritize productive land on their farms.

To achieve this aim, the following objective was established: The objective of this chapter was to determine the key factors driving variation in soil extractable Al_{CaCl2} concentrations on different landforms of similar parent material in a landform context.

Based on the climosequence and chronosequence studies cited above, the sampling strategy was designed to allow testing of the following hypotheses regarding landscape scale patterns of Al toxicity:

Hypothesis 1: Extractable Al_{CaCl2} increases with increasing soil and landform age

Hypothesis 2: Extractable Al_{CaCl2} increases with increasing mean annual rainfall

4.2 Materials and methods

4.2.1 Study area

The Ashburton Lakes catchment is located on the eastern side of New Zealand's Southern Alps, inland from Mt Somers (Figure 4.1). The catchment encompasses two inter-montane basins, Lake Heron and Lake Clearwater and is flanked by high mountain ranges. The valley floor varies in elevation from 500-800 m.a.s.l. The Lake Heron basin is 25 km long and runs north-south. At its southern end is the Lake Clearwater basin (Mabin, 1984). There are several main lakes which include Lake Heron (NNE of the catchment), Lake Clearwater, Lake Camp, Lake Emily and Lake Emma. The Southern branch of the Ashburton River runs through the centre of the catchment, south of Lake Heron and the Maori Lakes (Figure 4.1). Lake Stream, drains Lake Heron and flows into the Rakaia River, as does the Cameron River which joins Lake Stream from the west. To the west of Lake Heron lie the Arrowsmith and the Potts Mountain ranges (Burrows, 2002). High mountainous peaks rise above 1800 m, in particular Mt Arrowsmith is the highest point at 2795 m.a.s.l (Burrows, 2002).

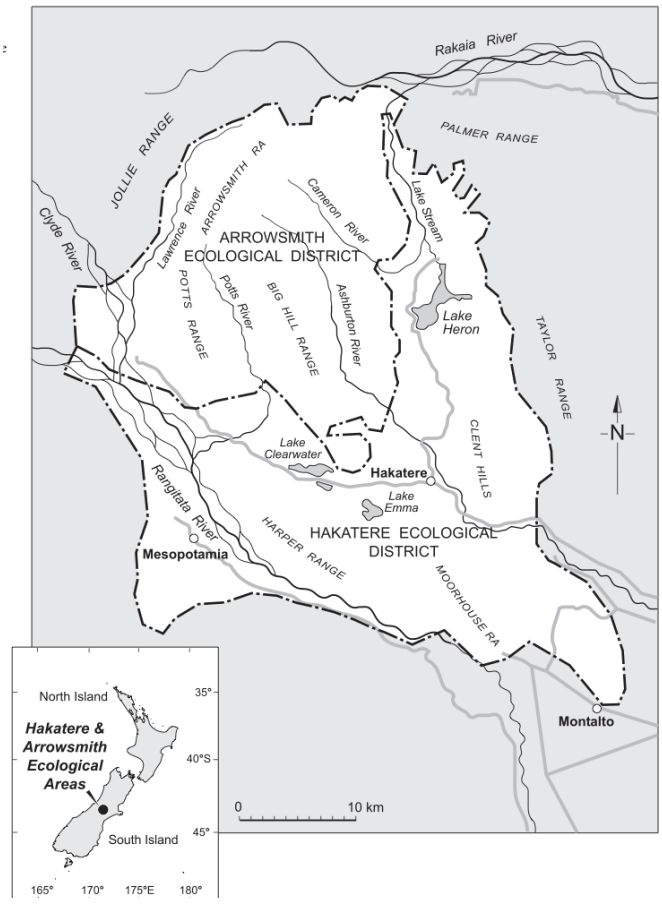

Figure 4.1 Boundaries of the Arrowsmith and Hakatere Ecological districts and some of the predominant landscape features (Burrows, 2002).

The dominant lithologies are Mesozoic greywacke and argillite (Oliver & Keene, 1990). which form the main mountains in this area (Gair, 1967). Cretaceous andesite and some rhyolite are present in the Clent Hills, in the south east of the catchment, south of Lake Heron (Oliver & Keene, 1990). Soils in the catchment are predominantly Brown Soils (Acidic or Typic Orthic subgroups). The valley floor includes young Fluvial Recent Soils and in waterlogged/swampy areas, Gley Soils (Harvey, 1974; Lynn *et al.*, 2015).

Before the arrival of Maori (700-800 years ago) and European settlers (mid 19th century) the South Island was dominated by beech forests (*Nothofagus spp*. including *N. fusca, N. menziesii* and *N. solandri*) (McGlone *et al.*, 1993) in the wetter and higher elevation areas, and podocarp species (predominantly *Dacrydium cupressinum*, *Prumnopitys spp*., *Halocarpus spp*. and *Phyllocladus alpinus*) in drier areas at lower elevation, based on pollen records (McGlone & Wilmshurst, 1999). Early Maori cleared around 40% of the native forests by burning (McGlone, 1983; McGlone & Wilmshurst, 1999; McWethy *et al.*, 2009) and much of the forest that was burned never recovered (McGlone & Wilmshurst, 1999; McWethy *et al.*, 2009). Europeans established grazing on open tussock grasslands and converted shrubland to pastures with non-native vegetation (McWethy *et al.*, 2010; Pawson & Brooking, 2002). McWethy *et al.* (2010) confirmed, using paleoenvironmental data in the sediments of 16 small closed basin lakes throughout the South Island spanning different environmental conditions, that human-set fires were responsible for the loss of New Zealand's forests, rather than climate variations, but the severity of fires varied with geography and local climate.

Vegetation has changed considerably over time. Remnants of beech forest remain along the eastern foothills (Department of Conservation, 2013) and second growth forest is present in parts along the foothills. Red tussock (*Chionochloa rubra)* is found predominantly in wetland areas. Species such as snow tussock (*Chionochloa rigida*), mountain daisy (*Celmisia spectabilis var. magnifica*), tall tussocks (*Chionochloa rigida, C. macra*), matagouri (*Discaria toumatou*) and spaniard grass (*Aciphylla aurea*), which were common in this rugged hill country environment, are now rare in the grazed or previously grazed areas (Burrows *et al.*, 1993).

Pastoral farming in this area, particularly sheep and beef grazing, has been a dominant land-use since the early European settlement (Department of Conservation, 2013). Over the past 40 years, farming has diversified with the planting of wind breaks, fence subdivision of land, fertiliser application and cultivation for fodder and pasture species (Department of Lands and Survey, 1990). The Hakatere and Arrowsmith, Department of Conservation (DoC) parks, cover many areas of the basin, which includes areas of Kettlehole wetlands.

The sites sampled near Lake Clearwater are from Hakatere Station (9110 ha), which was purchased by the Nature Heritage Fund to add to the Hakatere Conservation Park, which was opened in 2007. In 2008, Hakatere Conservation Park was expanded by a further 17, 500 ha from Mt Potts and Redcliffes pastoral leases to bring the park to over 85,000 ha (New Zealand Government, 2004, 2008). Other properties sampled on include Mt Arrowsmith, Lake Heron Station and Strathallan Farm, which are privately owned properties.

4.2.2 Geomorphology

The Ashburton lakes catchment was glaciated in the late Pleistocene from ice tongues from the Rakaia and Rangitata valley glaciers. Glaciation of this area produced different landforms including lateral (at the sides of the glacier) and terminal (end of the glacier) moraines, kame terraces and outwash terraces. The landforms in the Ashburton Lakes catchment have been given broad age ranges based on the glacial advances in the area and were mapped by Barrell *et al.* (2013). The most emphasis is placed on the Latest Otira Glaciation (about 45 kyr and 14.5 kyr), which was the last major glacial event in the Southern Alps (Barrell *et al.*, 2011). The ages of landforms in the Otira Glaciation include the Early Otiran or Older (45 kyr-360 kyr), the Late Otiran (14.5 kyr- 45 kyr) and the Latest Late Otiran (14.5 kyr-19 kyr). Holocene landforms in the catchment formed 11.7 kyr years ago- present day (Barrell *et al.*, 2011; Barrell *et al.*, 2013).

Recent work conducted by Rother *et al.* (2014) has refined the timing of glacial advances using cosmogenic nuclide exposure ages. A suite of 58 ages from the 17 last-glacial ice limits in the Rangitata Valley captured a record of glacial oscillations between 28-16 kyr. Their work on the chronology of the Rangitata glacial system has established four distinct phases of the last glacial maximum (LGM) and deglaciation. The LGM in this region occurred shortly before 28 kyr, followed by several successively less extensive ice advances between 26 and 19 ka. An overall trend of diminishing ice volume was documented between 28-20 kyr, and gradual deglaciation until at least 15 kyr (Rother *et al.*, 2014). The ages reported in the new study by Rother *et al.* (2014), are broadly consistent with the mapping of Barrell *et al.* (2013). Barrell *et al.* (2013) identified moraines of the Late Otira Glaciation to at least 14.5 kyr and Rother *et al.* (2014), using the cosmogenic datation method, identified the end of the glaciation period at 15 kyr. Due to the extensive geomorphic map produced from the study of Barrell *et al.* (2013), this thesis uses thresholds from the latter to define landform ages.

After glacier recession lakes formed, such as Lake Clearwater and Lake Emma (Barrell *et al.*, 2011), valley floors were modified by the accumulation of alluvial materials as fans in the Holocene and peat swamps also formed. Deglaciated mountain slopes were mantled by scree.

The processes involved in the formation of landforms influence the composition of sediments that make up the landforms. Moraine belts are common throughout this valley and have formed by the deposition of glacial sediments. Debris (till) and large boulders carried down by the glacier, build up at the margins of glaciers to form moraines (Barrell *et al.*, 2011). Moraines are made up of larger materials and are less ordered/stratified (Benn *et al.*, 2003). Outwash plains are developed on outwash river bed deposits, where the water source was glacial meltwater, highly charged with sediment which accumulates down valley. The meltwater stream carries predominantly sand and gravel (finer materials than moraines) which is deposited near moraines and is stratified (Benn *et al.*, 2003). Alluvial fans are developed on stream deposits (gravel, sand and silt), which have been formed by fluvial processes (Barrell *et al.*, 2011). In many parts of this landscape, deposits of wind-blown material/dust called loess have mantled surfaces (Schmidt *et al.*, 2005).

The sites sampled were selected to encompass many commonly farmed landforms such as moraines (10 sites), alluvial fans (five sites) and outwash surfaces (six sites). A geomorphic map of the 21 sites is presented in Figure 4.2.

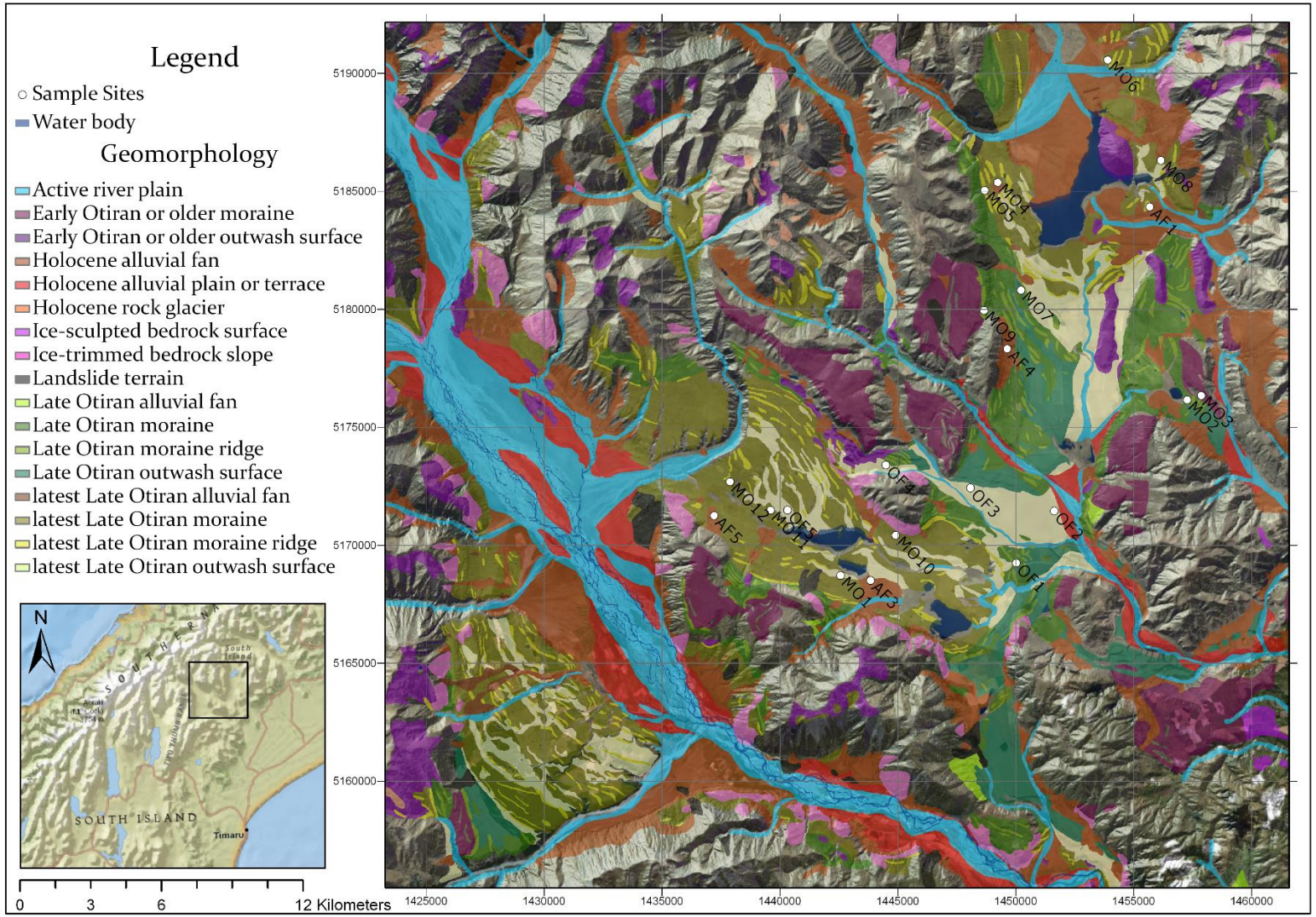

Figure 4.2 Geomorphic map (Barrell *et al.*, 2013) of the field survey area, Ashburton Lakes, Canterbury, showing sites sampled .

The landform and estimated geologic age of the sample sites, are presented in Table 4.1. Most landforms date from the LGM in the latter part of the Otira Glaciation within the period between 45 and 14.5 kyr (Barrell *et al.*, 2013). Landforms from the last glacial-interglacial transition, about 14.5-11.7 kyr, and younger Holocene landforms (<11.7 kyr) were also sampled in this study.

The majority of moraines sampled in this catchment were on latest late (19 - 14.5 kyr) or late Otiran (45kyr- 14.5 kyr) surfaces. There was one site (MO3) located on an older early Otiran surface, with an estimated age of 360 kyr-45kyr (Barrell *et al.*, 2013). The alluvial fans were formed on younger surfaces from the Holocene epoch, after the last glaciation (11.7 kyr- 1 kyr).

Table 4.1 The landform and geological age of the 21 sites in the Ashburton Lakes catchment.

Site code*	Landform	Geologic Age
AF1	alluvial fan	Holocene (<11.7 kyr)
MO8	alluvial fan	Holocene (<11.7 kyr)
AF3	alluvial fan	Holocene (<11.7 kyr)
AF4	alluvial fan	Holocene (<11.7 kyr)
AF5	alluvial fan	Holocene (<11.7 kyr)
OF2	outwash surface	Latest Late Otiran (19- 14.5 kyr kyr)
MO1	outwash surface	Latest Late Otiran (19- 14.5 kyr)
MO7	outwash surface	Late Otiran (45 kyr-14.5 kyr)
OF3	outwash surface	Latest Late Otiran (19- 14.5 kyr)
OF4	outwash surface	Latest Late Otiran (19- 14.5 kyr)
OF5	outwash surface	Latest Late Otiran (19- 14.5 kyr)
OF1	moraine	Late Otiran (45 kyr-14.5 kyr)
MO2	moraine	Late Otiran (45 kyr-14.5 kyr)
MO3	moraine	Early Otiran or Older (360 kyr-45 kyr)
MO4	moraine	Latest Late Otiran (19- 14.5 kyr)
MO5	moraine	Latest Late Otiran (19- 14.5 kyr)
MO6	moraine	Latest Late Otiran (19- 14.5 kyr)
MO9	moraine	Late Otiran (14.5 kyr- 45 kyr)
MO10	moraine	Latest Late Otiran (19- 14.5 kyr)
MO11	moraine	Latest Late Otiran (19- 14.5 kyr)
MO12	moraine	Latest Late Otiran (19- 14.5 kyr)

In the Geologic age column the number in brackets refers to the estimated age (approximate 1000 years, kyr) of the land surface (Barrell *et al.*, 2011) . * For several site codes, the lettering does not correspond correctly to the landform the site was on. This was due to plotting the GPS co-ordinates of the site after sampling and the landform changing in some circumstances.

4.2.3 Sampling sites

Twenty one sites, in total, were selected across the catchment (Figure 4.2) with the aim of sampling a range of landforms (age and type) and mean annual rainfalls (MAR). Sites also differed in elevation, aspect and land-use. All sites were on either farmland, which has had no lime or fertilizer application in the past, or DoC land. This area has undergone tenure review and if areas have been incorporated

into DoC land after tenure review, there may have been some inputs to the site. Site specific information is presented in Table 4.2.

Table 4.2 Site information for the 21 sites sampled in the Ashburton Lakes catchment.

Site code*	Landform	MAR (mm y^{-1})	MAT (°C)	Elevation	Land-use
AF1	alluvial fan	1512	8.7	745	DoC
MO8	alluvial fan	1529	8.4	771	Farmland
AF3	alluvial fan	1169	8.8	693	Farmland
AF4	alluvial fan	1449	8.5	819	Farmland
AF5	alluvial fan	1207	8.7	741	Farmland
OF2	outwash surface	1137	9.2	624	DoC
MO1	outwash surface	1180	8.8	702	Farmland
MO7	outwash surface	1479	8.7	752	Farmland
OF3	outwash surface	1282	8.6	747	DoC
OF4	outwash surface	1403	7.9	860	DoC
OF5	outwash surface	1288	8.8	689	DoC
OF1	moraine	1128	9.0	657	DoC
MO2	moraine	1290	8.9	703	DoC
MO3	moraine	1332	8.8	749	DoC
MO4	moraine	1502	8.4	811	Farmland
MO5	moraine	1522	8.0	929	Farmland
MO6	moraine	1558	8.7	734	Farmland
MO9	moraine	1500	7.6	988	Farmland
MO10	moraine	1260	8.4	736	DoC
MO11	moraine	1273	8.8	678	DoC
MO12	moraine	1271	8.8	696	DoC

Note: The MAR and MAT were determined using NIWA annual 50th percentile climate data for each of the sites using GPS coordinates (National Institute of Water and Atmospheric Research, 2015). Aspect was derived from a DEM with 8 m resolution (LINZ, 2012). * For several site codes, the lettering does not correspond correctly to the landform the site was on. This was due to plotting the GPS co-ordinates of the site after sampling and the landform changing in some circumstances.

4.2.4 Rainfall

Sites were chosen at different locations in the catchment to cover a range of yearly rainfalls for each landform. Sample site locations were intersected with the latest NIWA climate grids (National Institute of Water and Atmospheric Research, 2015) in a GIS environment to give estimates of the median annual total rainfall and temperature at each site (Table 4.2). Median annual rainfall is lowest in the south and highest is in the E and NE of the catchment. The sampled sites covered a rainfall range of 1128 mm y^{-1} to 1558 mm y^{-1} (Figure 4.3).

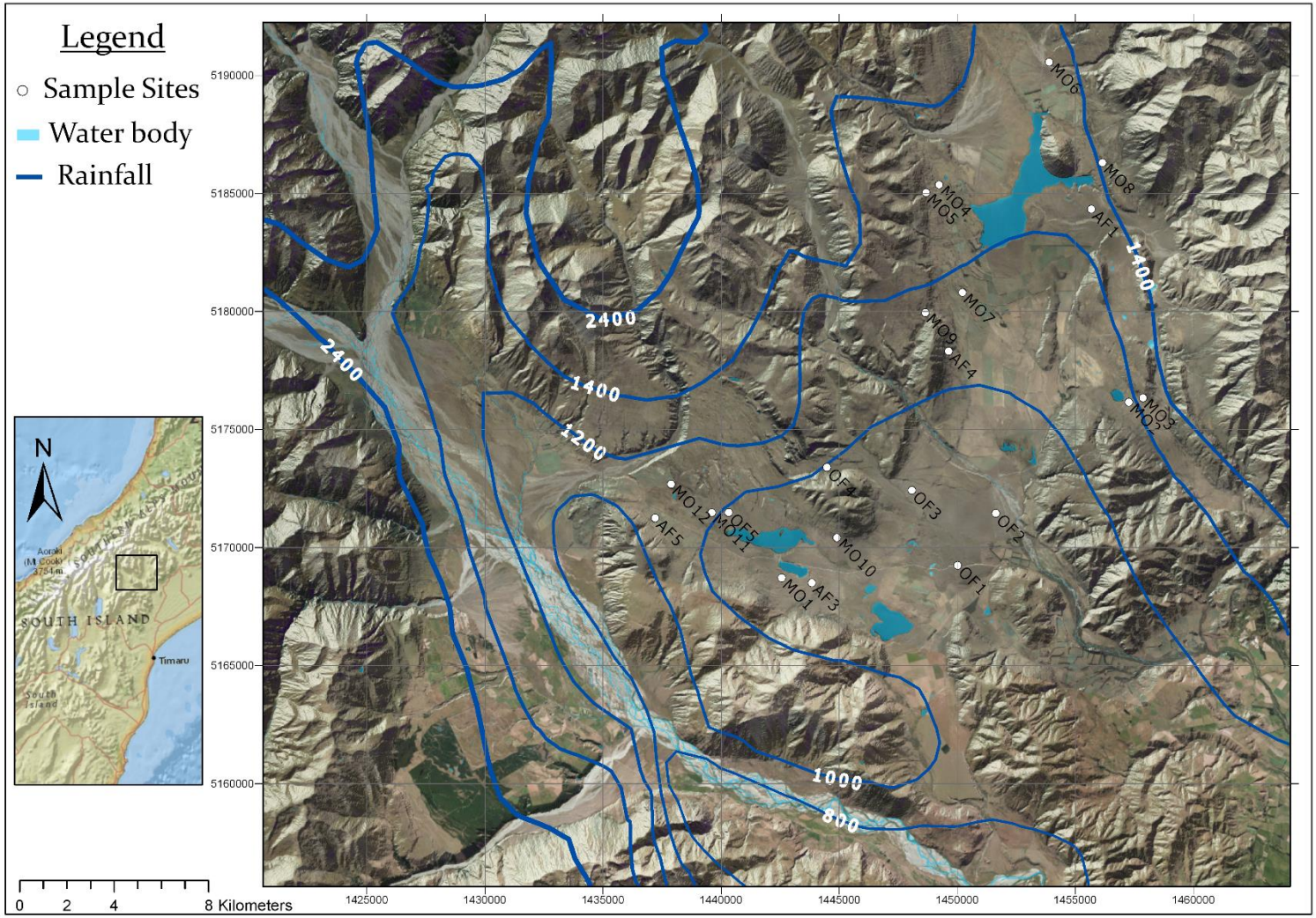

Figure 4.3 A map of the field survey area, Ashburton Lakes, Canterbury, showing sites sampled, the geomorphology of the area (Barrell *et al.*, 2013) and the Median Annual Rainfall (National Institute of Water and Atmospheric Research, 2015).

4.2.5 Soil description and sampling

At each site, details were recorded including location (GPS), landform, aspect, slope, elevation, vegetation and land-use. A pit was dug at a site visually representative of the local area from which a soil description was made according to the methods of Milne *et al.* (1995b) and Schoeneberger *et al.* (2012) (Plate 4.1 and Plate 4.2). Photographs were also taken at each pit. Soil pits were sampled in 5 cm vertical increments. The soils at each site were classified according to the New Zealand Soil Classification (NZSC) (Hewitt, 2010), based on profile morphology and informed by a recent soil map of the Ashburton Lakes catchment (Lynn *et al.*, 2015). The aspect (° deviation from north east or west), slope (percentage rise) and elevation (m.a.s.l) were later derived for each site from a digital elevation model (DEM) with 8 m resolution (LINZ, 2012).

Plate 4.1 Soil profile exposed in a pit at MO2 site. Plate 4.2 Soil descriptions.

4.2.6 Chemical analysis

Soil samples were taken back to Lincoln University, air dried (at 25-30°C) and sieved to yield a <2 mm fraction ready for chemical analyses. All soil samples were measured for soil pH_{H2O} and $CaCl_2$ extractable Al (Al_{CaCl2}). Soil pH was measured using the water: soil ratio of 2.5: 1 (Blakemore *et al.*, 1987). Soil Al_{CaCl2} was extracted using the 0.02 $CaCl_2$ extraction method, which is the common soil test used in New Zealand (Edmeades *et al.*, 1983) then measured by inductively coupled plasma optical emission spectrometry (Varian 720-ES ICP-OES; Varian Inc, Victoria, Australia).

A subset of 76 samples were selected from the original 21 profiles for further chemical analyses. The criterion for selection was that a significant change in soil pH_{H2O} or Al_{CaCl2} (either an increase or decrease) was observed in the profile. A sample from the top 10-15 cm from each profile pit was also included. This subset of soil samples will be referred to as 'testing subset' in subsequent sections. These samples underwent additional analyses including: soil pH in 0.01 M $CaCl_2$ using the extractant: soil ratio of 2.5: 1 (Blakemore *et al.*, 1987); cation exchange capacity (CEC), soil extractable ions and base saturation (BS) determined by the 0.01 M $AgTU^+$ method (Blakemore *et al.*, 1987; Rayment & Lyons, 2011b; Searle, 1986); and total C and N content as determined by the Dumas method of combustion (Horneck & Miller, 1998) with the use of an Elementar 'Vario' MAX CN Analyzer (Elementar Analysensystane, GmbH). The variables found to be significantly correlated to Al_{KCl} concentrations in the New Zealand database chapter were investigated further in this catchment study.

4.2.7 Statistical analysis

All statistical tests were conducted in GenStat version 16. For the soil profile samples (258), a series of linear models were constructed to determine if there was a significant difference in mean pH_{H2O} and Al_{CaCl2} across different rainfall zones, landform type and landform age. For the effect of depth, a linear mixed model was constructed because, unlike the other three factors, depth differed among soil samples taken at a single site. Site was used as a random variable to remove the effect of site similarities, therefore depth can be compared across sites. In order to carry out analyses on Al_{CaCl2}, this measure was log transformed to achieve a normal distribution and to satisfy model assumptions. Residual plots, *P* values and the Akaike Information Criterion were produced for each linear model. The Akaike Information Co-efficient value (AIC) was used to determine the fit and explanatory value (quality and information loss) of each model. A lower AIC implies a better fitting model and a higher explanatory value (Akaike, 1974; Sokal & Rohlf, 2012c). Plots of the observed values and fitted values for each model were constructed to show the relationship between both pH_{H2O} and $logAl_{CaCl2}$ for each factor. A single, master linear model was created after an evaluation of the performance of models with different factor combinations for pH_{H2O} and $logAl_{CaCl2}$. AIC values produced for each model were used to determine if a single factor, or a combination of factors, was best for predicting pH_{H2O} and Al_{CaCl2} in this catchment (lowest AIC).

The same methods were used on the testing subset. First, linear models were conducted for the four main factors for pH_{H2O} and $logAl_{CaCl2}$. Secondly, the extra factors measured, including soil pH_{CaCl2}, total N, total C, CEC and BS, were used in linear mixed models (with site as a random variable) to determine their effect on pH_{H2O} and Al_{CaCl2} individually. A single master linear model was created after an

evaluation of the performance of models with different factor combinations for pH_{H2O} and $logAl_{CaCl2}$. AIC values were used to determine the best fitting model.

Finally, decision trees (non parametric test) created in R (version 3.3.1,package rpart;Therneau *et al.*, 2017), were used to derive a set of rules for predicting the classes of pH_{H2O} or Al_{CaCl2} from values of predictor variables for the full dataset (Loh & Shih, 1997). A tree-like diagram is constructed by recursive binary splits of the data in which the class label and the values for the predictor variables are known. Each partition is represented on the tree as a node (Loh & Shih, 1997). The tree starts at the root nodes at the top and moves down to the leaf nodes at the base of the tree. Each split by a factor (rule) and the mean (estimated value) was shown on a separate branch of the tree. Depth was identified as an important factor in the main models for pH_{H2O} and Al_{CaCl2} and therefore the dataset was split into three depth zones in order to investigate factors driving pH_{H2O} and Al_{CaCl2} at different depths in the profile. The depth zones were 0-20 cm, 20-50 cm and >50 cm. The factors included in the pH_{H2O} and Al_{CaCl2} models were: age, landform, aspect, rainfall, elevation and slope. These trees were used to determine the importance of each factor for pH_{H2O} and Al_{CaCl2}. This technique was used as a tool to help to establish the relative importance of the predictor variables in explaining the response, and the relationships between the response and predictor variables. The legend for the variables used in the decision trees are presented in Table 4.3. Maps of soil pH_{H2O} and Al_{CaCl2} in the 20 cm depth zone were constructed using ArcPro software (version 2.0.1) using the rules established in the decision trees.

Table 4.3 Legend for the factors from the decision trees for pH_{H2O} and Al_{CaCl2}.

Age code	**Age**
1	Holocene
2	Latest Late Otiran
3	Late Otiran
4	Early Otiran or earlier
Landform code	**Landform**
AF	alluvial fan
OF	outwash surface
MO	moraine

4.3 Results

4.3.1 Soil profile descriptions

Detailed soil profile descriptions of the 21 sites sampled in the Ashburton Lakes catchment are provided in the Appendix in Tables 2.1-2.4.

4.3.1.1 Alluvial fans

Of the five sites excavated on alluvial fans, four were Acidic Orthic Brown Soils (AF1, AF4, AF5 and MO8) and AF3 was a Typic Orthic Brown Soil. Sites ranged in elevation from 693 m.a.s.l (AF3) to 819 m.a.s.l (AF4) and all sites were in native tussock grassland with matagouri and hieracium present. The slope of each site ranged from flat to 17°. The soil structure was either granular or subangular blocky, varying in size and grade. The texture differed among profiles and often within profiles and included silt loams, sandy loam and loamy silt. The soil colour ranged between the horizons within a profile and across sites. The consistence differed between horizons within a profile and among profiles. AF1, MO8 and AF4 had worms present and AF5 was in a cultivated paddock with loose soil prone to erosion. Plant roots were abundant in many profiles and ranged from very fine to medium in size to a depth ranging from 55 to 84 cm. Alluvial gravels were present at all sites except site MO8. These gravels were angular or subrounded and medium to coarse in size.

4.3.1.2 Outwash surfaces

Of the six sites excavated on outwash surfaces, all soils were Acidic Orthic Brown Soils (MO1, MO7, OF2, OF3, OF4 and OF5). The sites range in elevation from 624 m.a.s.l (OF2) to 860 m.a.s.l (OF4) and the slope from flat through to 26°. Most sites were low gradient but one (MO7) high gradient slope occurred on a terrace riser. At all sites the vegetation was native tussock grassland with snow tussock, matagouri and hieracium present at some sites. Roots were very fine to medium and were mostly common or many in abundance, however, some horizons had few roots. Maximum depth of profile sampling ranged from 40 cm (OF3) to 105 cm (MO7). The texture was silt loam in the MO7, OF3, OF4 and OF5 profiles. MO1 transitioned from silt loam to loamy sand with depth and the OF2 soil was a sandy loam. The OF2 and MO1 soils had a granular structure, fine-coarse in size and differed in degree of development. All other soils had a subangular blocky structure, which ranged from very fine to coarse in aggregate size and weak to strong in development. The consistence differed within and among profiles. Clasts were present at all sites, generally at depth in the profile, mainly subrounded in shape, although angular in the OF5 profile. Cobbles and gravels were common (fine-coarse) and boulders were present at the OF3 and OF4 sites. The MO1, MO7, OF4 and OF5 sites showed evidence

of worm mixing and the OF4 site had no clasts in the top 30 cm of the profile, which suggests loess deposition. In the Ah horizon of the OF2, MO1 and MO7 sites, the colour was 10YR 3/2, changing in the Bw horizon to 10YR 3/4, 10YR 4/4 and 2.5Y 5/4 in the respective profiles. The colour of the Ah horizon of the OF3, OF4 and OF5 profiles was 10YR 4/3, the OF3 and OF4 B horizons were 2.5Y 6/4 and the OF5 B horizon was 2.5Y 5/4.

4.3.1.3 Moraines

Of the ten sites sampled on moraines, all soils were Acidic Orthic Brown Soils (OF1, MO3, MO4, MO5, MO6, MO9, MO10, MO11, MO12), except for MO2, which was a Typic Orthic Brown Soil. Sites ranged in elevation from 657 m.a.s.l (OF1) to 988 m.a.s.l (MO9) and in slope from flat to 34°. The maximum sampling depth ranged from 50 cm (MO10) to 106 cm (MO4). At all sites, native short tussock grassland was the dominant vegetation. Many sites had a combination of hieracium, matagouri and spaniard. The MO4 site was semi-developed pasture. Roots were very fine to coarse in size and ranged from many to few in abundance. Worm mixing was evident in the soil profiles at MO2, MO5, MO6, MO9 and MO10. A thick root mat was present at MO2, MO3 MO4, MO9 and MO12 sites. Loess deposition was evident at the MO4 site and at 35 cm in the MO12 site was a hard pan (fragipan), not observed in any of the other seven profiles. The soil texture was silt loam at MO5, MO6, MO10, MO11 and MO12 sites. At the other five sites, the texture differed within the profile from silt loam, to loamy sand, silty sand, sandy loam and silty clay loam. Structure was either granular or subangular blocky, ranged in size from very fine to coarse and differed in degree of development. Consistence differed within single profiles and among sites. The soil colour ranged between the horizons within a profile and across sites (Appendix Table 2.4). In the Ah horizon of the MO2, MO3, MO4, MO11 and MO5 sites the colour was 10YR 4/3 and the OF1 and MO10 sites were 10YR 3/2. The colour in the Ah horizon of the MO6, MO12 and MO9 profiles was 10YR 5/3, 10YR 3/3 and 10YR 4/4 respectively. In the Bw horizon, the hue for most soils was 2.5Y with a value of 5 or 6 and a chroma of 4 or 6. In the MO4 and MO12 profiles, the hue remained the same, however, the value and chroma differed between the Bw1 and Bw2 horizons. The colour of the B horizon of the OF1, MO6 and MO10 profiles had a hue of 10YR, with values ranging from 4-6 and chroma was 3, 4 or 6. For the OF1 profile the hue remained the same, however, the chroma differed between the Bw1 and Bw2 horizons. The MO5 site transitioned from 10YR 4/4 in the Bw1 horizon to 2.5Y 5/4 in the Bw2 horizon. Clasts were present at all sites except MO6 and MO9, and ranged in shape from subrounded to rounded and angular. The size ranged from medium-coarse gravels to stones, cobbles and boulders and the location in the profile differed, but generally were present deeper in the profile. An absence of clasts at MO6 and MO9 could indicate loess parent material.

4.3.1.4 Summary of soil profile forms

The soils sampled in this catchment were all Brown Soils, either Acidic Orthic Brown or Typic Orthic Brown despite the contrasts in landform age. Vegetation at the sites was predominantly native tussock grassland. Worm activity was a common occurrence and several sites indicated loess deposition. The structure was either granular or subangular blocky and differed in aggregate size and grade of development. Texture was mostly silt loam across the sites, however, sandy loam, loamy silts and loamy sand were present in several profiles, both throughout the profile and in certain horizons. There did not appear to be any patterns in terms of the depth of texture change in the profile on particular landforms.

4.3.2 Effects of environmental variables and depth on soil chemistry

4.3.2.1 Rainfall

The soil pH_{H2O} ranged from 4.7 to 6.0 and showed a linear trend (P<0.05) of increasing soil pH_{H2O} with increasing rainfall (Figure 4.4). At any given rainfall, pH_{H2O} varied by as much as 0.7 pH units, although there was no trend of increasing or decreasing variance with changing rainfall. The variance in soil pH_{H2O} at a given rainfall implies there is likely to be another factor involved.

The Al_{CaCl2} concentration showed a linear trend (P<0.01) of decreasing Al_{CaCl2} concentration with increasing rainfall (Figure 4.5). The Al_{CaCl2} concentration ranged from 0.5 to 39.1 mg kg^{-1} and at a given rainfall the Al_{CaCl2} concentration varied by as much as 30.4 mg kg^{-1}, although there was no trend of increasing or decreasing variance with changing rainfall. The variance in Al_{CaCl2} concentration at a given rainfall implies there is likely to be another factor involved.

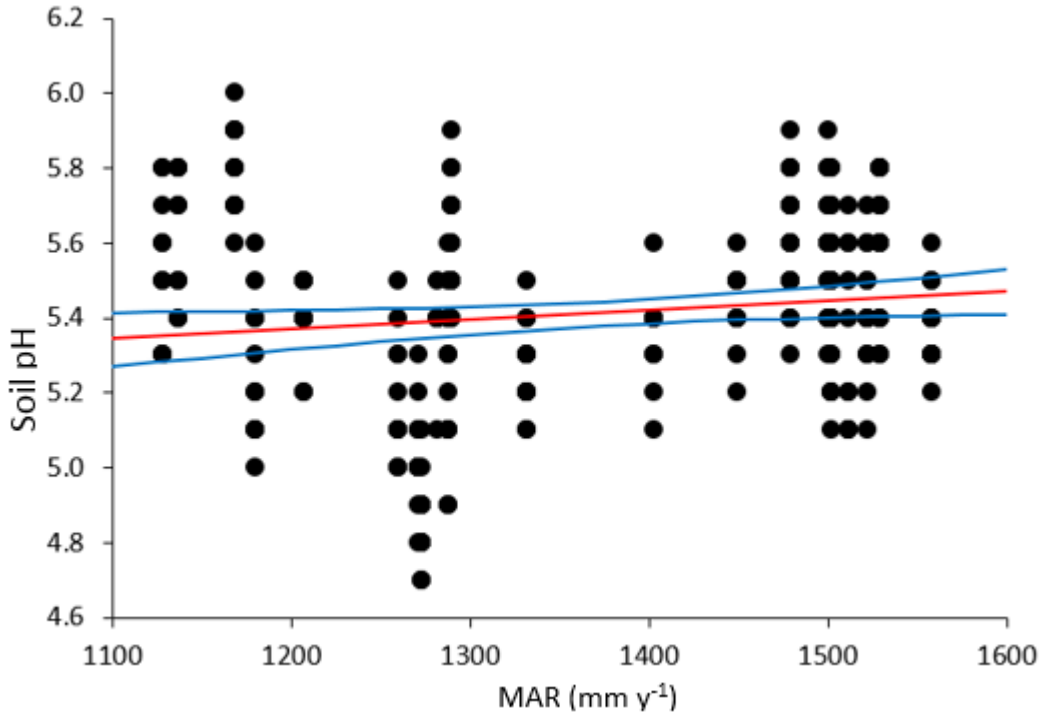

Figure 4.4 Median annual rainfall (mm y^{-1}) and the soil pH_{H2O} for the 21 sites from the Ashburton Lakes catchment, Canterbury, New Zealand. Fitted and observed relationships are shown with 95% confidence intervals.

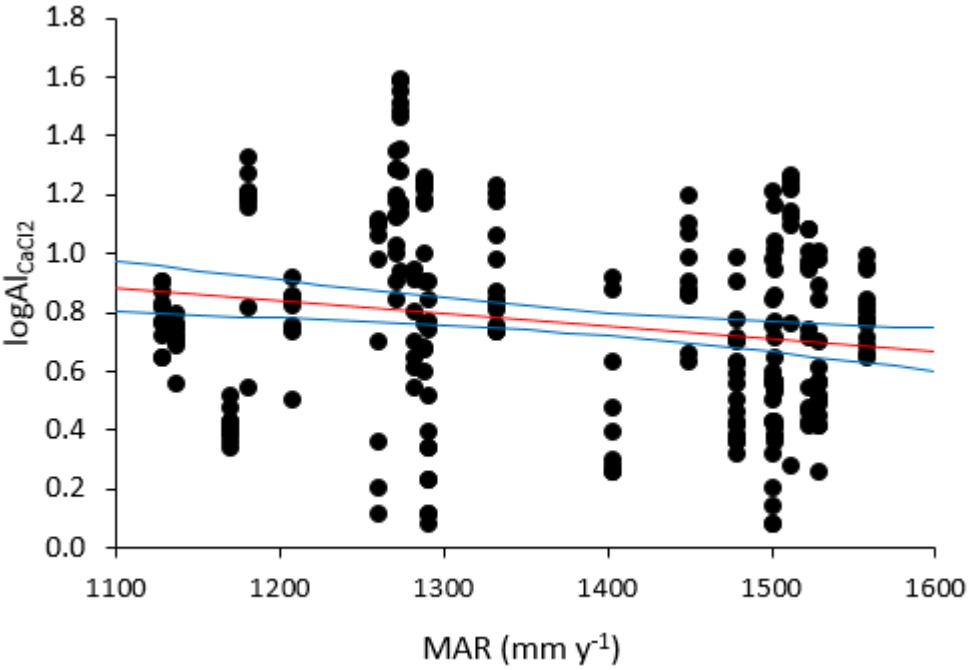

Figure 4.5 Median annual rainfall (mm y^{-1}) and the Al_{CaCl2} for the 21 sites from the Ashburton Lakes catchment, Canterbury, New Zealand. Fitted and observed relationships are shown with 95% confidence intervals.

4.3.2.2 Landform

There was a difference (P<0.001) in the mean soil pH_{H2O} among the different landforms from which soils were sampled (Table 4.4). The mean pH_{H2O} was highest on the alluvial fan at 5.5 and the mean pH_{H2O} on the outwash surfaces and moraines was 5.4.

There was no difference (P>0.05) in the mean Al_{CaCl2} among the different landforms from which soils were sampled (Table 4.4).

Table 4.4 Mean soil pH_{H2O} and Al_{CaCl2} (mg kg^{-1}) across different landforms in the Ashburton Lakes catchment and the significance of landform for predicting soil pH_{H2O} and Al_{CaCl2} from the 21 sites sampled in this catchment.

	Expected values (mean)				
Landform	pH_{H2O}	SEM	$LogAl_{CaCl2}$	SEM	Al_{CaCl2} (mg kg^{-1})
alluvial fan	5.5	0.03	0.74	0.04	5.5
outwash surface	5.4	0.03	0.76	0.04	5.8
moraine	5.4	0.02	0.79	0.03	6.2
P value (Landform)	<0.001		0.612		

Values in the table are from a general linear model which determined the significance of landform for soil pH_{H2O} and Al_{CaCl2} concentrations in this catchment. The Al_{CaCl2} is the back-transformed Al_{CaCl2} concentration from the log output.

4.3.2.3 Age

There was a strong difference (P<0.001) in the soil pH_{H2O} with age of the surface (Table 4.5). The means were higher on the Holocene and the Late Otiran surfaces, with a mean soil pH_{H2O} of 5.5 and 5.6 respectively. The mean pH_{H2O} for both the Latest Late Otiran and the Early Otiran, or older, were the lowest at 5.3. There was not a systematic trend with increasing landform age.

Table 4.5 Mean soil pH_{H2O} and Al_{CaCl2} concentrations (mg kg^{-1}) across different aged surfaces in the Ashburton Lakes catchment and the significance of geologic age for predicting soil pH_{H2O} and Al_{CaCl2} from the 21 sites sampled in this catchment.

	Expected values (mean)				
Age of surface	pH_{H2O}	SEM	$LogAl_{CaCl2}$	SEM	Al_{CaCl2} (mg kg^{-1})
Holocene	5.5	0.03	0.74	0.04	5.5
Latest Late Otiran	5.3	0.02	0.86	0.03	7.3
Late Otiran	5.6	0.02	0.56	0.04	3.7
Early Otiran or older	5.3	0.04	0.94	0.05	8.6
P value (Age)	<0.001		<0.001		

Note: Values in the table are from a general linear model which determined the significance of age for soil pH_{H2O} and Al_{CaCl2} concentrations in this catchment. The Al_{CaCl2} is the back-transformed Al_{CaCl2} concentration from the log output.

There was a strong difference (P<0.001) in the Al_{CaCl2} concentration measured on different aged surfaces (Table 4.5). The mean Al_{CaCl2} concentrations were lower on the Holocene and Late Otiran surfaces at 5.5 mg kg^{-1} and 3.7 mg kg^{-1} respectively. The Latest Late Otiran and the early Otiran, or Older surfaces, had the highest means of 7.3 mg kg^{-1} and 8.6 mg kg^{-1} respectively. There was not a systematic trend with increasing landform age.

4.3.2.4 Depth

Depth had a significant (P<0.001) effect on soil pH_{H2O} (Figure 4.6; Table 4.6). Soils are more acidic in the top of the soil profile, with an expected value of 5.3 at 5 cm and 10 cm, increasing with depth to 5.4 at 50 cm and 5.6 at 80 cm. Soil pH_{H2O} at any given depth varied by as much as 1.1 pH units.

Depth had a significant (P<0.001) effect on Al_{CaCl2} concentrations (Table 4.6; Figure 4.7). The soil Al_{CaCl2} concentration was higher in the top of the soil profile, with expected values of 8.4 mg kg^{-1} and 7.1 mg kg^{-1} at 5 cm and 10 cm, declining with depth to 5.1 mg kg^{-1} at 50 cm and 3.6 mg kg^{-1} at 80 cm. The Al_{CaCl2} concentration at any given depth varied by as much as 36.5 mg kg^{-1}.

Table 4.6 Mean soil pH_{H2O} and Al_{CaCl2} (mg kg^{-1}) across different depths in soils in the Ashburton Lakes catchment and the significance of depth for predicting soil pH_{H2O} and Al_{CaCl2} from the 21 sites sampled in this catchment.

		Expected values (mean)	
Depth (cm)	pH_{H2O}	$LogAl_{CaCl2}$	Al_{CaCl2} (mg kg^{-1})
5	5.3	0.92	8.4
20	5.3	0.85	7.1
50	5.4	0.71	5.1
80	5.6	0.56	3.6
P value (Depth)	<0.001	<0.001	
A Linear mixed model with site as a random variable.			

Note: Values in the table are from a General Linear Mixed Model (with site as a random variable) which determined the significance of depth for soil pH_{H2O} and Al_{CaCl2} concentrations in this catchment. The Al_{CaCl2} is the back-transformed Al_{CaCl2} concentration from the log output.

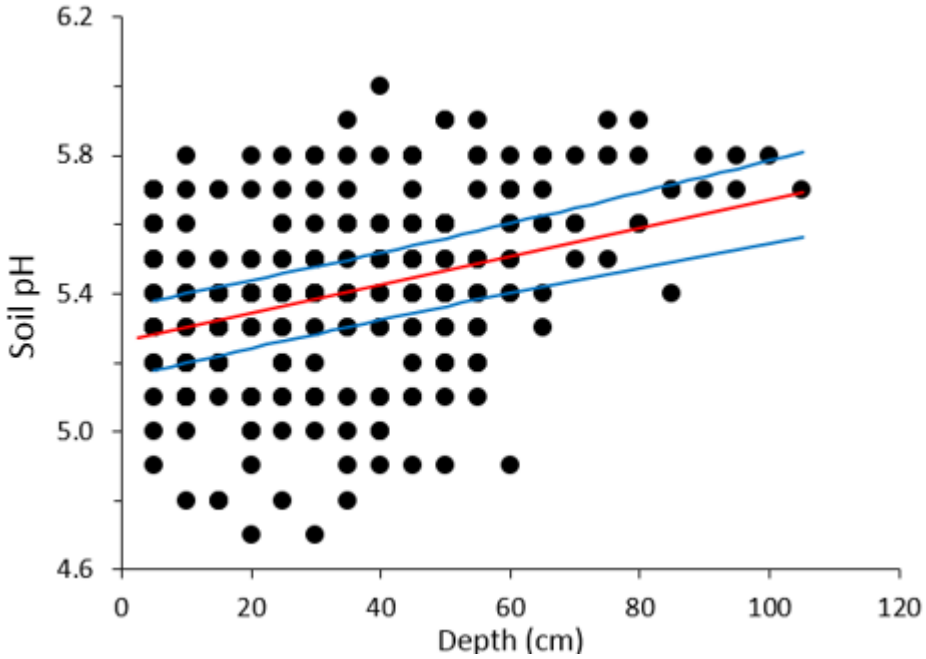

Figure 4.6 Soil pH_{H2O} and depth for the soils sampled in the Ashburton Lakes catchment. Fitted and observed relationships are shown with 95% confidence intervals.

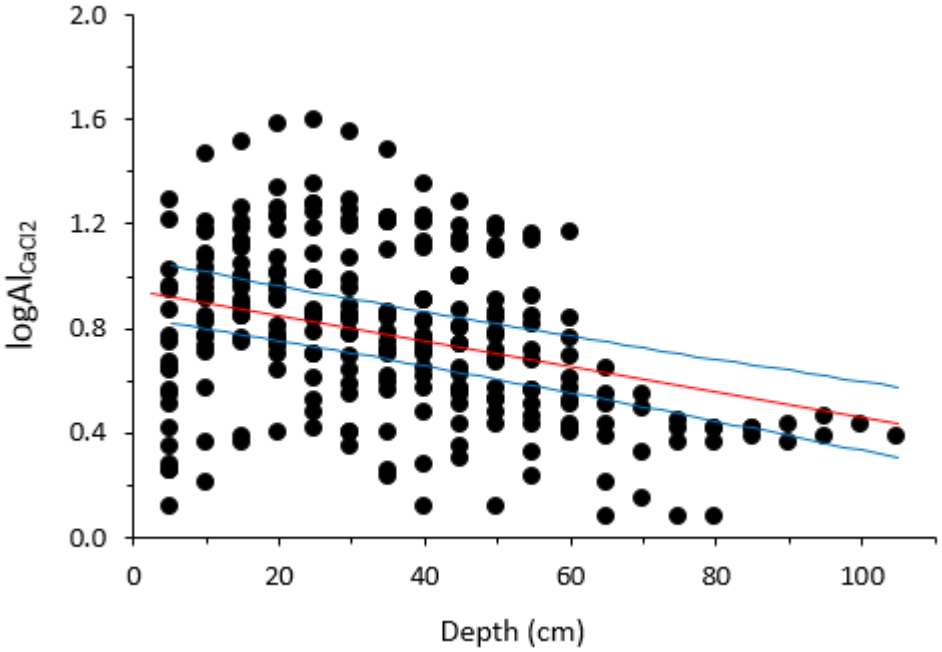

Figure 4.7 Log soil Al_{CaCl2} and depth for the soils sampled in the Ashburton Lakes catchment. Fitted and observed relationships are shown with 95% confidence intervals.

4.3.2.5 The relationship between pH_{H2O} and extractable Al_{CaCl2} on different landforms

The soil pH_{H2O} and Al_{CaCl2} relationship for the 258 samples (from 21 sites at different depths) showed a moderate (R^2=0.56) exponential decay across the pH_{H2O} range of 4.3 to 6.0 (Figure 4.8). Soil Al_{CaCl2} varied significantly at a single pH_{H2O} value and conversely, at a single Al_{CaCl2} measurement, the soil pH_{H2O} varied. The Al_{CaCl2} from the sites sampled in this catchment ranged from 0.5 mg kg^{-1} to 39.1 mg kg^{-1}.

Figure 4.8 shows that the moraine soils had higher concentrations of Al_{CaCl2} and more acidic soils. The relationship of pH_{H2O} and Al_{CaCl2} for the 131 moraine samples had the highest R^2 of 0.70. The 61 alluvial fan samples had an R^2 of 0.56 and the 67 outwash surface samples had the poorest relationship (R^2= 0.34).

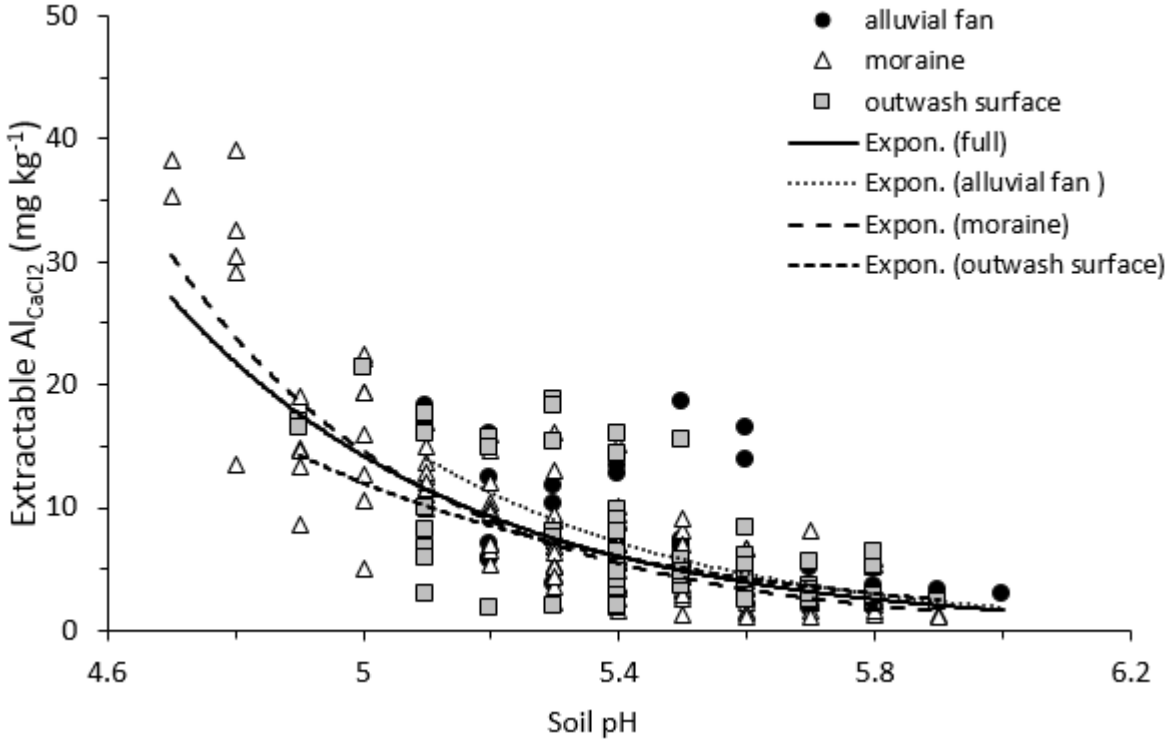

Figure 4.8 Soil Al_{CaCl2} (mg kg^{-1}) against soil pH_{H2O} measured for 258 samples from 21 soil sites, across four different landforms in the Ashburton Lakes catchment, Canterbury, New Zealand. Forms of the equations are: Exp(full) $y = 642357e^{-2.144x}$($R^2 = 0.56$); Exp(alluvial fan) $y = 1E+06e^{-2.22x}$ ($R^2 = 0.56$); Exp (moraine) $y = 3E+06e^{-2.453x}$ ($R^2 = 0.70$ and Exp (outwash surface) $y = 66482e^{-1.723x}$ ($R^2 = 0.34$).

4.3.2.6 Factors driving pH_{H2O}/ Al_{CaCl2}: Mixed linear models with individual and multiple factors (Main dataset)

A mixed linear model was constructed to determine which factors were most important for pH_{H2O} and Al_{CaCl2}. The mixed models performed no better than the simple linear model and adding other variables reduced the fit of the model and the explanatory power. Depth as a single factor was the best predictor for soil pH_{H2O} and Al_{CaCl2} (see AIC parameter in Appendix Tables 2.5 and 2.6). The relationship between depth and soil pH_{H2O} and depth and Al is described in Section 4.3.2.4.

4.3.2.7 Individual factors driving pH / Al: linear model (testing subset)

The same result for the testing dataset as the main dataset was found when rainfall, landform, depth and age were used in linear models to determine their importance for soil pH_{H2O} and Al_{CaCl2} (Appendix Table 2.7) variability. Overall, in the individual linear models, depth was the most important of the four factors for both variables. For the testing subset, pH_{CaCl2}, carbon, nitrogen, cation exchange capacity (CEC) and base saturation (BS) were used as additional factors to determine their relationship with soil pH and Al_{CaCl2} (Appendix Table 2.8). Mixed models performed no better than the simple linear model. Of the five additional factors, total N was the most important for soil pH_{H2O} and soil pH_{CaCl2} for Al_{CaCl2} for the testing subset (Appendix Table 2.8).

4.3.2.8 Factors driving pH / Al: Mixed linear models with multiple factors (testing subset)

The soil pH_{H2O} ranged from 4.8 to 6.0 and showed a linear trend (P<0.05) of decrease in soil pH_{H2O} with increasing total soil total N (Figure 4.9). The variance in soil pH_{H2O} at a given soil N content implies there is likely to be another factor involved. The Al_{CaCl2} concentration showed a strong linear trend (P<0.001) of decreasing Al_{CaCl2} concentration with increasing pH_{CaCl2} (Figure 4.10). The Al_{CaCl2} concentration ranged from 0.5 to 39.1 mg kg^{-1} and the highest Al_{CaCl2} concentrations occurred in soils with a pH_{CaCl2} of ≤4.2.

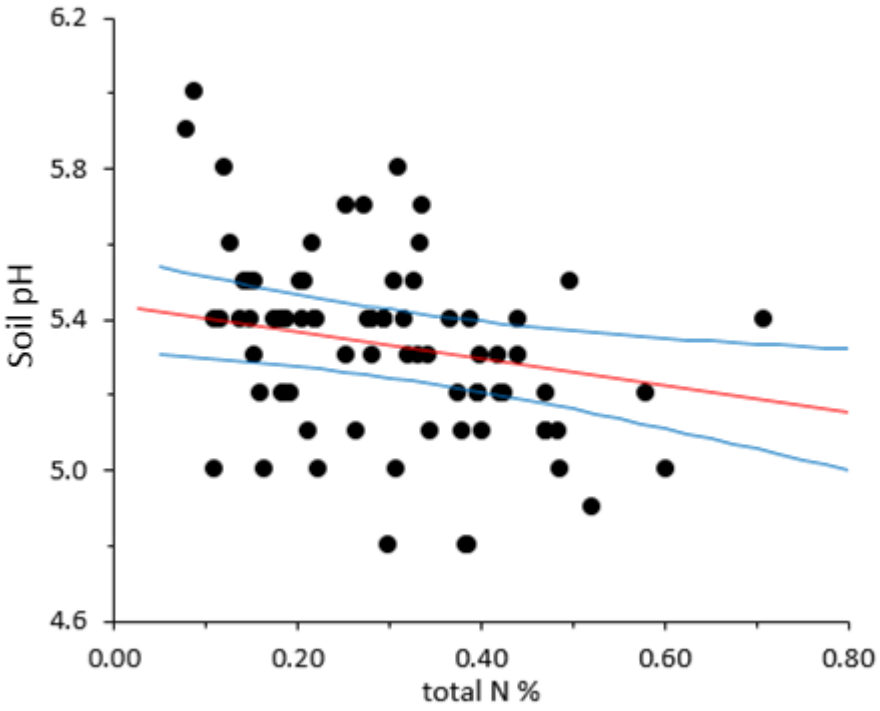

Figure 4.9 Soil pH_{H2O} and total N % for soils sampled in the Ashburton Lakes catchment, Canterbury. Fitted and observed relationships are shown with 95% confidence intervals.

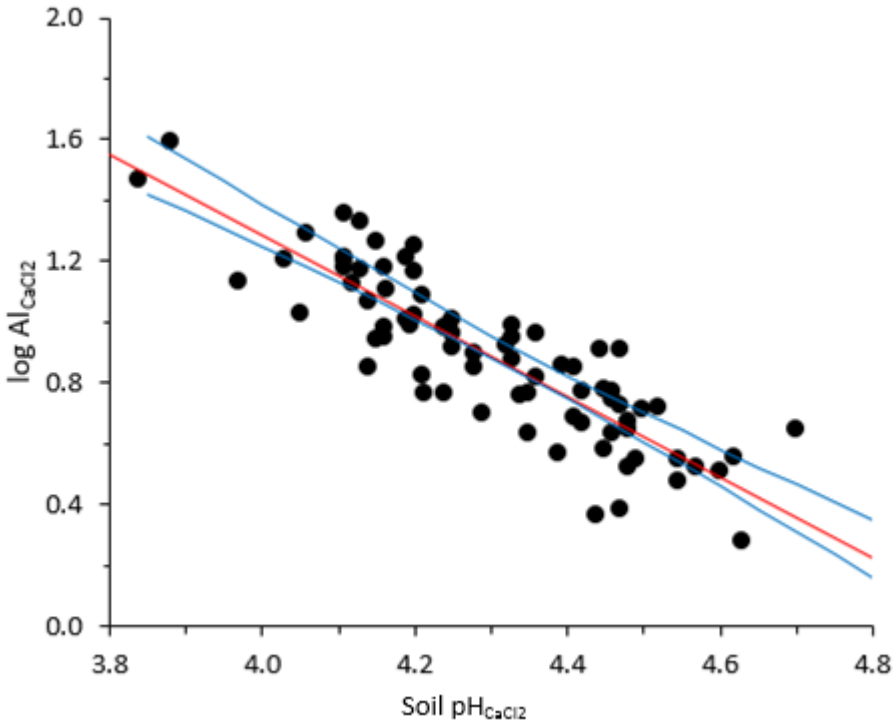

Figure 4.10 Log Al_{CaCl2} and soil pH_{CaCl2} for soils sampled in the Ashburton Lakes catchment, Canterbury. Fitted and observed relationships are shown with 95% confidence intervals.

4.3.3 Decision tree for soil pH_{H2O}

The decision trees defining the combination of factor levels determining soil pH_{H2O} for each of the depth zones, 20 cm (0-20 cm), 50 cm (20-50 cm) and > 50 cm (50 cm to base of pit) are shown in Figures 4.11-4.13 respectively. A set of rules defined on the combinations of factor levels for each depth zone is given in Table 4.7 as well as the number of leaf nodes produced for each decision tree.

4.3.3.1 20 cm depth zone

Rainfall was the most important factor for soil pH_{H2O} in the 20 cm depth zone, followed by elevation and aspect (Figure 4.11). The expected pH_{H2O} of 5.0 was found in soils with a median annual rainfall of ≥1174 mm y^{-1} and positioned at an elevation of <699.1 m.a.s.l in the catchment, which corresponds to the valley floor. In contrast, the expected highest soil pH_{H2O} of 5.6 was in soils with a median annual rainfall of <1174 mm y^{-1}.

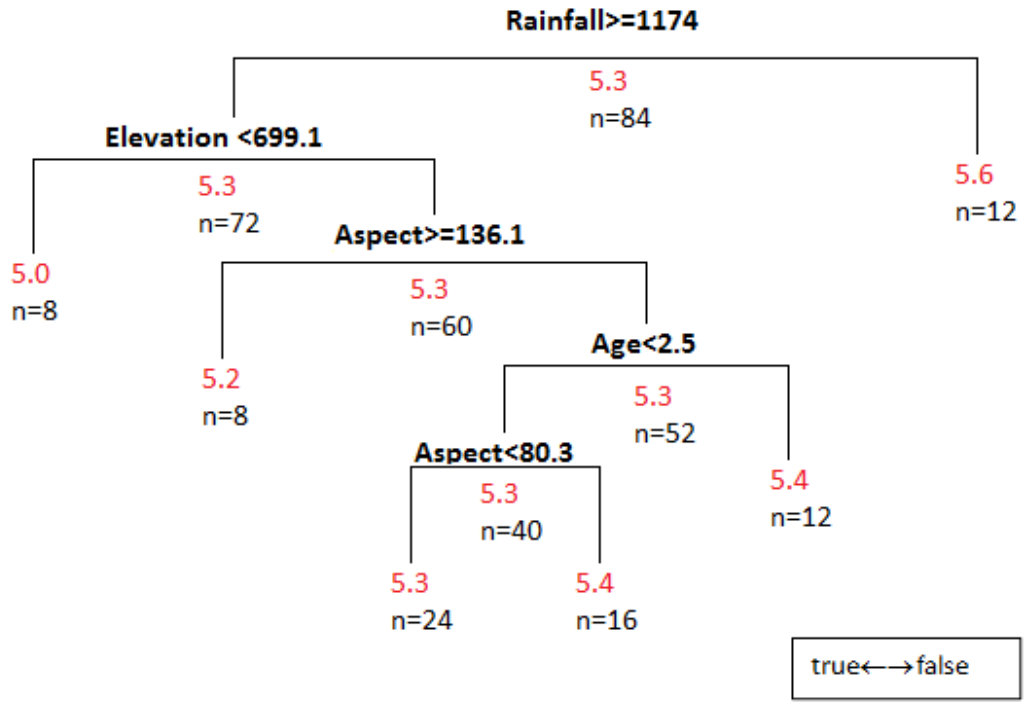

Figure 4.11 A decision tree to predict soil pH_{H2O} including age, landform, aspect, rainfall, elevation and slope for 258 soils from the Ashburton Lakes catchment in the 20 cm depth zone.

4.3.3.2 50 cm depth zone

Rainfall was the most important factor for soil pH_{H2O} in the 50 cm depth zone, followed by elevation and aspect (Figure 4.12). The expected pH_{H2O} of 5.0 was found at sites with ≥1174 mm y^{-1} of rainfall located at an elevation of <699 m.a.s.l. The expected highest pH_{H2O} of 5.7 was in soils with a median annual rainfall of <1174 mm y^{-1}. Above 699 m.a.s.l., aspect was also an important discriminator of pH_{H2O}. At south facing sites the expected pH_{H2O} was lower and the northern facing sites were associated with higher pH_{H2O} in this depth zone.

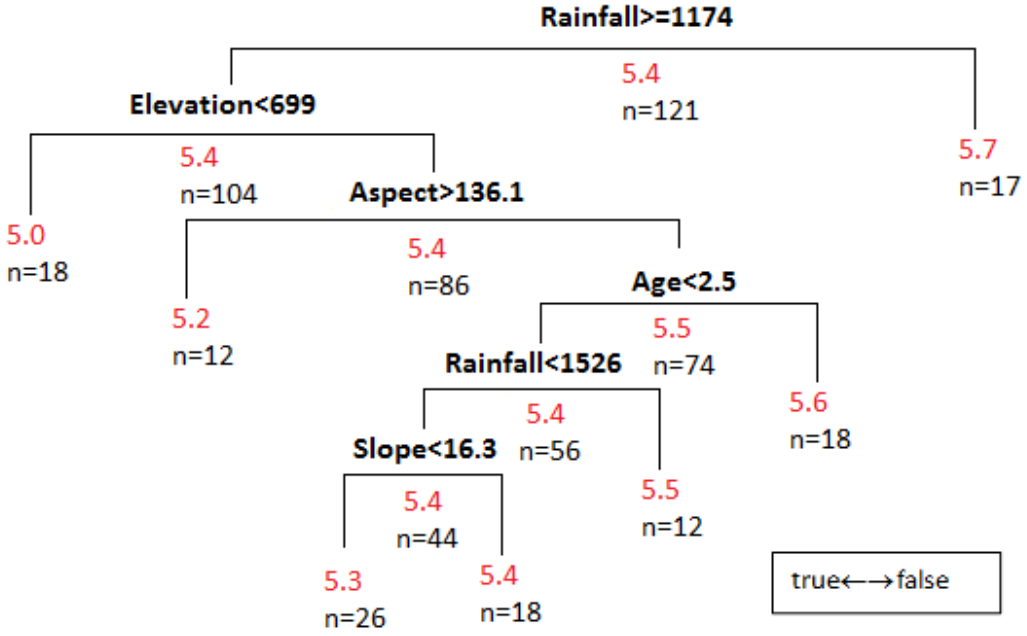

Figure 4.12 A decision tree to predict soil pH_{H2O} including age, landform, aspect, rainfall, elevation and slope for 258 soils from the Ashburton Lakes catchment in the 50 cm depth zone.

4.3.3.3 >50 cm depth zone

In the >50 cm depth zone, elevation was the most important factor for soil pH_{H2O}, followed by slope (Figure 4.13). The expected lowest pH_{H2O} of 5.4 was located at sites in the catchment at <750 m.a.s.l. The expected highest pH_{H2O} at 5.7, was located at an elevation of >750.4 m.a.s.l and at sites with a slope of >7.8 %, which correspond to valley margins. The expected soil pH_{H2O} was notably higher in the >50 cm depth zone compared to the two shallower depth zones.

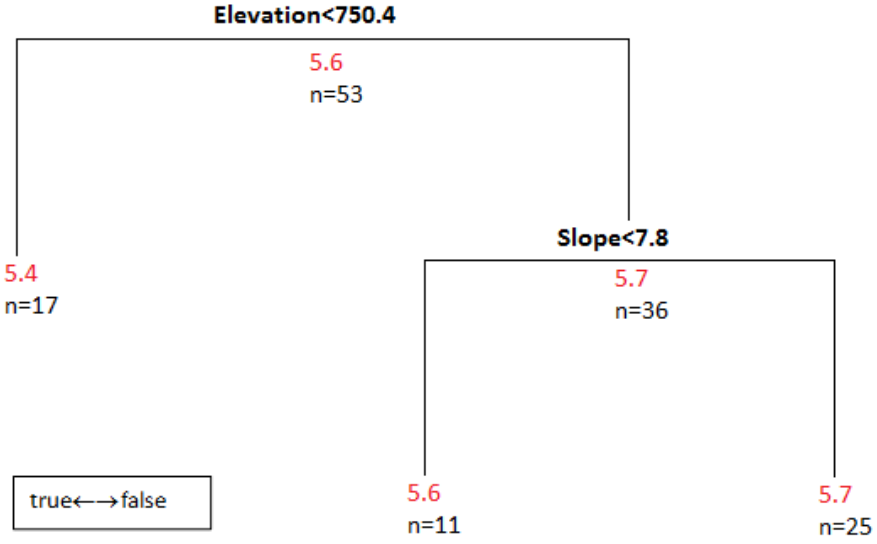

Figure 4.13 A decision tree to predict soil pH_{H2O} including age, landform, aspect, rainfall, elevation and slope for 258 soils from the Ashburton Lakes catchment in the >50 cm depth zone.

Table 4.7 List of rules associated with each node of the decision tree for soil pH_{H2O} in the Ashburton Lakes catchment for the three depth zones, 20 cm, 50 cm and >50 cm.

Nodes	Age	Aspect (° from N)	Rainfall (mm y^{-1})	Landform	Elevation (m.a.s.l)	Slope % rise	Mean pH_{H2O}
20 cm							
1	NA	NA	X≥1174	NA	X<699.1	NA	5.0 (n=12)
2	NA	X≥136.1	X≥1174	NA	X>699.1	NA	5.2 (n=8)
3	X<2.5	X<80.3	X≥1174	NA	X>699.1	NA	5.3 (n=24)
4	X<2.5	80.3<X<136.1	X≥1174	NA	X>699.1	NA	5.4 (n=16)
5	X>2.5	X<136.1	X≥1174	NA	X>699.1	NA	5.4 (n=12)
6	NA	NA	X<1174	NA	NA	NA	5.6 (n=12)
50 cm							
1	NA	NA	X≥1174	NA	X<699	NA	5.0 (n=18)
2	NA	X≥136.1	X≥1174	NA	X>699	NA	5.2 (n=12)
3	X<2.5	X<136.1	1174≤X<1526	NA	X>699	X<16.3	5.3 (n=26)
4	X<2.5	X<136.1	1174≤X<1526	NA	X>699	X>16.3	5.4 (n=18)
5	X<2.5	X<136.1	X>1526	NA	X>699	NA	5.5 (n=12)
6	X>2.5	X<136.1	X≥1174	NA	X>699	NA	5.6 (n=18)
7	NA	NA	X<1174	NA	NA	NA	5.7 (n=17)
>50 cm							
1	NA	NA	NA	NA	X<750.4	NA	5.4 (n=17)
2	NA	NA	NA	NA	X>750.4	X<7.8	5.6 (n=11)
3	NA	NA	NA	NA	X>750.4	X>7.8	5.7 (n=25)

Note: (n=X) represents the number of samples at each node of the decision tree. NA) not applicable in the tree Age 1) Holocene, 2) Latest late otiran, 3) Late otiran and 4) Early Otiran or older.

4.3.4 Decision tree for soil Al_{CaCl2}

The decision tree defining the combination of factor levels determining soil Al_{CaCl2} are presented in Figures 4.14-4.16. A set of rules defined on the combinations of factor levels is given in Table 4.8 and the number of leaf nodes produced in each tree.

4.3.4.1 20 cm depth zone

Rainfall was the most important factor determining soil Al_{CaCl2} concentration in the top 20 cm, followed by aspect (Figure 4.14). The expected lowest soil Al_{CaCl2} concentration (3.8 mg kg^{-1}) was found at a rainfall <1266 mm y^{-1} and at sites with an aspect of ≥105.4° (south facing). The expected highest Al concentration (20.9 mg kg^{-1}) was found in soils with a median annual rainfall of >1266 mm y^{-1} but < 1278 mm y^{-1}. At rainfalls above 1278 mm y^{-1} aspects >136° (south-facing) had the expected highest Al_{CaCl2}, easterly or westerly aspects the lowest and more northerly aspects intermediate values.

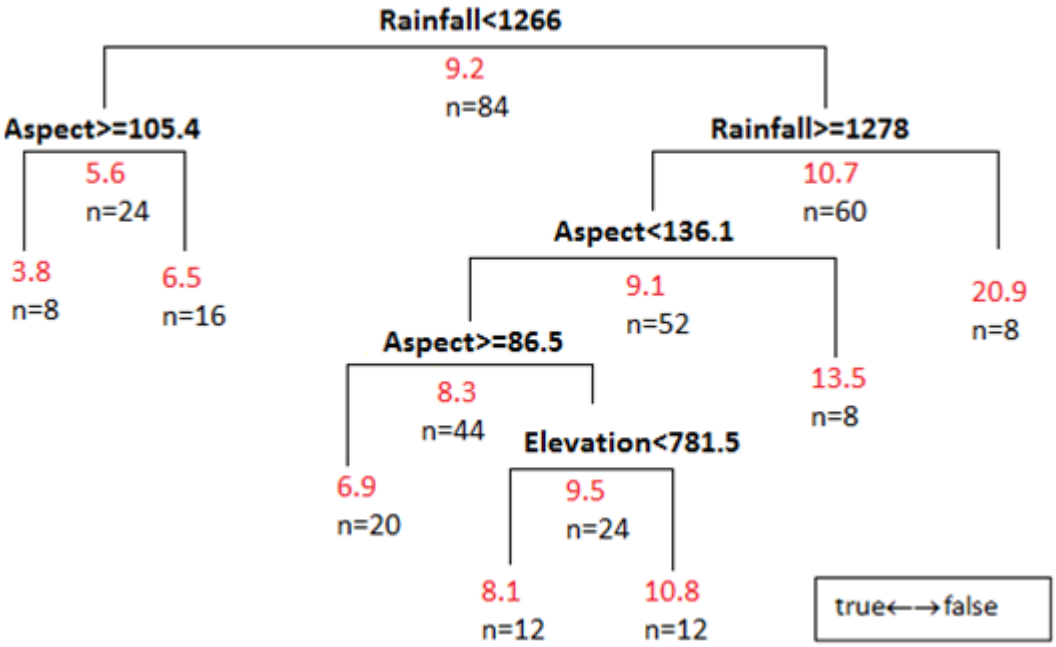

Figure 4.14 A decision tree to predict soil Al_{CaCl2} including age, landform, aspect, rainfall, elevation and slope for 258 soils from the Ashburton Lakes catchment in the 20 cm depth zone.

4.3.4.2 50 cm depth zone

Elevation was most important for Al_{CaCl2} concentrations in the 50 cm depth zone (lower elevations, higher Al_{CaCl2}), followed by slope and rainfall (Figure 4.15). The expected lowest Al_{CaCl2} concentration of 4.3 mg kg^{-1} was in soils with a slope of <22.5 %. An expected Al_{CaCl2} concentration of 4.7 mg kg^{-1} was found in soils with a median annual rainfall <1174 mm y^{-1} and an elevation of <745.7 m.a.s.l. The

expected highest Al_{CaCl2} concentrations of 17.9 mg kg^{-1} were at sites with rainfall >1174 mm y^{-1} and with an elevation of <699 m.a.s.l.

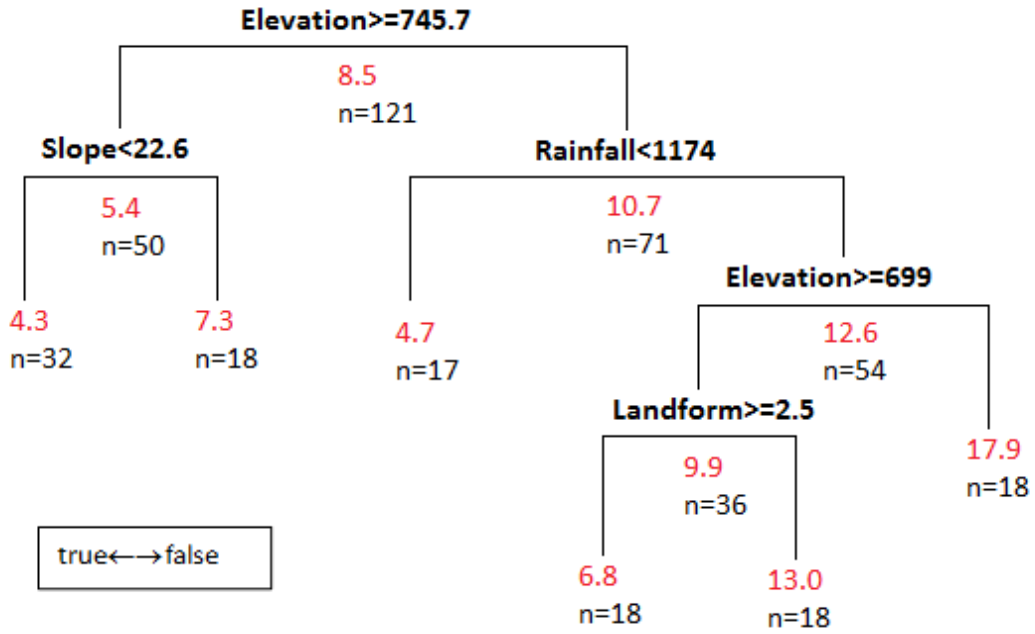

Figure 4.15 A decision tree to predict soil Al_{CaCl2} including age, landform, aspect, rainfall, elevation and slope for 258 soils from the Ashburton Lakes catchment in the 50 cm depth zone.

4.3.4.3 >50 cm depth zone

For Al_{CaCl2} concentrations in the >50 cm depth zone, rainfall was most important, followed by elevation (Figure 4.16). Expected lowest Al_{CaCl2} concentrations (2.6 mg kg^{-1}) were found in soils with a rainfall of ≥1280 mm y^{-1} and an elevation of ≥750.4 m.a.s.l. The expected highest Al_{CaCl2} concentration at 9.7 mg kg^{-1} were at sites with a median annual rainfall of <1280 mm y^{-1}. The Al_{CaCl2} concentration was notably lower in the >50 cm depth zone compared to the shallower depths.

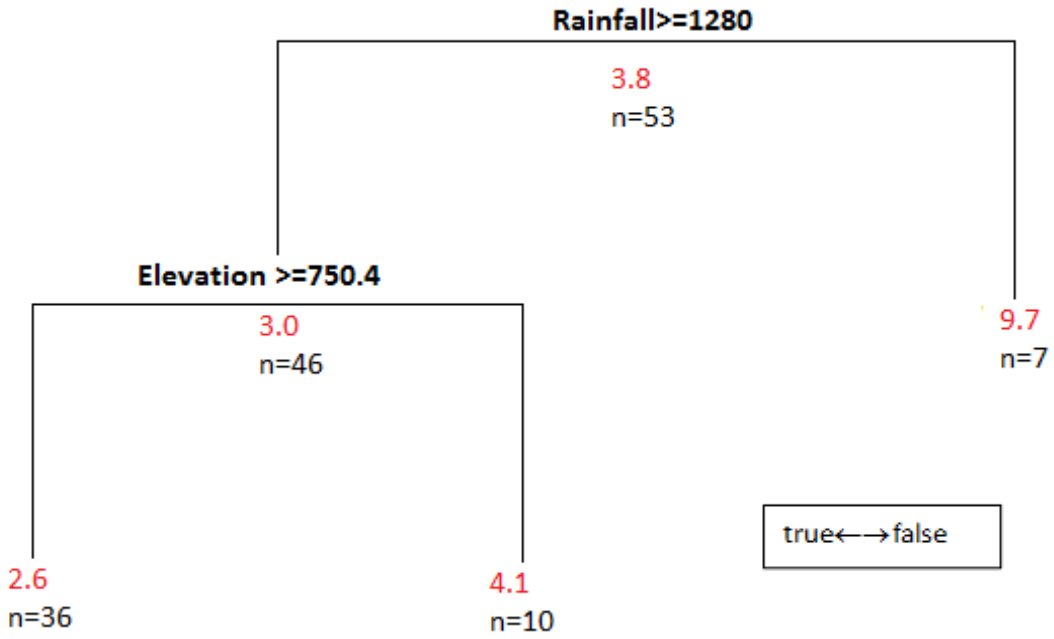

Figure 4.16 A decision tree to predict soil Al_{CaCl2} including age, landform, aspect, rainfall, elevation and slope for 258 soils from the Ashburton Lakes catchment in the >50 cm depth zone.

Table 4.8 List of rules associated with each node of the decision tree for soil extractable Al_{CaCl2} in the Ashburton Lakes catchment for the three depth zones, 20 cm, 50 cm and >50 cm.

Nodes	Age	Aspect (° from N)	Rainfall (mm y^{-1})	Landform	Elevation (m.a.s.l)	Slope % rise	Mean Al_{CaCl2} mg kg^{-1}
20 cm							
1	NA	X≥105.4	X<1266	NA	NA	NA	3.8 (n=8)
2	NA	X<105.4	X<1266	NA	NA	NA	6.5 (n=16)
3	NA	86.5≤X<136.1	X≥1278	NA	NA	NA	6.9 (n=20)
4	NA	X<86.5	X≥1278	NA	X<781.5	NA	8.1 (n=12)
5	NA	X<86.5	X≥1278	NA	X>781.5	NA	10.8 (n=12)
6	NA	X>136.1	X≥1278	NA	NA	NA	13.5 (n=8)
7	NA	NA	X<1278	NA	NA	NA	20.9 (n=8)
50 cm							
1	NA	X≥745.7	NA	NA	NA	X<22.6	4.3 (n=32)
2	NA	X≥745.7	NA	NA	NA	X>22.6	7.3 (n=18)
3	NA	X<745.7	X<1174	NA	NA	NA	4.7 (n=17)
4	NA	X<745.7	X>1174	MO	X≥699	NA	6.8 (n=18)
5	NA	X<745.7	X>1174	Not MO	X≥699	NA	13.0 (n=18)
6	NA	X<745.7	X>1174	NA	X<699	NA	17.9 (n=18)
>50 cm							
1	NA	NA	X≥1280	NA	X≥750.4	NA	2.6 (n=36)
2	NA	NA	X≥1280	NA	X<750.4	NA	4.1 (n=10)
3	NA	NA	X<1280	NA	NA	NA	9.7 (n=7)

Note: (n=X) represents the number of samples at each node of the decision tree. NA) not applicable in the tree Landform AF) alluvial fan, OF) outwash surface and MO) moraine.

4.3.5 Maps of soil pH_{H2O} and Al_{CaCl2} in the Ashburton Lakes catchment

The rules established in the decision tree analysis of pH_{H2O} and Al_{CaCl2} were used to create a map of the catchment for the 20 cm depth zone, broad patterns within the catchment were observed (Figures 4.17 and 4.18).

4.3.5.1 Soil pH_{H2O}

There was a large area in the south of the catchment, with soils that have a mean pH_{H2O} of 5.6 (Figure 4.17), which corresponds to the drier areas (<1174 mm y^{-1}). The most acidic soils were identified as a band through the centre of the catchment from east to west, and an area surrounding Lake Heron. These acidic soils are found in the wettest areas (≥1174 mm y^{-1}) and are positioned on the valley floor (<699.1 m.a.s.l). Variability on mountain slopes at higher elevation arises as a result of the aspect criterion: south facing slopes have lower pH_{H2O}.

4.3.5.2 Extractable Al_{CaCl2}

There were distinct areas identified which had contrasting Al_{CaCl2} concentrations in the catchment (Figure 4.18). The higher concentrations in the catchment were found at the wettest sites in the catchment (≥1266 mm y^{-1}). The pink and purple areas with higher concentrations of Al_{CaCl2} seem to mirror the areas that were identified as most acidic in the soil pH_{H2O} map above. The lowest Al_{CaCl2} areas, with a mean Al of 3.8 mg kg^{-1} are more difficult to differentiate on the map, as they don't appear as a coherent area. These are drier areas of the sites sampled (<1266 mm y^{-1}) and south facing sites.

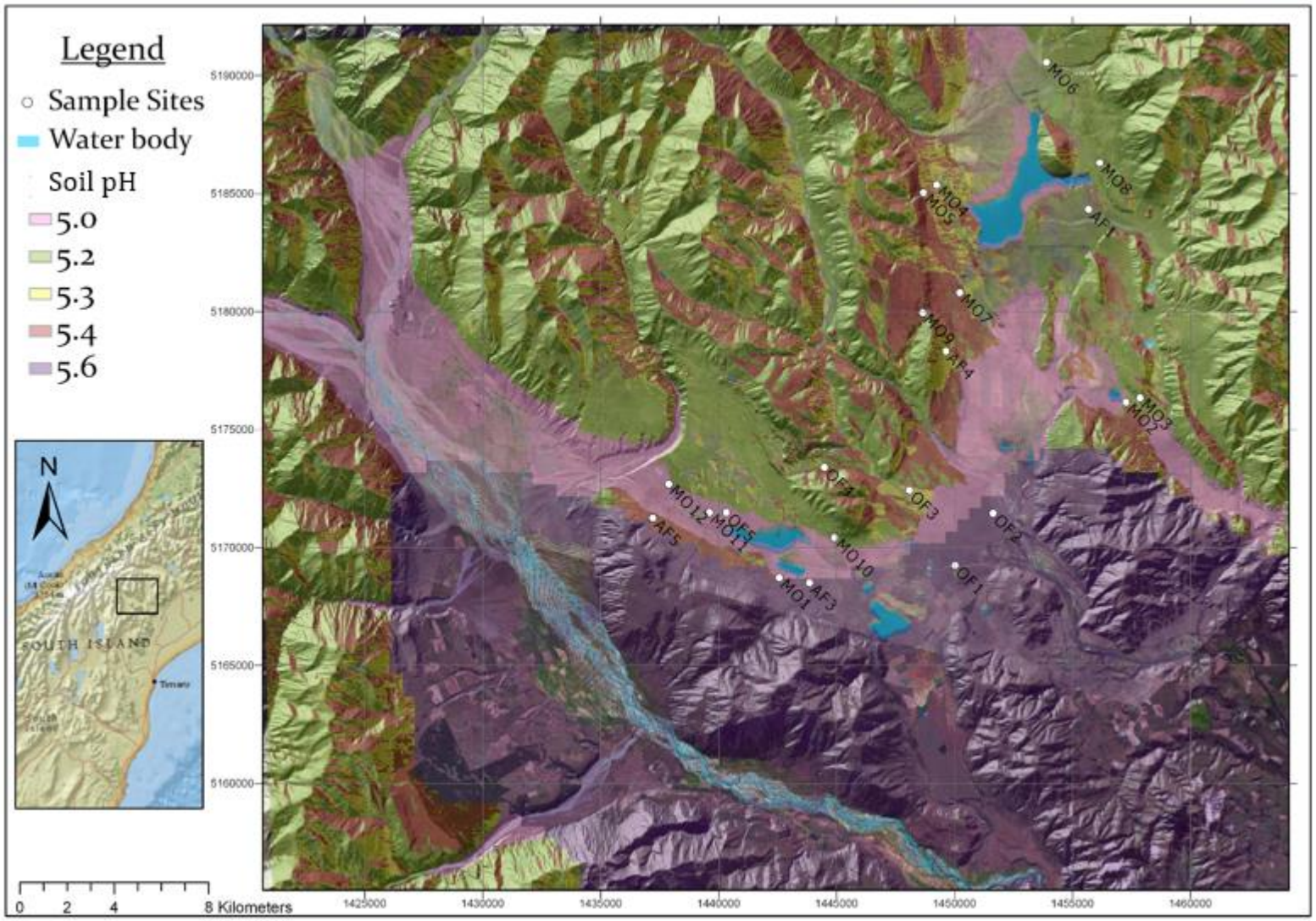

Figure 4.17 A map of mean soil pH_{H2O} for 0-20 cm depth in the Ashburton Lakes catchment, constructed using rules from the decision tree analysis.

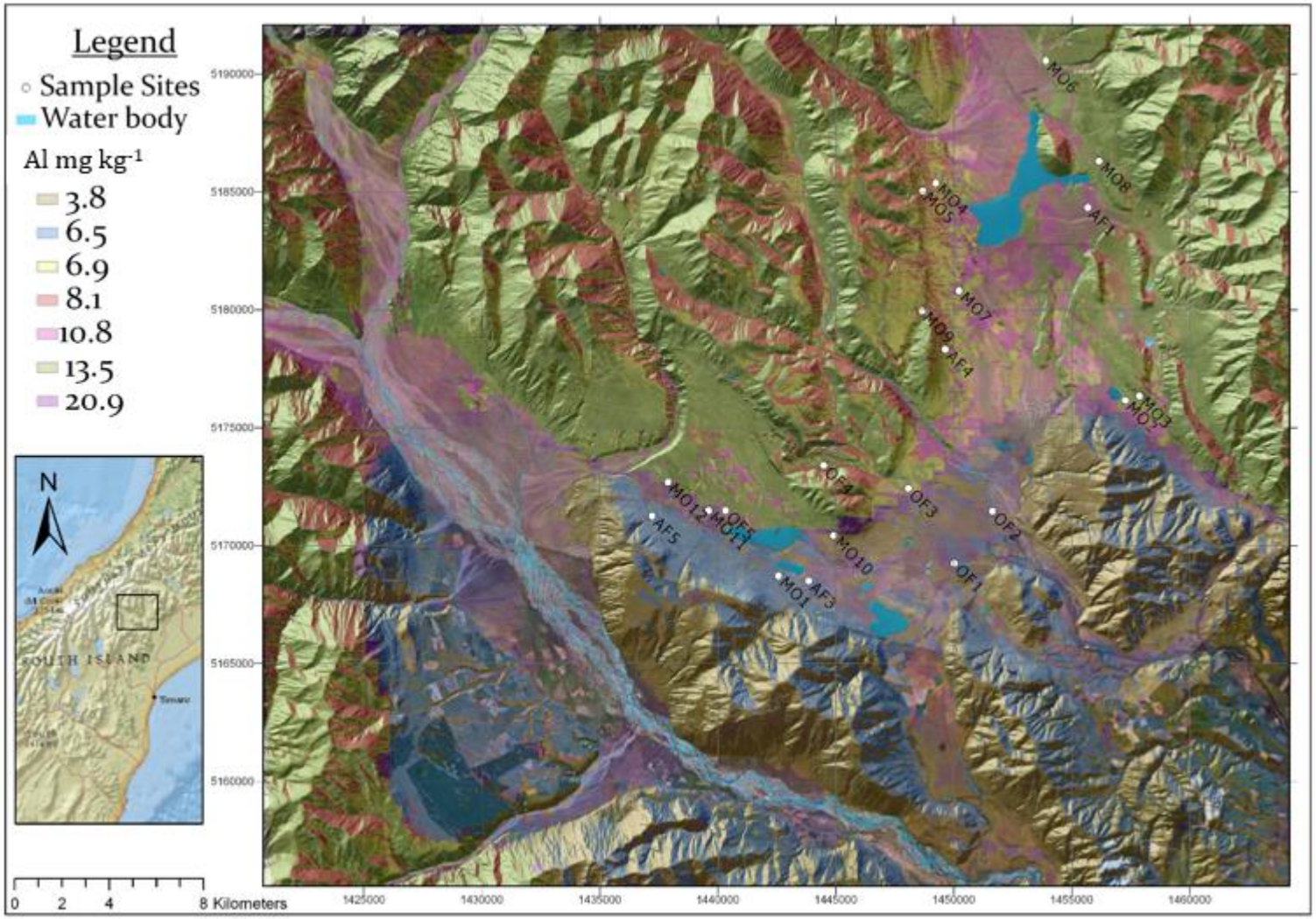

Figure 4.18 A map of mean Al_{CaCl2} concentration for 0-20 cm depth in the Ashburton Lakes catchment, constructed using rules from the decision tree analysis.

4.4 Discussion

4.4.1 Soil morphology

The soils sampled in this catchment were all Brown Soils, either Acidic Orthic Brown (19 soils) or Typic Orthic Brown (two soils), despite the contrasts in landform age. The Ashburton Lakes area is conducive to the formation of Brown Soils, which in the South Island are typically formed in higher rainfall (>800 mm y^{-1}) areas (Hewitt, 2013). Brown Soils are more leached and weathered, often more acidic with lower fertility than Semiarid or Pallic Soils (found at lower rainfalls), and are older and more weathered and leached than Recent Soils (Hewitt, 2013). Orthic Brown Soils usually occur on steep or hilly slopes or on Holocene surfaces (Hewitt, 2010). Soil pH_{H2O} in the B horizon is used as a diagnostic characteristic for identifying Acid groups or Acidic subgroups of Brown Soils. The criteria for Acid Brown Soils are soils with a pH_{H2O} of 4.8 or less in some part between 20-60 cm from the mineral surface or a placic horizon (Hewitt, 2010). If the soil pH_{H2O} was <5.5 in at least one part of the B horizon to 60 cm from the mineral surface, then the soil was classified as an Acidic Orthic Brown Soil. Soils that did not meet this criterion were classified as Typic Orthic Brown Soils. These were found at sites AF3 and MO2. The AF3 and MO2 sites were similar in elevation, at 696 and 703 m.a.s.l respectively and at slopes of 2.9% and 3.9%. However, sites differed in the age of the surface, the landform, rainfall, aspect and the land-use (Tables 4.1 and 4.2). Acidic Orthic Brown and Typic Orthic Brown Soils span different aged surfaces, rainfall, landforms, aspect, elevations and slope classes and there appeared to be no pattern in their distribution in this catchment. Lynn *et al.* (2015) mapped in S-Map predominantly Brown Soils in this area, however, there were also some Recent Soils on the fans. The classification of soils sampled in this study were consistent with findings of Lynn *et al.* (2015); that majority of sites were Brown Soils. There was one alluvial fan site (AF4) which they classified as a Recent Soil, however, in this study it was classified as an Acidic Orthic Brown Soil. Two sites were not mapped in their survey (OF4 and MO6), which were classified as Acidic Orthic Brown Soils.

These soils can be compared with the climosequence of Webb *et al.* (1986) and the two chronosequences in different climates of Harrison *et al.* (1990). Webb *et al.* (1986) observed the development of Podzols at highest rainfall sites of 1800 mm y^{-1} and 2000 mm y^{-1}, despite the 2000 mm y^{-1} site being on the youngest landform. This indicates that the effects of soil age on soil development are overwhelmed by the effects of climate, in this case rainfall and the leaching it promotes. The rainfall was above the range for sites sampled in our study. The soil in that sequence of comparable rainfall (on a landform of late Otiran age) was a Brown Soil but belongs to Acidic Allophanic subgroup. This is a contrast to the soils in the Ashburton Lakes catchment. However, P retention in the

McKenzie basin soil was at the threshold between allophanic and non-allophanic. The allophanic properties of the soils in this study were not explored, but it is possible some had allophanic character. The Harrison *et al.* (1990) terrace chronosequence was at a lower rainfall (c. 830 mm y^{-1}) but the soils had Brown Soil morphology consistent with soils found at similar rainfalls in Webb *et al.* (1986) study. Harrison *et al.* (1990) also found Podzols at the two older moraine sites in the chronosequence and increased weathering and development, reflective of a forest history. The age of the sites was similar to the Latest Late Otiran sites in our study, however, the Late Otiran and Early Otiran or Older sites are older than their M2 and M3 sites. The rainfall at their sites was in the same range as ours and elevation, however, there was no evidence of podzolisation in our study. This may be explained by past vegetation patterns, with the Craigieburn area covered in mountain beech forest and the Ashburton Lakes catchment with Podocarps (Landcare Research New Zealand Ltd, 2012). In contrast, the youngest moraine site in their study was classified as an Allophanic Brown Soil, and the authors suggest that the morphology of this soil indicates long periods of time under grassland. This site was younger than the sites in the Ashburton Lakes catchment, however, it represents a similar soil morphology and is within the same rainfall range.

Recognising the presence of a loess component to soil is important because when a loess sheet mantles landforms of different age the effects of the age difference for soil development are negated. Loess was recognised at different sites in the catchment in which there were no clasts present and a silt loam texture, either in the topsoil or throughout the whole profile. The OF4 site had no clasts in the top 30 cm and the MO4 site had no clasts above 100 cm in the profile. There were no clasts throughout the profiles at MO6 and MO9 sites, which could indicate loess parent material. There was no apparent pattern in its occurrence, although it was not present on alluvial fans. The sites spanned across different aged surfaces, landforms, elevations, slope and aspects in the catchment. The expectation is that older surfaces should have more loess present because of the greater opportunity for loess to accumulate. The soils were on pre Holocene surfaces, either Latest Late Otiran or Late Otiran and were on both moraines and an outwash surfaces. However, loess was not present at the oldest site sampled. These sites had rainfall ≥1403 mm y^{-1} (higher rainfall sites), with the elevation of sites ≥734 m.a.s.l and slope ranging from 4 - 10°. Given other factors affecting loess accumulation being equal, the preservation of loess is likely dependent on a complex erosional history. According to maps by Schmidt *et al.* (2005), loess cover in the Ashburton Lakes catchment is present as both patchy and continuous, with 25-50% thin loess (0.25-1 m) and 50-85% very thin (0.05-0.25 m) loess cover. Most loess cover groups are represented ranging from low to high.

4.4.2 Soil chemistry (pH_{H2O} and Al_{CaCl2})

4.4.2.1 Depth effects

Depth was the most important single factor for soil pH_{H2O} in this catchment. Soil pH_{H2O} differed (P<0.001) among depths in the soil profile. The mean soil pH_{H2O} was lower (more acidic) in the top soil and increased with increasing depth in the soil profile (Figure 4.6). Harrison *et al.* (1990) found that the pH_{H2O} of the topsoil decreased along a development sequence of terrace soils. The total exchangeable bases declined, due to a greater susceptibility to erosion and leaching and therefore a decrease in soil pH was observed. Their study reported an increase in soil pH with depth in the soil profile and reached constant pH values in the C horizons for a Recent Soil and four Allophanic Brown Soils on a sequence of terrace soils near Puffer Stream. Eger and Hewitt (2008) found that the soil pH_{H2O} values increased from the surface to the base of the soil profile for eight hillslope sites in the Canterbury high country, which ranged in depth from 75 cm-135 cm+. Across all soil orders in the NSD analysis in the previous chapter, the mean soil pH_{H2O} increased with an increase in depth. The same trend was observed in this catchment study.

A strong difference (P<0.001) in soil Al_{CaCl2} concentration was observed among different depths in the soil profile. The Al_{CaCl2} concentration was highest in the top soil and reduced with increased depth (Figure 4.7). Harrison *et al.* (1990) found for two high country soil sequences that exchangeable Al_{KCl} was highest in the top 40 cm (terrace soils) and top 50 cm (moraine soils). However, the concentration at each site differed as a response to landform age, rainfall and weathering. All sites sampled in their study had Al_{KCl} concentrations throughout the profile which were above the toxicity threshold for white clover $\geq$1.0-2.0 $cmol_c/kg$ Al_{KCl} (Edmeades *et al.*, 1983). Our data were similar. Contrastingly in a study by Singleton *et al.* (1987), the opposite trend was measured in a Ruawaro clay loam (Typic Yellow Ultic Soil) and the Te Kauwhata clay loam (Orthic Granular Soil). Both soils increased in extractable Al concentration from 2.3 mg kg^{-1} and 3.9 mg kg^{-1} in the 0-20 cm zone to 14.1 mg kg^{-1} and 6.9 mg kg^{-1} in the 60-80 cm zones respectively. However, the soils in their experiment were derived from pumiceous alluvium and volcanic ash parent materials, in contrast to the Brown Soils formed in greywacke-derived regoliths in the Ashburton Lakes catchment. Across all soil orders in the NSD analysis (Chapter 3), mean soil pH_{H2O} increased with an increase in depth, as did the KCl extractable Al concentration. This was a different trend to the catchment study, however, many soil orders from across New Zealand were included in the database study. This highlights that the depth at which Al is present at high concentrations in the soil, varies among sites and soil types, and that local assessments on profile variability of Al_{CaCl2} is necessary.

Even though there was an overall decrease in the Al_{CaCl2} concentration with increased depth in soils of the Ashburton Lakes catchment even at 80 cm in the soil profile, the mean Al_{CaCl2} was 3.6 mg kg^{-1}, concentrations that could be toxic to sensitive legumes such as white clover and lucerne (Moir *et al.*, 2016). In the top 20 cm, the mean Al_{CaCl2} concentrations range between 7.1 mg kg^{-1} and 8.4 mg kg^{-1}, which even for the most hardy and tolerant legumes, will likely represent 'toxic' conditions (Moir *et al.*, 2016).

4.4.2.2 Environmental controls on pH_{H2O} and Al_{CaCl2}

4.4.2.2.1 Landform type

There was a strong difference (P<0.001) in the soil pH_{H2O} measured among the three landforms. The mean soil pH_{H2O} was highest on the alluvial fan, while the moraine and outwash surface soils were more acidic (Table 4.4). However, the difference between the mean pH_{H2O} on the alluvial fan, compared to the moraine and outwash surfaces, was only 0.1 pH units. Harrison *et al.* (1990) previously reported lower soil pH_{H2O} in the topsoil of moraines compared to alluvial fans. This was attributed to the greater weathering, increased soil development and the leaching of basic cations from the soil at the moraine sites. Age and climate in our study were likely to be more important factors influencing the pedogenesis and chemistry, rather than the landform the soil formed on. It is important to note that not all soils of the same landform are located on the same age surface, with the exception of the alluvial fans, which are all on Holocene aged surfaces. Overall, the soil pH_{H2O} was generally lowest on the moraines in the Ashburton Lakes catchment (Figure 4.8), however, there was minimal difference in the mean pH_{H2O}, even though it was statistically significant. There is substantial variation in the soil pH_{H2O} measured across a single landform sampled (Figure 4.8). This is likely to be the result of the interaction of other factors in this landscape.

There was no difference (P>0.05) in the mean extractable Al_{CaCl2} concentration among the three landforms sampled (Table 4.4). Overall, the Al_{CaCl2} concentrations appeared to be higher on the moraine soils (Figure 4.8), however, there was no difference in the mean Al_{CaCl2} concentrations across landforms. This was an unexpected result and is contrasting to other New Zealand studies across landforms.

Harrison *et al.* (1990), measured higher exchangeable Al_{KCl} concentrations in the sequence of moraine soils compared to the alluvial terrace soils. The alluvial terraces were located in a drier intermontane basin, while the moraines were in a higher rainfall zone in Craigieburn. The contrasting environments could be a contributing factor to the substantially greater difference in extractable Al concentrations

between landforms in their study, as there was a larger difference in rainfall between Harrison's terrace and moraine sequences than exists in the Ashburton Lakes study area. In the latter area the mean extractable Al concentration was within the same range on all landforms and was at concentrations that could be toxic to plants (Moir *et al.*, 2016). Other factors in this catchment have more influence on the extractable Al concentrations than the landform that the soil has formed on. These factors could include soil age, slope, elevation and aspect.

4.4.2.2.1 Landform Age

Soil pH_{H2O} differed strongly (P<0.001) among different aged surfaces in the catchment (Table 4.5) but there was not a systematic trend with increasing age. Soils on progressively older landforms did not show consistently declining pH_{H2O} as they did in the study of Harrison *et al.* (1990). For example the mean pH_{H2O} was the same on early and Latest Late Otiran surfaces. The difference in mean soil pH_{H2O} between each surface was 0.2-0.3 pH units, although, there was large variation in the pH_{H2O} measured for soils on each land surface. A potential explanation for the contrasts between this study and that of Harrison *et al.* (1990) is the lack of uniformity of other soil forming factors (such as slope, aspect), which are more tightly controlled in chronosequence studies such as Harrison *et al.* (1990). Even in Harrison's moraine chronosequence the youngest moraine soil had lower pH_{H2O} than the soil on the oldest moraine, confirming that other factors, which are also likely at play in the Ashburton Lakes area, can be more important than soil age in determining soil pH and other chemical properties. In this instance the higher elevation of Harrison's youngest moraine soil and the associated increase in rainfall probably was involved especially if thresholds in water balance were crossed (See Dixon *et al.*, 2016).

Eggleston (1989) found for sites sampled on depositional lobes of different age on alluvial and debris flow fans that there was not a clear sequential trend in soil pH_{H2O}, despite systematic trends in soil morphology; a similar finding to our study. The lowest soil pH sites were located on the oldest deposit, however, an intermediate age deposit had no pH values below 5.6. This was interpreted by the author as a result that reflected a period of renewed nutrient cycling and soil development, and the elevation of pH and base saturation superimposed upon an older soil complex. Higher pH on this part of the fan could represent a more buffered system compared to the older deposit. The youngest deposits had lower soil pH values than the intermediate age deposit. Eggleston (1989) suggested that the acidification was a result of land-use.

A substantial difference (P<0.001) in the soil Al_{CaCl2} was observed among different aged surfaces across this catchment. The mean extractable Al_{CaCl2} was highest on the two surfaces that had the lowest mean pH_{H2O}, including the oldest surface (Early Otiran or older) and the Latest Late Otiran (Table 4.5). The

Al_{CaCl2} concentration was highest on the oldest surface, however, mean extractable Al_{CaCl2} concentrations did not follow a sequential increase with age of the surface sampled on. This was an unexpected outcome, as other studies along soil development sequences have reported an increase in soil extractable Al concentrations with increasing age.

In the previous NSD chapter, Recent Soils were found to have the lowest Al_{KCl} concentrations, which was uniform throughout the profile. This finding suggests that the younger and less weathered soils have undergone less acidification and have lower concentrations of Al present. Eggleston (1989) found, on geomorphic surfaces of different age within fans in the Cass Basin, that the Al_{KCl} concentration was highest for soils on the older surfaces, with Cass and Katrine soil morphology. In an afore mentioned study, Harrison *et al.* (1990) found that exchangeable Al_{KCl} increased with soil age and development. The moraine sites had higher rainfall and overall extractable Al concentrations compared to the alluvial terrace soils, however, Al increased along both development sequences with increasing age. The highest Al was generally located in the top 50 cm of each of the soil profiles. The age of a surface is related to both time and location in the landscape. An increase in soil acidification along these sequences and an increase in Al were similar findings to our study. However, the environmental conditions, the catchment area and many of the soil types were different.

It must be emphasized that, although the highest mean extractable Al concentration was measured on the older surface, there was only one site sampled on this older land surface. An increase in extractable Al_{CaCl2} sequentially with age was not observed in our study, however, this could be related to different numbers of sites sampled on the four geologic surfaces. Further sampling on these sites would give more confidence in the result and may show a clearer pattern in soil Al_{CaCl2}. The non-intuitive order to the extractable Al concentrations across the age sequence also implies that factors other than the age of the surface are influencing the soil extractable Al concentrations in this catchment.

4.4.2.2.2 Rainfall

Rainfall was identified as a factor that affected (P<0.05) the soil pH_{H2O} in this catchment. The overall trend was an increase in the mean pH_{H2O} with an increase in median annual rainfall (Figure 4.4). This was an unexpected result, as other New Zealand studies have reported different trends for soil pH across a rainfall gradient. Leamy *et al.* (1974) found that the soil pH_{H2O} decreased as the rainfall increased for a climosequence of soils in Central Otago. Sites differed in elevation in the landscape and soils ranged from Pallic to Allophanic Brown Soils. An increase in both leaching and weakly decomposed organic matter were suggested as reasons for the observed acidification with increased

rainfall in their study. Webb *et al.* (1986) reported similar trends for Allophanic Brown Soils along a transect of moraines near Lake Pukaki; the soil pH_{H2O} declined with an increase in rainfall from 640 mm y^{-1} to 2000 mm y^{-1}. Increased soil acidification was observed at the higher rainfall site as a result of the leaching of basic cations from the soil.

A smaller rainfall range across sites in the Ashburton Lakes catchment (1128 mm y^{-1} to 1558 mm y^{-1}), a difference of 230 mm y^{-1} annually, could be a reason why no overall decrease in pH_{H2O} was observed with an increase in rainfall. In contrast, the studies by Leamy *et al.* (1974) and Webb *et al.* (1986) ranged from 460 to 1500 mm y^{-1} and 640 mm y^{-1} to 2000 mm y^{-1} respectively, a difference of 1040 mm y^{-1} and 1360 mm y^{-1}, which showed stronger trends of acidification. It is possible that the rainfall range may not be large enough to allow the trends found by other authors to be observed in this field study. Moreover, the rainfall range covered by the sites in this study may not cross important thresholds of rainfall over which significant change might be expected. Dixon *et al.* (2016) sampled 28 soil profiles in the McKenzie basin with a rainfall gradient of around 400 to 4700 mm y^{-1}. Their study determined an important pedogenic threshold at ~800 mm y^{-1} rainfall and between 400 mm y^{-1} and 800 mm y^{-1} there was a trend of decreasing pH with increasing rainfall. There was a rapid loss of exchangeable cations (Ca, Mg and K) within this rainfall range and soil exchange sites were depleted of cations at sites with a rainfall >800 mm y^{-1}. The pH_{H2O} and rainfall trend shown in our study is not inconsistent with the zone of reduced pH sensitivity in their study at a mean annual rainfall >800 mm y^{-1} (Dixon *et al.*, 2016). Both the Leamy *et al.* (1974) and Webb *et al.* (1986) studies had sites that were below this threshold and showed a decrease in pH_{H2O} with an increase in rainfall, whereas in this catchment study the lowest rainfall is above this range at 1128 mm y^{-1}. As such, it is likely that our study did not capture the transition across this pedogenic threshold. Dixon *et al.* (2016) also found that the consistent changes in precipitation overprinted the differences in age and weathering in their sequence of 28 soil profiles, following a north to south increase in weathering along the rainfall gradient, rather than a north to south decrease with relative age. A key finding was, at this threshold (800 mm y^{-1} rainfall,) there is a transition point from a water deficit to a positive water balance. Moisture availability and leaching intensity are responsible for changes in the soil weathering and development along this sequence.

Unlike the studies cited above it is important to note that in the present study the difference in mean pH_{H2O} between the lowest rainfall (1000 mm y^{-1}) and highest predicted rainfall (1600 mm y^{-1}) was only 0.2 pH units. The difference was statistically significant and rainfall was identified as a significant factor, however, it could be a correlation rather than causation of pH_{H2O} differences in the catchment. The

model did not fit the observed data as well as some of the other factors did. The variation in the soil pH_{H2O} at a single rainfall and the pattern of the data (Figure 4.4), shows that there are likely other factors involved.

Rainfall was determined as a factor that affects (P<0.01) the extractable Al_{CaCl2} concentration in the catchment. There was an overall decrease in the mean extractable Al concentration with an increase in median annual rainfall (Figure 4.5), which was an un-expected result. Other studies have measured an increase in soil Al at higher rainfall sites. Webb *et al.* (1986) found, in the previously mentioned study across a rainfall gradient, an increase in extractable Al_{KCl} between the lowest and highest rainfall sites. The increase in extractable Al concentration was linked to higher leaching and the reduction in bases on the cation exchange sites. An increase in clay content under higher rainfall, which was attributed to more intense weathering, could result in more exchange sites for acidic cations to bind to. Harrison *et al.* (1990) also reported an increase of soil extractable Al_{KCl} with soil development and increased leaching, which corresponded to higher rainfall environments. Both studies sampled sites with a much wider range in rainfall than was sampled in the Ashburton Lakes catchment study. Dixon *et al.* (2016) found that the pedogenic threshold of at ~800 mm y^{-1} rainfall coincided with associated cation weathering, acidification, reduced buffering capacity and the release and rapid mobilisation of trivalent metallic ions. Subsurface peaks in Al_o concentrations increased across the climate gradient with increased rainfall and weathering. In their study, the strong variation in rainfall across the range overwhelmed any other potential confounding factors. The Ashburton Lakes catchment rainfall fell above the pedogenic threshold identified by Dixon *et al.* (2016) and, as such, a different trend in extractable Al concentrations was observed. Moreover, given the range in rainfall and the relationship with extractable Al (Figure 4.5), it is likely that there are confounding factors such as elevation and aspect in our study, which were not present in their study.

Walker and Adams (1959) found that organic matter (OM) increased with rainfall to a maximum at 1500-2000 mm y^{-1}. At mean annual rainfall >2000 mm y^{-1} the OM declined as these soils were strongly leached and weathered. With increasing rainfall, the plant growth improves and more OM accumulates in the soil. It is possible that the decrease in Al with increased rainfall in the Ashburton Lakes study, is a result of Al binding to OM and removal from soil solution. The ability of OM and low molecular weight organic acids to complex soluble Al and reduce bioavailability, has been well documented in the literature (Ismail *et al.*, 1994; van Hees *et al.*, 2000). However, measurement of total C and N concentrations could not be conducted for the full dataset of samples and therefore this explanation

remains speculative. Moreover, this process would likely only affect soil at depths in the profile that are influenced by plants (top soil).

4.4.2.3 Soil chemical variables

Total nitrogen was the most important factor that affected (P<0.05) soil pH_{H2O} across the 76 soil samples (Figure 4.9). There was a linear decrease in soil pH_{H2O} with an increase in soil total N. This was a different trend to those observed in other studies. Walker and Adams (1959) found that an increase in leaching in each weathering zone (a sequence from weakly to strongly weathered soils), led to an increase in the C:N ratio (reduction in total N and increase in total C) in the A horizon with increased soil acidification. This result was attributed to increased plant growth, more carbon and OM accumulation. Leamy *et al.* (1974), also found an increase in C: N ratio in the A horizon across soils in a rainfall sequence in Central Otago, which was coupled with a decline in soil pH_{H2O}. This result was explained by increased plant growth (increased C) and a reduction in biological decomposition under high leaching and low temperature conditions (Leamy *et al.*, 1974). These studies suggest that the pH affects the total N in the soil, rather than the nitrogen determining the soil pH. However, the trends were the opposite to those found in our analysis. An explanation could be that more organic carbon drives both acidification (organic acids) and more N, i.e. overwhelms any shifting C:N ratio effect.

Interestingly, in the previous chapter, a significant relationship between total N and Al_{KCl} concentrations in the top 20 cm of the soil profile was identified. There was a linear decrease in extractable Al concentrations with an increase in total N. The relationship was interpreted as high Al_{KCl} restricting N fixation by legumes, with low soil N as a consequence. There is the assumption in the NSD study that soil pH_{H2O} increases with decrease in Al_{KCl}. The opposite trend in the present study is difficult to explain, although it should be noted that there was a much smaller range of total N in the Brown Soils in the Ashburton Lakes catchment compared to the NSD analysis**.** This relationship between soil pH_{H2O} and nitrogen was significant for the 76 soils in this catchment across a range of soil pH_{H2O} (4.8 to 6.0) and total N. However, there was a large variance in soil pH_{H2O} at a given N content (Figure 4.9). The relationship may not be causative, instead reflecting interactions among other correlated variables. Total N was significantly negatively related to soil pH_{H2O} (pH declines- total N increases), and remembering the strong negative relationship between soil pH_{CaCl2} and Al_{CaCl2} (see below), it is possible that the N-Al relationship derives from a pH control. Moreover, for the raw data in the NSD, when the pH_{H2O} and total N are plotted there is a distinct decrease in pH_{H2O} with an increase in total N, the same trend as was observed in this analysis.

Soil pH_{CaCl2} was the best predictor (P<0.001) of Al_{CaCl2}. There was a strong linear decrease in the Al_{CaCl2} concentration with an increase in the pH_{CaCl2} (Figure 4.10). This was an expected trend, as there is a known relationship between soil pH and Al_{CaCl2} (Edmeades *et al.*, 1983; Kinraide, 1991; Moir & Moot, 2010, 2014; Whitley *et al.*, 2016). Soil pH influences the form of Al present in the soil and at a soil pH of <5.5, the Al in the soil solution (Al^{3+}) is much higher, which means that it is extractable by $CaCl_2$ (Kinraide, 1991). Expected toxic levels of Al_{CaCl2} (>3 mg kg^{-1}) occurred at pH_{CaCl2} of 4.6 and below. Soil pH_{CaCl2} performed better than soil pH_{H2O} as a predictor for Al_{CaCl2}, which was also found by Eggleston (1989) for 12 soil profiles in the Cass Basin.

4.4.2.4 Environmental controls based on decision trees

Splitting the dataset into depth zones and including the main factors (rainfall, age and landform) plus additional variables including aspect, slope and elevation, enabled controls on, and landscape patterns of, pH_{H2O} and Al_{CaCl2} to be explored. General trends indicated that the higher rainfall (≥1174 mm yr^{-1}) sites, located at lower elevations in the catchment (<699 m.a.s.l) and south facing in aspect, had more acidic soils with more Al_{CaCl2} in the 20 cm and 50 cm depth zones (Table 4.9).

Table 4.9 Summary of the pH_{H2O}, Al_{CaCl2} concentration, and characteristics of the 21 sites sampled in the Ashburton Lakes catchment.

Variable	Mean	Minimum	Maximum
pH_{H2O}	5.4	4.7	6.0
Al_{CaCl2} (mg kg^{-1})	7.8	1.2	39.1
rainfall (mm yr^{-1})	1368	1128	1558
slope (% rise)	13.2	2.1	44.5
aspect (° related to N)	92.9	6.1	156.4
elevation (m.a.s.l)	761.6	624.1	988.3

The dichotomy of a high pH_{H2O} class at low rainfall and a low pH_{H2O} class at high rainfall appears intuitive but contrasts with the trend identified in the linear models, of increasing pH_{H2O} with increasing rainfall (Section 4.4.2.2.2). Considering Figure 4.4 it appears the algorithm has separated a small group of soil pHs at rainfalls of < 1200 mm y^{-1}, for both 20 and 50 cm depth zones, which are higher than the larger remaining group at higher rainfalls. A trend of increasing pH_{H2O} with increasing rainfall in the larger group has obviously dominated the regression analysis and caused the apparent contradiction between the two analyses. The larger low-pH group at rainfalls above 1174 mm y^{-1} is subdivided by altitude (low pHs < 699 m and higher pHs above) for both the 20 cm and 50 cm depth zones. This pattern has not been reported elsewhere, but may relate to older more strongly developed soils on the more stable valley floor. Spatially this area corresponds to a band through the centre of the catchment from east to west, and an area surrounding Lake Heron (Figure 4.17). At higher elevations,

southerly aspects have lower pHs, consistent with the findings of Eger and Hewitt (2008). They found aspect-induced microclimatic differences promoted stronger leaching, enhanced weathering and acidification on south facing slopes. Similarly, Archer and Cutler (1983) found stronger leaching and podsolization on south facing slopes in the Ben Ohau Range.

The identification of rainfall, elevation and aspect as factors that discriminate soil pH_{H2O} in this catchment, particularly in the 20 cm and 50 cm depth zone, strongly suggests the influence of water balance and weathering on the soil pH_{H2O}. Dixon *et al.* (2016) found that around the 800 mm y^{-1} rainfall threshold identified, there was a transition to a positive water balance, and that the soil pH between 400 and 800 mm y^{-1} rainfall declined as the annual water deficit declined. They stated that the transition from negative to positive water balance can enable rapid weathering. Webb *et al.* (1986) found that the lowest rainfall sites (640 mm y^{-1}) along the climate sequence had a large moisture deficit and measured higher pH_{H2O} and base saturation and a lower extractable Al concentration. The rainfall of their site was much lower than the lowest rainfall in our catchment study, however, these studies emphasise the role that moisture and water balance have in the chemistry of soils and support our findings. The fact that rainfall, aspect and elevation (inference to water balance effects) are the most important factors in the decision tree, indicates that pH_{H2O} and Al_{CaCl2} are more sensitive to factors affecting leaching rate rather than duration (i.e. landform age).

Approximately the same rainfall criterion as for pH_{H2O} (<1266 mm y^{-1}) identifies a low Al_{CaCl2} group in the 20 cm depth zone, but not deeper zones. Again, the apparent contradictory patterns of Al_{CaCl2} in relation to rainfall identified by the regression and decision tree analyses can be explained as for pH_{H2O} above. The large group at rainfalls above 1266 mm y^{-1} Al_{CaCl2} appears to be insensitive to altitude but it is affected by one or two soil profiles (eight data points) in the narrow rainfall band 1266-1278 mm y^{-1} with anomalously high values (mean 20.9 mg kg^{-1}). Ignoring these outliers and focusing on the remainder of the group (MAR > 1278 mm y^{-1}), southerly aspects have higher Al_{CaCl2}, consistent with their lower pHs. Distinct areas with higher extractable Al_{CaCl2} concentrations were identified that seem to mirror the areas identified as most acidic in the catchment (Figure 4.18). For the 50 cm depth zone, elevations below 746 m, corresponding to valley margins and valley floors yielded higher Al_{CaCl2}, consistent with their lower pHs. In these areas, the 1174 mm y^{-1} MAR threshold gives rise to much higher Al_{CaCl2} when it is exceeded, and the highest of these in the valley floor positions (altitude < 699 m).

Elevation and aspect influences on extractable Al concentrations have been reported in other studies. Adams *et al.* (1999) measured the solution Al (free Al and organically bound) and capacity of humic substances and OM to bind Al (Al-CC) at a depth of 0-7.5 cm in the South Canterbury high country. Sites representing both sunny and shady aspects, and at three altitudes (730, 945 and 1190 m.a.s.l), were sampled. Their study found that the four lowest elevation sites had a lower Al-CC, and therefore higher free Al in solution, which would equate to a high Al concentration extracted by $CaCl_2$, than the two highest elevation sites. The Al-CC decreased with a decrease in soil solution pH_{H2O} and the authors inferred that there could be an onset of Al toxicity through acidification at the lower elevation sites in the catchment. However, the depth of their sampling was only to 7.5 cm, compared to the samples taken every 5 cm in the Ashburton Lakes study. Moreover, their study contained sites with higher elevations than our study, although, the two lower elevation sites are within the range we sampled. Their study found that shady (south facing) sites had lower Al-CC values, lower OM bound and higher free Al present and, therefore, are likely more prone to Al toxicity. Eger and Hewitt (2008) found that Al_o concentrations in the subsoil were highest on south facing slopes compared to north facing. This indicates, coupled with the pH trends, that there was enhanced leaching, weathering and acidification on south facing slopes, which enhanced Al_o and translocation in the profile. Their measure of Al was different to the Al_{CaCl2} used in our study, however, one would expect the Al_{CaCl2} to be higher at the lower pH sites (south facing) sites under greater weathering conditions, although this was not tested.

The >50 cm depth zone showed relatively small variation in pH_{H2O} and Al_{CaCl2} and little can be gleaned from the simple decision trees, however, the lowest mean pH_{H2O} (Al_{CaCl2}) in the >50 cm zone was higher (lower) than the two shallower depth zones. This parallels the trend of increasing pH_{H2O} and declining Al_{CaCl2} with depth identified in the main dataset study (Figure 4.6 and Figure 4.7).

Finally, although the decision trees point to landscape controls on soil pH_{H2O} and Al_{CaCl2} the spatial representation in Figures 4.17 and 4.18 need to be viewed with caution. The data set of 21 profiles represents a reconnaissance level exploration only. Nonetheless, it appears that Al_{CaCl2} and pH_{H2O} have spatial structure and that a more rigorous exploration of spatial patterns of these important soil properties is warranted.

4.5 Conclusions

- Soil types sampled were Acidic Orthic Brown and Typic Orthic Brown Soils.
- Sporadic loess occurrence was identified at four sites, at varying depths which suggested that loess preservation was likely dependent on a complex erosional history, and its occurrence appeared to have no influence on soil pH_{H2O} or Al chemistry.

- Depth was the strongest explanatory variable for pH_{H2O} and Al_{CaCl2}.
- Soil pH_{H2O} increased with depth in the soil profile and Al_{CaCl2} declined. The pH_{H2O} trend was consistent with the trend found in the NSD chapter, however, the Al_{CaCl2} trend was opposite to that of $Al_{KCl,}$ revealed in the NSD analysis.
- Rainfall was a significant factor for Al_{CaCl2} in this catchment, however, there was not an overall trend of an increase in Al_{CaCl2} concentration with an increase in rainfall as was hypothesised.
- There were no systematic patterns of Al_{CaCl2} and pH_{H2O} on different age surfaces in contrast to chronosequence studies which show increased Al_{CaCl2}, and decreased pH_{H2O} with increasing soil age.
- Differences in pH_{H2O} among landform types were found, in contrast to no difference in Al_{CaCl2} concentrations.
- The relationships between pH_{H2O} and Al_{CaCl2} and soil chemical and environmental variables were explored using mixed linear models and decision trees.
- Linear mixed models emphasised that the addition of other factors complicated the model and did not improve the outcome from a single factor model.
- In respect to the soil chemical variables, total N was the best explanatory variable of soil pH_{H2O} and was negatively correlated with soil pH, whereas the opposite trend was revealed in the large scale NSD analysis. The reason for this remains uncertain but may involve the interaction of other correlated factors. The pH_{CaCl2} was the best predictor of Al_{CaCl2}.
- Unlike the NSD analysis, total C was not a significant variable for the Al_{CaCl2} in this study. This could be an aspect worth exploring further for soils in this catchment.
- Al_{CaCl2} was measured at concentrations that could be toxic to legumes, which ranged from 1.2 mg kg^{-1} to 39.1 mg kg^{-1}.
- Relationships were explored further using decision trees on three tiers of the soil profile to limit the dominance of the depth effect.
- At higher rainfall sites, there was consistently an increase in Al_{CaCl2} and decreased pH_{H2O}.
- Elevation occurred high in the trees: low elevation decreased pH_{H2O} and increased Al_{CaCl2}.
- Aspect was important, but occurred lower in the trees, often with south facing sites showing a decrease in pH_{H2O} and increase in Al_{CaCl2}, which in some cases was modulated by the rainfall.
- Age was only present in the pH_{H2O} tree, whereby older sites had higher pH_{H2O}, which was unexpected.
- This field study focused on a single catchment and the results cannot be extrapolated to other catchments in New Zealand, as the combination of soil forming factors such as climate, relief and parent material differ from those in the Ashburton Lakes catchment.

- The maps showed that soil pH_{H2O} and Al_{CaCl2} are strongly related and areas with higher Al_{CaCl2} concentrations in the top 20 cm of the profile were identified.
- The reconnaissance level study presented here suggests spatial structure to soil pH_{H2O} and Al_{CaCl2} variability in the high country of the South Island, which may be of agronomic importance, and as such warrants further exploration through denser and more rigorous sampling.

5 A glasshouse experiment growing legumes as bioindicators of Al toxicity in a suite of acidic New Zealand soils

5.1 Introduction

High and hill country farmers often face challenges with acidic and low fertility (N, P and S) soils (Langer, 1990). Aluminium toxicity is associated with acidic soils, which can severely restrict the establishment and growth of legume species (Haynes & Williams, 1993; Moir & Moot, 2010, 2014). Legumes are key drivers of productivity for these areas and provide an important source of N through biological N fixation (Haynes & Williams, 1993). With high costs associated with lime application via aerial topdressing in high and hill country areas, soils are continuing to naturally acidify and Al toxicity is becoming more widespread. Previous chapters have focussed on soil extractable Al concentrations and factors influencing them, however, this was not linked back to plant growth. This experiment explores the effect of the soil extractable Al concentration measured on plant growth.

This study involved a glasshouse experiment which ran for 10 months in 2015. A total of 10 high and hill country sites, predominantly in the South Island, were sampled to encompass a range of New Zealand soil orders including; Pumice, Allophanic, Brown, Recent and Pallic Soils (Hewitt, 2013). The criteria for selection were that the soils were acidic ($pH_{H2O} \leq 5.6$) and had a range of extractable Al_{CaCl2} concentrations, many at levels which could be toxic to plants. Two legume species were grown, lucerne (*Medicago sativa L.*, cv. 'Kaituna') and Caucasian clover *(Trifolium ambiguum L.*, cv. 'Cossack'). The focus was on lucerne, a more sensitive species to acidity and soil Al (Hoyt & Nyborg, 1972; Rechcigl *et al.*, 1988; Su & Evans, 1996). Caucasian clover was used as a benchmark as it is a more tolerant species, which has been shown to grow well in low fertility, acidic and low P environments (Berenji *et al.*, 2017; Caradus *et al.*, 2001). This is a finer scale experiment than the catchment study in the previous chapter and examines the growth of plants in different soils with lime and P fertiliser inputs under controlled conditions.

The aim of this chapter was to study the effects of soil type and pH_{H2O} on soil extractable Al_{CaCl2} concentrations for these 10 New Zealand soils. In particular, this section used the growth of legumes to quantify the relative biotoxicity of Al in different New Zealand soils. This glasshouse investigation examined how Al toxicity affects legumes and whether this differed for key New Zealand soil orders. This also determined if the current soil Al test reflects the conditions that the legumes experience in the soil.

To achieve this aim, the following objective was established: To compare and contrast key New Zealand soils with known acidity and soil extractable Al_{CaCl2} issues using plants (legumes) as bioindicators. The experiment was designed to allow testing of the following hypotheses regarding soil extractable $Al_{CaCl2.}$

1: Legume yield is strongly associated with soil extractable Al_{CaCl2} concentrations.

2: The relationship between soil Al_{CaCl2} and plant growth differs among soils at the same pH.

5.2 Materials and methods

5.2.1 Soil Collection and Preparation

Soil was collected from 10 high and hill country sites across New Zealand in October and November 2014 (Figure 5.1). Details of location, soil order, rainfall and elevation for each site are given in Table 5.1.

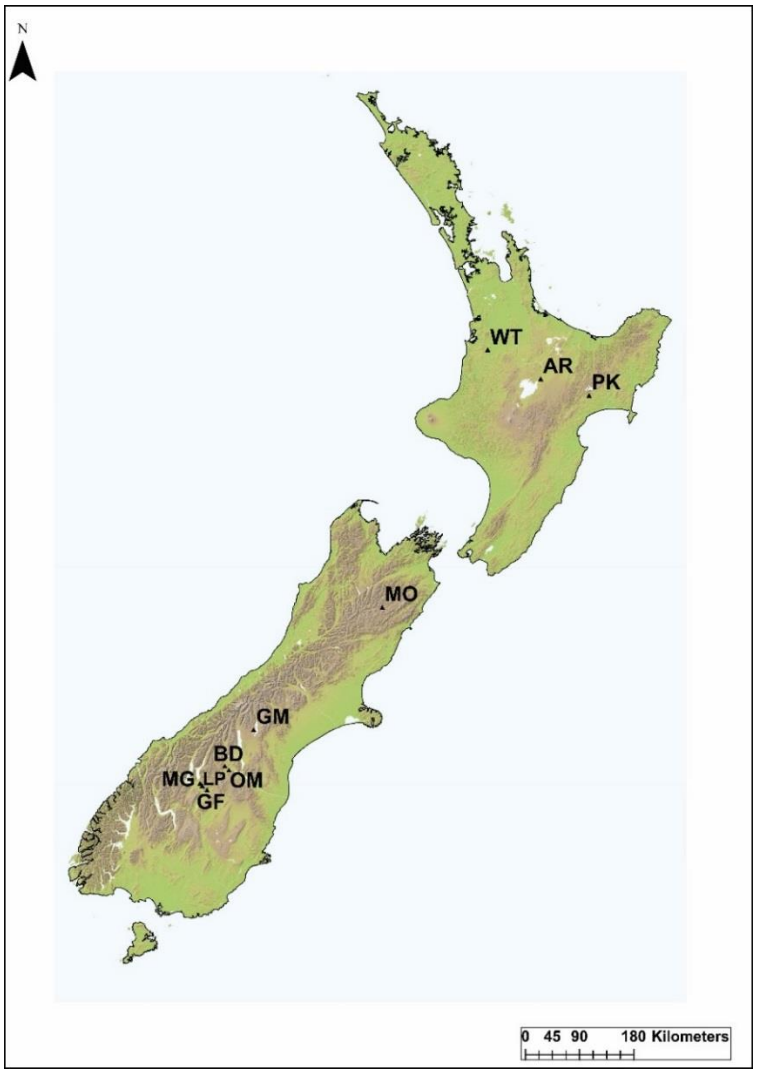

Figure 5.1 The location of the 10 sites where the soil was collected from across NZ.

Soil (0-15 cm) was collected along a transect (minimum of 50 m) at each of the 10 sites. The soil was prepared by passing it through a 6 mm sieve while field moist, removing all plant material and then mixing thoroughly. Subsamples were prepared for chemical analysis by air-drying (30°C, 2 mm sieved).

Table 5.1 A description of the 10 sites where soils were sampled from across New Zealand including the designated site code, name of the property and location, GPS coordinates, soil order, median annual rainfall and elevation.

Site	Property	GPS location (NZTM)	Soil order	Median annual rainfall (mm yr^{-1})	Elevation (m.a s.l).
WT	Waitomo, Waikato.	E1785412 N5765099	Allophanic	1704	84
AR	Aratiatia Station,Taupo	E1873143 N5717186	Pumice	1081	495
PK	Panekiri Station, Gisborne.	E1953459 N5690007	Pumice	1492	495
MO	Molesworth Station, Marlborough	E1610410 N5338595	Brown	1289	914
GM	Glenmore Station, Tekapo.	E1396953 N5135905	Brown	599	765
BD	Ben Dhu Station, Omarama	E1349149 N5075266	Brown	665	545
OM	Omarama Station, Omarama	E1355541 N5068666	Recent	518	489
LP	Lindis Peaks Station, Tarras	E1319574 N5036046	Pallic	616	530
GF	Glenfoyle Station, Hawea	E1311504 N5042987	Brown	624	786
MG	Mount Grand Station, Hawea	E1308183 N5046963	Brown	668	564

Note: Rainfall is the 50th percentile Mean Annual rainfall derived from (National Institute of Water and Atmospheric Research, 2015) at each GPS location. Sites are listed by their geographic location from North to South.

5.2.2 Soil Chemical Analysis

Soil chemical analyses, both standard and advanced, were conducted on the 'native' soil samples after they were air dried and sieved (Table 5.2). Soil pH was measured at a water: soil ratio of 2.5:1 (Blakemore *et al.*, 1987). Available P in the soil (Olsen P on a volumetric basis; Drewry *et al.*, 2013; Taylor *et al.*, 2018) was measured using the method of Olsen *et al.* (1954) with 0.5 M $NaHCO_3$, as a standard method by commercial laboratories in New Zealand. The ability of the soil to retain phosphorus (anion storage capacity) was determined using methods from both Blakemore *et al.* (1987) and Murphy and Riley (1962). The Resin P test, determined the phosphate ions extracted from soil slurry using anionic exchange resin strips (Saggar *et al.*, 1990). The pool of extractable sulphate sulphur and extractable organic S were determined using methods outlined by Watkinson and Kear (1996). A modification of the method by Rayment and Higginson (1992) (1:20 compared to 1:10) was used to test soil extractable cations (Ca^{2+}, $Mg^{2+,}$ K^+ and Na^+), the cation exchange capacity and the base saturation were determined using methods by Brown (1943). The Dumas method of combustion (Horneck & Miller, 1998) determined soil total C and N content using an Elementar 'Vario' MAX Cube Analyzer (Elementar Analysensystane, GmbH). Soil organic matter content was also determined by

combustometric analysis for total carbon using the Elementar Vario Max Cube Analyser. Soil extractable aluminium was determined using 0.02 M $CaCl_2$ with an extraction time of 60 minutes (Analytical Research Laboratory, 2014; Edmeades *et al.*, 1983) then measured on the ICP-OES (Varian 720-ES ICP-OES; Varian Inc, Victoria, Australia). Reserve magnesium was measured using methods of Metson and Brooks (1975) and Blakemore *et al.* (1987), while the reserve potassium was determined by methods of Carey and Metherell (2003). The soil anaerobic mineralisable N was determined through the incubation of soil under anaerobic conditions at 40°C for seven days. Available ammonium was leached with 1.7 M KCl, filtered and determined through the reaction of ammonia with hypochlorite and phenol (catalysed by sodium nitroprusside) to form a blue compound, Indophenol (Hinds & Lowe, 1980). The value obtained gives an indication of the soil's nitrogen availability index (Keeney & Bremner, 1966). Total P was determined using the sum of a detailed set of P fractionation analyses, detailed in Tian *et al.* (2017), according to a scheme described by Condron *et al.* (1996). For completeness, total metal analysis and particle size analysis (texture) were conducted for each soil and are presented in Appendix Tables 3.1 and 3.2.

Table 5.2 Initial soil chemical analyses for the 10 New Zealand 'native' soils used in this experiment (as described in Table 5.1), before the addition of treatments.

	Soil code									
Soil Analysis	WT	AR	PK	MO	GM	BD	OM	LP	GF	MG
pH_{H2O}	5.2	5.2	5.1	5.2	5.0	5.5	5.3	5.6	5.3	5.2
Olsen P (μg mL^{-1})	7	37	16	13	18	12	9	13	12	12
Resin P (mg kg^{-1})	12	54	30	24	31	31	10	18	19	27
P retention (%)	99	46	57	59	42	40	23	21	38	25
Sulphate sulphur (μg g^{-1})	17	6	7	9	15	1	<1	11	5	5
Ext.org sulphur (μg g^{-1})	20	8	7	11	10	6	3	5	5	6
Reserve K ($cmol_c$/kg)	0.69	0.71	0.78	6.45	2.10	3.86	2.29	5.10	5.72	5.74
Anaerobic Min N (kg ha^{-1})	183	87	156	102	169	90	50	175	131	175
Organic matter (% w/w)	22.2	8.5	11.5	8.5	10.6	6.9	4.3	4.7	7.1	6.2
Extractable Al_{CaCl2}* (mg kg^{-1})	5.0	4.8	7.4	13.1	6.6	2.5	3.8	0.9	7.1	2.6
Total P (mg P kg^{-1})	1982	1069	1014	1130	1469	768	593	761	985	914
Total N (% w/w)	1.02	0.43	0.57	0.38	0.53	0.26	0.19	0.24	0.31	0.31
Total C (% w/w)	12.89	4.93	6.70	4.91	6.18	3.99	2.49	2.74	4.14	3.60
Carbon/Nitrogen	12.6	11.5	11.8	12.9	11.7	15.3	13.1	11.4	13.4	11.6
CEC ($cmol_c$/kg)	21	11	15	14	17	13	11	13	13	15
Ca (QTU)	3	5	4	<1	5	3	2	8	3	5
Mg (QTU)	16	10	8	7	14	20	9	20	11	21
K (QTU)	6	7	6	7	7	11	6	7	7	9
Na (QTU)	6	8	6	1	2	6	<1	5	4	2
Base saturation (%)	26.9	37.4	35.9	12.9	34.1	27.6	20.4	53.6	24.6	42.0

Note: Soil codes are listed by their geographic location from north to south. * 0.02 M $CaCl_2$ extractable. QTU stands for quick test unit, indicating plant available Ca, Mg, K and Na. For sheep and beef farms in New Zealand all soils were acidic (pH_{H2O}<5.8-6.0); WT, GM and LP soils were within the target range for Sulphate S, and WT soil was in the target range for organic S, all other soils were below the range required for maximum pasture growth. All soils were below the target Olsen P for their particular soil type (Sedimentary, Pumice and Allophanic), except for AR, which was close to the value for maximum pasture production. All soils were within the target range for K for optimal pasture growth except WT and PK, which were just below. All soils have sufficient Mg (Morton & Roberts, 2004).

5.2.3 Experimental Design and Project Management

5.2.3.1 Trial Design and set up

This pot experiment was conducted under glasshouse conditions at Lincoln University, growing two legume species in the selected 10 New Zealand high and hill country soils. Five rates of lime were applied (Table 5.3) and there were two phosphorus (P) treatments to determine if extractable Al could be reduced by P application alone (Table 5.4). An overall rate of 120 kg S ha^{-1} in the form of gypsum ($CaSO_4 \cdot 2H_2O$) was applied as basal sulphur (S) to each pot (Table 5.5). Each of the two plant species and eight treatments were replicated four times for each of the 10 soils to give a total of 560 pots, which formed a fully replicated randomised block design (Table 5.6). No nitrogen (N) fertiliser was applied, therefore plants depended on fixation for N.

Pots, 2010 cm^3 volume (16 cm deep and 13 cm diameter) were used. Field moist soil (1.9 L) was measured for each pot. Next, the appropriate treatment of lime or P was added to the soil and mixed thoroughly. The soil was then tipped into the appropriate labelled pot. Pots were placed on saucers to collect any leachate from the soil during the experiment to ensure no nutrients were lost from the system and any collected was tipped back into the pot. The set up was conducted on the 28th and 29th January 2015 and pots were placed in the Aluminex glasshouse at Lincoln University Campus, Canterbury, New Zealand. Soils were lightly watered. The final level of the soil was 1-2 cm from the rim of the pot.

Table 5.3 Lime (100% $CaCO_3$, laboratory grade) rates and treatment codes used in the glasshouse experiment.

*Lime rate (t lime ha^{-1})	Lime rate (mg $CaCO_3$ mL^{-1} soil)	Surface applied rate (g $CaCO_3$ pot^{-1})	Pot treatment code
0	0	0	L0
2	1.4	2.6	L1
4	2.8	5.2	L2
8	5.5	10.5	L3
12	8.3	15.7	L4

*Note: The lime was fully incorporated within the soil volume and the application was based on a surface area rate.

Table 5.4 Phosphorus rates (Ca $(H_2PO_4)_2 \cdot H_2O$ Monohydrate, 24.6% P) and treatment codes used for the glasshouse experiment.

Surface applied (kg P ha^{-1})	Phosphorus rate (mg P L^{-1} soil)	Total applied (g P pot^{-1})	Pot treatment code
0 (0)	0	0	P0
43.5 (484)	30	0.06	P1
217.5 (2416)	150	0.28	P2

* Note: The P was incorporated within the volume of the soil and calculated on a soil volume basis. The number in column A that is within the parentheses is the amount of single superphosphate this represents at 9% P by w/w.

Table 5.5 Sulphur rate (Gypsum, 18.6% S) applied to all pots in the glasshouse experiment.

Sulphur rate (kg S ha^{-1})	Sulphur rate (mg S mL^{-1} soil)	Total applied (g S pot^{-1})
120	0.09	0.16

*Note: The gypsum was fully incorporated within the soil volume and application was based on a surface area rate.

Table 5.6 The glasshouse experimental design and treatment codes.

Treatment combinations	P0	P1	P2
L0	L0P0	L0P1	L0P2
L1	L1P0	-	-
L2	L2P0	-	-
L3	L3P0	-	-
L4	L4P0	-	-

5.2.3.2 Glasshouse conditions

The glasshouse was temperature controlled; the heating system was initiated when the temperature dropped below 17°C and cooling vents and fans operated when the temperature reached 23°C. During the summer, the outside of the glasshouse was coated with a white wash to reduce heating from the sun.

5.2.3.3 Plant Establishment

Uncoated Caucasian clover and lucerne seeds (no lime or trace element coating) were sown at a rate of 14 per pot on the 3rd February at a depth of 1-2 mm and lightly watered. All pots were thinned to a final density of five plants on the 18th March.

5.2.3.4 Inoculation

Liquid inoculants were used for both lucerne and Caucasian clover to encourage nodulation and N fixation. The rhizobium strain used for Caucasian clover was ICC105 (~9.4×10^6 cells mL^{-1}; commercial strain) (Pryor *et al.*, 1998) and for lucerne RRI128 (~4.1×10^6 cells mL^{-1}; commercial strain)((Wigley *et*

al., 2017). The liquid inoculant was applied (10 mL per pot) on the 20th February, equivalent to 10^6-10^7 bacteria per mL and was delivered in a 0.85% saline (NaCl).

5.2.4 Trial Management

Plant counts were conducted in February, April and July 2015 and the pots which had fewer than five plants were re-sown. The lower plant numbers were potentially a treatment effect, so re-sowing was used to confirm a response. Pots were continually weeded throughout the experiment. The glasshouse occasionally had insect pests and fungi. Sticky traps were set up to catch flying insects such as white fly (*Trialeurodes vaporariorum*). Pyrethrum, which contains 14 g L^{-1} pyrethrum and 56.5 g L^{-1} piperonyl butoxide in the form of emulsifable concentrate, was used to control aphids (*Myzus persicae*) and sprayed on the 17 March and 6th May. Talstar 80SC, a synthetic pyrethroid with the active ingredient bifenthrin (applied on the 4th and 24th June), Orthene WSG (29th June) and Success naturalyte (applied on the 6th of July) were used to control/exterminate thrips (*Heliothrips haemorrhoidalis*). Alto and Thiram fungicides were used to control fungi on the 3rd August.

5.2.4.1 Soil and air temperatures

The monthly mean air temperature inside the glasshouse was 18.3°C, ranging from 16.3°C in July to 20.2 °C in February. The mean monthly soil temperature was 17.5°C, ranging from 14.6°C in July to 20.5°C in October (Figure 5.2).

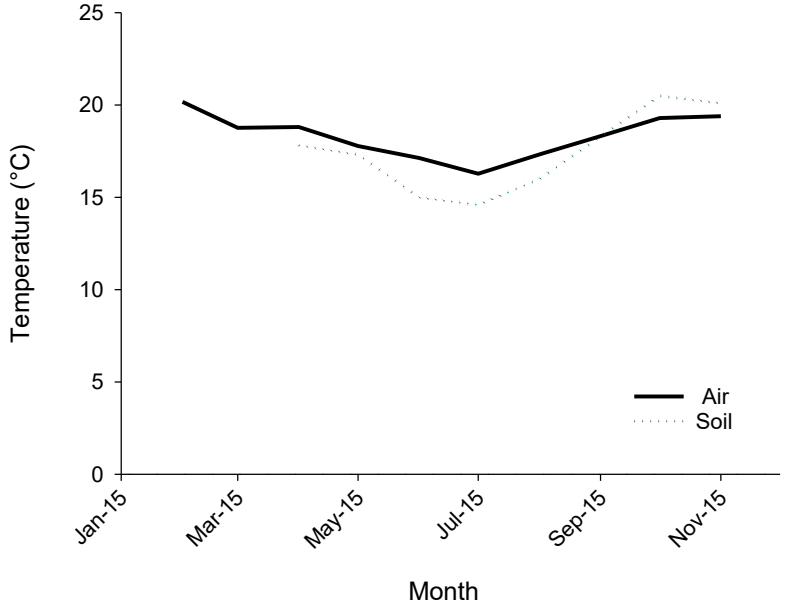

Figure 5.2 The average monthly air temperature inside the glasshouse and soil temperatures (°C) experienced by Caucasian clover and lucerne growing in the Aluminex glasshouse from February- November 2015. Air temperature was recorded every two hours by an automated system run by a computer program. Soil temperatures were recorded from the moisture sensors throughout the growth period.

5.2.4.2 Soil Moisture and Water Management

Soil moisture content must be sufficient to cope with the increasing demands of plant growth and, at the same time, avoid water logging and nutrient leaching from the pots. The irrigation regime was designed to provide water so that there was no significant limitation to plant growth due to water stress throughout the experiment. An automated irrigation system was set up, which consisted of sub mains supplying water for potted plants on each table under a consistent irrigation regime (Appendix Figure 3.1). Water from each sub main was supplied to the pots via pressure compensated Antelco Pinch Drip Emitters (Antelco Murray Bridge South Australia) at a rate of two L hr^{-1} through small water distribution tubes. The irrigation control system consisted of a Campbell Scientific CR23X data logger and relays. Pressure to the system was controlled with a regulator and monitored by a pressure switch connected to the data logger. Strategic pots were instrumented with Decagon 5TM volumetric water content (VWC) / temperature sensors (Decagon 5TM, Decagon Devices LTD, USA) and monitored by the data logger. The data logger kept soil moisture in the pots within specified ranges and applied water, when necessary, by controlling the irrigation system's solenoid valves (controlled Hunter 9Volt latching solenoid valve). If either the pressure switch or the power supply showed a fault, then the data logger prevents the system from attempting to apply irrigation. The target soil moisture content (VWC) for this experiment was 26% and the variation programmed in was ± 2%. The mean daily VWC was 27.7% across the seven sensors and the ranges are presented in Appendix Table 3.3. Sensors were placed in randomly selected pots of the same replicate containing the contrasting soil types, AR, PK, WT, MO, LP, OM and MG soils. It was important to represent the soils with no sensors by having sensors in pots with a similar texture/soil order (Appendix Table 3.2). Manual checks with the HydroSense were conducted frequently to ensure that the moisture was balanced across the pots. If individual pots were dry, water was applied to raise the moisture content to within the range of the other measured pots.

The sensors were calibrated in the 10 soils prior to the experiment, to calculate the relationship between the soil VWC and the dielectric probe reading, using the output of the moisture sensors (5TM, Decagon Devices, Pullman, WA USA) and the HydroSense. The moisture sensors were then inserted into the soil, water was applied in 10% VWC increments, and readings taken from the moisture sensor to calculate the calibration curve. The Topp equation, (Θ (m^3 m^3) = 4.3 X 10^{-6} * ε^3 - 5.5 X 10^{-4} * ε^2 + 2.92 X 10^{-2} * ε -5.3 X 10^{-2}) is a general equation used to convert dielectric measurements to soil moisture values (Topp *et al.*, 1980). However, this equation did not work for these soils and therefore the sensors were calibrated and the resulting equation was used rather than the Topp equation.

5.2.5 Measurements

5.2.5.1 Shoot Yield

Plants were harvested every five to six weeks, to maximise shoot yield but prevent flowering. A total of six harvests were taken, on 4th May, 22nd/23rd June, 10th /11th August, 17th /18th September, 16th/17th October and 23rd November 2015 respectively (Plate 5.1). The herbage was lifted and trimmed to 3 cm above the soil surface. Both species were cut to the same height, however, the youngest leaf on each Caucasian clover plant was left behind, as it is slow to establish and the plants must be able to regenerate after each harvest. Once harvested, herbage samples were dried at 70 °C for 48 hours and weighed to obtain the dry weight. Samples were finely ground (Retsch ZM 200, Retsch Gmbh, Haan, Germany) and bulked on an individual pot basis for all six harvests; for tissue analysis.

Plate 5.1 Harvest 1 (04/05/15).

5.2.5.2 Plant tissue analysis

Herbage (0.5000 g) was digested in 2.5 mL of nitric acid (HNO_3) and 2.5 mL of 30% hydrogen peroxide in microwave vessels and analysed using a Microwave Digestor (CEM MARS Xpress 0-1600 watts $\pm$ 15%). The temperature was increased to 90°C for 15 minutes, held for five minutes and then increased to 180°C for 10 minutes and held for a further 15 minutes. Samples were analysed for a complete range of elements (excluding N) using Inductively Coupled Plasma Optical Emission Spectrophotometer analysis (Varian 720-ES ICP-OES; Varian Pty Ltd, Melbourne, Australia). Samples were also measured for total N using Near-Infrared Spectroscopy (NIR) at Lincoln University (NIRS; MODEL: FOSS NIRSystems 5000, Maryland, USA).

5.2.5.3 Root nodule scoring

After the final shoot harvest, plants were removed from their pots and all soil was removed from the roots. The roots were gently washed under tap water, to prevent damage and loss of nodules, at the root washing facility at the Field Research Centre at Lincoln University. All plants from each pot were scored and an average nodule score calculated (25th November-11th December). Nodule colour, number, position on the roots and size were evaluated, according to criteria adapted from Rice *et al.* (1977); (Table 5.7). Pigmented nodules were assumed to be active and the green or white inactive. However, this was not tested.

Table 5.7 Nodule characteristics and criteria (Rice *et al.*, 1977) for lucerne and Caucasian clover nodulation assessment from the glasshouse experiment at Lincoln University, 2015.

Nodule characteristics	Criteria	Score
Colour	90-100% pink	6
	70- 89%	5
	50-69%	4
	30-49%	3
	0-29%	2
	No nodules-no colour	1
Number	>20/plant	4
	5-20/plant	3
	1-5/plant	2
	None	1
Position*	60-100% crown	4
	20-59% crown	3
	0-19% crown	2
	No nodules-no position	1
Size	>10 mm diameter	4
	3-10 mm diameter	3
	<3 mm diameter	2
	No nodules-no size	1

*plants with nodules on the first 50 mm of the taproot or lateral roots within 10 mm of this taproot were considered to be crown nodulated.

5.2.6 Final soil sampling and testing

5.2.6.1 Soil pH, Aluminium and Olsen P

Soils were removed from the pots, dried and 2 mm sieved (24th-27th November). Final chemical analyses were conducted on all pots, including pH_{H2O} (standard test in New Zealand), extractable Al_{CaCl2} and Olsen P, according to the methods described in Section 5.2.2. However, soil samples that were analysed for Olsen P were bulked randomly within replicates, therefore there were two samples (replicates) tested for Olsen P per treatment.

5.2.7 Statistical analysis

Statistical analyses were performed using 'Genstat 16.0' (VSN, International). Data were analysed using a three-way ANOVA in randomised blocks (replicate), to determine the effects of lime or P additions, soil type, and plant species on the main measurement in this experiment, the legume shoot yield. The effects of factors were also determined on other plant measurements (root yield, root: shoot ratio and nodule score), soil chemistry (soil pH_{H2O}, extractable Al_{CaCl2} and Olsen P) and herbage nutrient concentrations (macro and micronutrients) for the lime dataset. Plant shoot nutrient concentration and uptake data were also analysed and are presented in Appendix Tables 3.22- 3.25 and Tables 3.26-3.31. For the P dataset, the effects of factors on legume shoot yield and soil chemistry (soil pH_{H2O}, extractable Al_{CaCl2} and Olsen P) were determined.

Soil extractable Al_{CaCl2} concentration, soil Olsen P and shoot Al, S, Mn and Zn concentration data were log transformed for analysis, to satisfy the assumptions of the ANOVA, which were checked using residual plots and data distribution prior to analysis. In several parts of the chapter, these data means were back-transformed for figures and tables (Appendix Tables 3.5, 3.28, 3.29, 3.31, 3.34, 3.35 and Figure 5.3). For the soil extractable Al against soil pH_{H2O} (Figure 5.3) the error bars for the back-transformed means are the standard error of the mean (SEM), upper and lower, which were calculated using the general mean and standard error from the ANOVA outputs on the log transformed data. The SEM were also back transformed which is why the error bars are asymmetric (Nicholls, 2014).

To focus on the main component of the treatment sum of squares for each ANOVA, three-way interactions were ignored if their F value was an order of magnitude lower than the two-way interactions and main effects. Two-way interactions were ignored if their F value was an order of magnitude lower than the main effects. If the F value fell below the order of magnitude rule, but was close and the sum of squares (SS) was higher than one of the main effects (first or second most important-highest F values), then it was examined. The order of magnitude difference was always related to the most important main effect F value (highest F value) for each ANOVA output. These rules identified which were the most important effects and interactions in the model output and therefore which were presented graphically in the chapter. To further investigate key drivers, there were several exceptions to the rule including; the soil x lime interaction for soil Al_{CaCl2} concentration and the soil x lime x species for shoot yield for the lime dataset. The mean values produced by the ANOVA outputs, which were significant, were compared using Fisher's protected LSD (5%). Comparisons of means are presented in Appendix Tables 3.4- 3.5 and Tables 3.32-3.35 and numbers with letter subscripts in common are not different (α=0.05) based on Fisher's protected LSD. Key interactions identified

through the above criteria were plotted using 'Sigmaplot 13.0' (Systat Software Inc. GmbH.2014) to interpret results. Lines were fitted to the data and equations presented in Appendix Tables 3.6-3.12 and Tables 3.36-3.37. Figures for interactions that followed the criteria of the F value rule but, either did not show large differences, or were not considered to be relevant to the hypotheses, were excluded.

An ANCOVA (analysis of variance with covariates) was used to test for the effect of soil Al_{CaCl2} concentration on Caucasian clover and lucerne yields, to test the main hypothesis. A MANCOVA (multiple covariates) was used to determine the effects of soil chemical properties (soil pH_{H2O}, log Al and log Olsen P) and shoot nutrient concentrations (log Al, P%, N%, log S, Mo, B, log Mn, log Zn) on lucerne shoot yield, root yield, root: shoot ratio and nodule score for the low lime treatments and the effect on lucerne shoot yield for the P dataset. This analysis also determines if a relationship exists between the covariates and the dependent variable (Sokal & Rohlf, 2012a). The order of variates was determined by those that were most likely to affect the plant measurements. For the analysis on the lime dataset, soil pH_{H2O} was not included as a covariate and for the P dataset, Olsen P was not included as a covariate. This was because there is a strong correlation between these variates and the treatment factors lime and P. In addition, many other variables included as covariates were strongly correlated with soil pH_{H2O} (collinearity) (Sedcole, 2018; Statistics Solutions, 2018).

For the covariate analysis the lucerne growth measurements were focused on due to the significant effect (P<0.05) of soil Al_{CaCl2} concentration on the lucerne shoot yield. The covariate analysis also focused on the low lime dataset, as across the 0, 2 and 4 t lime ha^{-1} treatments were the greatest changes to soil Al_{CaCl2} concentration and shoot yield response to the lime applied. Moreover, we know from the literature and this study that at higher lime rates the availability of other nutrients such as P, B, Mo and Zn were affected. Relationships between the covariates identified as significant and the plant measurements were graphed and linear regression lines fitted (Sokal & Rohlf, 2012b). Linear regression equations are presented in the appendix Tables 3.15-3.21 and Tables 3.38-3.41.

5.3 Results

5.3.1 Lime treated soils

5.3.1.1 Soil Analyses

As expected, soil analyses completed at the end of the experiment indicated that soil pH_{H2O} increased with increasing lime rate. A soil type by lime rate interaction (P<0.001) was observed (Table 5.8). The soil pH_{H2O} ranged from 5.0 in the L0P0 treatment on the GM, GF and MO soils, to pH_{H2O} 7.5 at 12 t lime ha^{-1} on the AR soil (Appendix Table 3.4). The magnitude of the pH_{H2O} change from lime differed among the 10 soils and ranged from 0.13 to 0.18 pH units per t ha^{-1} equivalent of lime applied.

There was an overall decline in soil extractable Al_{CaCl2} across the range of lime rates applied, with the most noticeable differences between the L0P0 and 2 t lime ha^{-1} treatments on each soil. Extractable Al_{CaCl2} concentration was affected (P<0.001) by the interaction of soil type and lime rate (Table 5.8). Extractable Al_{CaCl2} ranged from 8.4 mg kg^{-1} on the MO L0P0 soil to 0.1 mg kg^{-1} in the 8 t lime ha^{-1} on the PK soil (Figure 5.3).

Soil Olsen P was affected (P<0.001) by the interaction of soil type and lime rate applied (Table 5.8). There was an overall decline in soil Olsen P with increasing lime between the L0P0 soil and the 2 and 4 t lime ha^{-1} treatments. The Olsen P ranged from 31 μg/mL in the L0P0 treatment on the AR soil (pH_{H2O} 5.4), to 5 μg/mL in the 4 t lime ha^{-1} (pH_{H2O} 6.5) on the OM soil (Appendix Table 3.5).

Table 5.8 Mean soil pH_{H2O}, extractable Al_{CaCl2} concentration (mg kg^{-1}) and Olsen P (μg mL^{-1}) measured at the completion of the experiment for legumes grown on 10 New Zealand high and hill country soils supplied with 0 (L0P0), 2 (L1P0), 4 (L2P0), 8 (L3P0) and 12 t lime ha^{-1} (L4P0).

		Soil pH_{H2O}	Log soil Al (0.02 M $CaCl_2$)	Log Olsen P
Lime (t lime ha^{-1})	*P* value	***$_i$	***$_i$	***
Soil type	*P* value	***	***	***$_i$
Species	*P* value	ns	ns	***
Interactions	Soil x Species	**	***	***
	Soil x Lime	***$_e$	***$_e$	***$_e$
	Species x Lime	***	*	***
	Soil x Lime x Species	***$_d$	***$_d$	***$_d$

Note: *** Significant at P<0.001 level, ** significant at P<0.01 level, * significant at P<0.05 level, ns- no significant difference. The i symbol refers to important effects (high F value), d refers to discounted effects and e refers to exceptions to the F value rule.

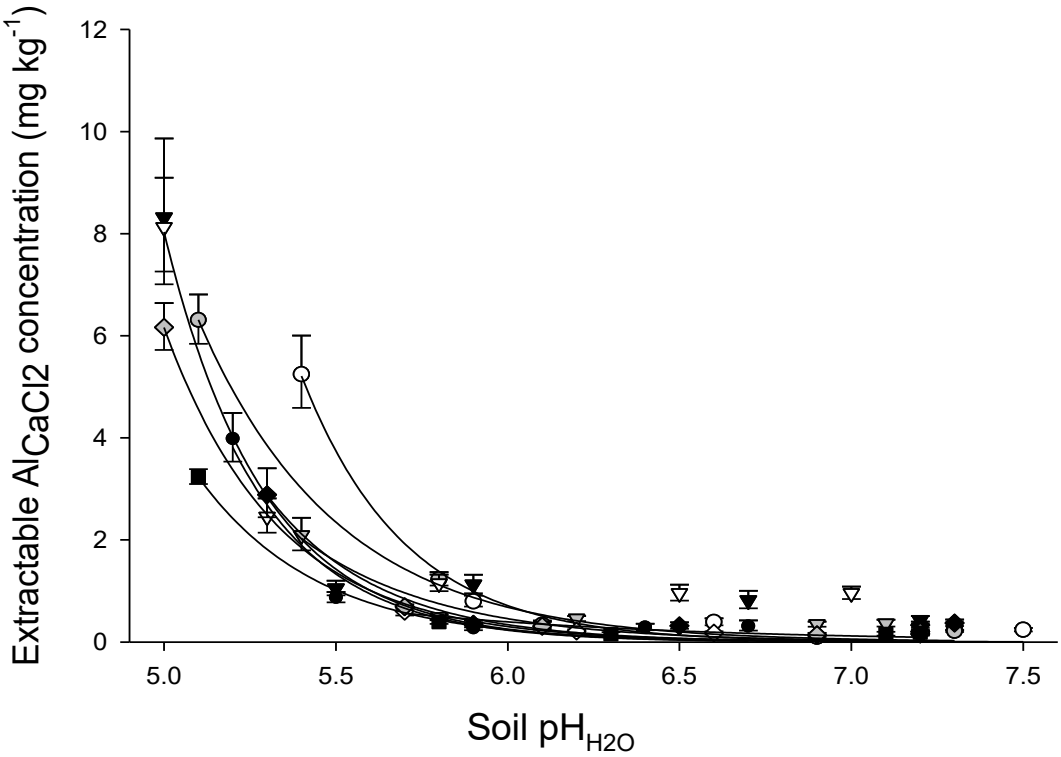

Figure 5.3 Back transformed mean soil extractable Al_{CaCl2} concentration (mg kg^{-1}) and soil pH_{H2O} at 0 (L0P0), 2 (L1P0), 4 (L2P0), 8 (L3P0) and 12 t lime ha^{-1} (L4P0) for the WT (●), AR (○), PK (●), MO(▼), GM (▽), BD (▼), OM (◆), LP (◇), GF (◆) and MG (■) soils. Soil acronyms are described Table 5.1. The standard error bars indicate upper and lower ± one SEM (n=8) for all means that have been back-transformed. The equations for fitted lines, R^2 and P value are presented in Appendix Table 3.6.

5.3.1.2 Shoot yield

Total shoot dry matter (DM) yield of sown legume species was affected (*P*<0.001) by the interaction of soil type, lime rate applied and plant species (Table 5.9). Caucasian clover yields ranged from 2.0 g pot^{-1} in the 12 t lime ha^{-1} treatment on the PK soil, to 16.8 g pot^{-1} at 12 t lime ha^{-1} on the BD soil. Caucasian clover yield responses to lime occurred on the MO, BD and GF soils, with an increase in yield with lime applied (Figure 5.4a and c). In the other soils, Caucasian clover yields appear to be unaffected by lime application (WT, AR, PK, GM, OM, LP and MG soils). Caucasian clover yields declined at the higher lime rates on the AR, PK, MO, LP, GF, and MG soils.

Lucerne yields were lowest on the WT L0P0 soil at 1.1 g pot^{-1} and highest at 23.7 mg kg^{-1} on the LP soil with 12 t lime ha^{-1} applied. Yields on the LP soil were higher than all other soil and lime combinations. Lucerne yield showed a strong response to lime rate applied on the WT, AR, MO, GM, BD, OM, LP and GF soils (Figure 5.4b,d and f). The yields generally peaked at the 2 t lime ha^{-1} or 4 t lime ha^{-1} rates, however, on the WT soil, lucerne yields peaked at 12 t lime ha^{-1} and for the BD and OM soils at 8 t lime ha^{-1} (Figure 5.4b, d and f). The lucerne yields on the MG soil were similar between the L0P0 and lower lime treatments, before declining above 4 t lime ha^{-1} (Figure 5.4d).

Table 5.9 Mean total accumulated (six harvests) shoot yield (g pot^{-1}), root yield (g pot^{-1}) and root: shoot ratio for legumes grown on 10 New Zealand high and hill country soils supplied with 0 (L0P0), 2 (L1P0), 4 (L2P0), 8 (L3P0) and 12 t lime ha^{-1} (L4P0).

		Shoot yield (g pot^{-1})	Root yield (g pot^{-1})	Root: shoot ratio
Lime (t lime ha^{-1})	*P* value	***	***	**
Soil type	*P* value	***	***	***
species	*P* value	***	***	**
Interactions	Soil x species	***$_i$	***	***
	Soil x lime	***	***	*
	Plant x lime	***$_i$	***	ns
	Soil x lime x species	***$_e$	**$_i$	**$_i$

Note: *** Significant at *P*<0.001 level, ** significant at *P*<0.01 level, * significant at *P*<0.05 level, ns- no significant difference. The i symbol refers to important effects (high F value), d refers to discounted effects and e refers to exceptions to the F value rule. This analysis was also carried out on nodule score data and is presented in Table 3.14 in the appendix.

5.3.1.3 Root yield

Legume root yield generally increased with an increase in lime applied among the 10 soils and a soil type by lime rate by plant species interaction was observed (*P*<0.001) (Table 5.9). Caucasian clover root yields were similar across the lower lime rates and declined with lime applied when above 2 t lime ha^{-1} or 4 t lime ha^{-1}, on the PK, MO, GM, LP and GF soils (Figure 5.5 a, c and e). The root yields on the WT and BD soil peaked at the higher lime rates of 8 t lime ha^{-1} and 12 t lime ha^{-1}, respectively (Figure 5.5a and e). Caucasian clover root yields decreased with an increase in the lime rate applied on the MG soil (Figure 5.5c). Lucerne root yields increased with applied lime and peaked at 2 t lime ha^{-1}, 4 t lime ha^{-1} or 8 t lime ha^{-1}, and declined with further lime application on the AR, PK, MO, GM, BD and GF soils (Figure 5.5b, d and f). Lucerne root yield increased with an increase in lime rate applied on the WT and OM soils (Figure 5.5d and f). In contrast, on the MG soil, the root yields decreased with lime applied up to 8 t lime ha^{-1}.

5.3.1.4 Root: shoot ratio

The root to shoot ratio was variable for Caucasian clover and lucerne plants with applied lime and a soil type by lime rate by plant species interaction was observed (*P*<0.01; Table 5.9). The Caucasian clover root: shoot ratio ranged from 0.40 on the OM soil, with 4 t lime ha^{-1}applied, to 0.84 on the AR soil with 12 t lime ha^{-1} applied (Appendix Figure 3.2c and e). Root: shoot ratios were generally within a similar range of 0.40-0.80 except for the AR soil at 12 t lime ha^{-1} applied where the root: shoot ratio was higher. The root: shoot ratio of lucerne plants ranged from 0.24 on the AR soil, with 12 t lime ha^{-1} applied, to 0.97 on the WT L0P0 soil (Appendix Figure 3.2f). The root: shoot ratio was higher in the L0P0 and 2 t lime ha^{-1} treatment for the WT soil and the MG L0P0 soil, while the AR soil root: shoot ratios of lucerne were lower than on all other soils with 2 t lime ha^{-1} applied and greater.

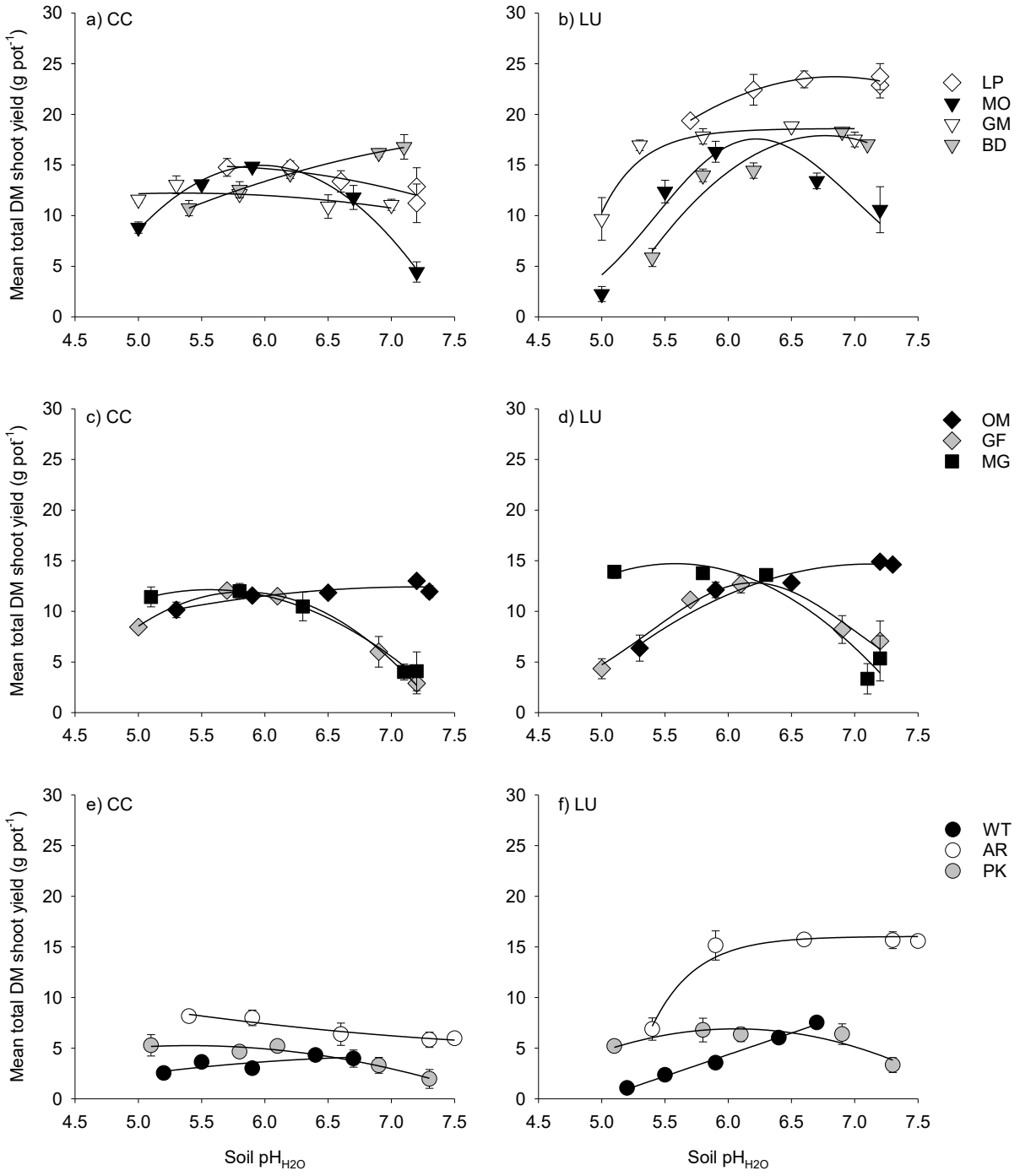

Figure 5.4 Mean total accumulated (six harvests) shoot dry matter yield (g pot^{-1}) for Caucasian clover (a, c, e) and lucerne (b, d, f) and soil pH_{H2O} obtained with the application of 0 (L0P0), 2 (L1P0), 4 (L2P0), 8 (L3P0) and 12 t lime ha^{-1} (L4P0) for the WT (●), AR (○), PK (●), MO(▼), GM (▽), BD (▼), OM (◆), LP (◇), GF (◆) and MG (■) soils. Soil acronyms are described in Table 5.1. The standard error bars indicate the standard error of the mean ± one SEM (n=4). The equations for fitted lines, R^2 and P value are presented in Appendix Tables 3.7 and 3.8.

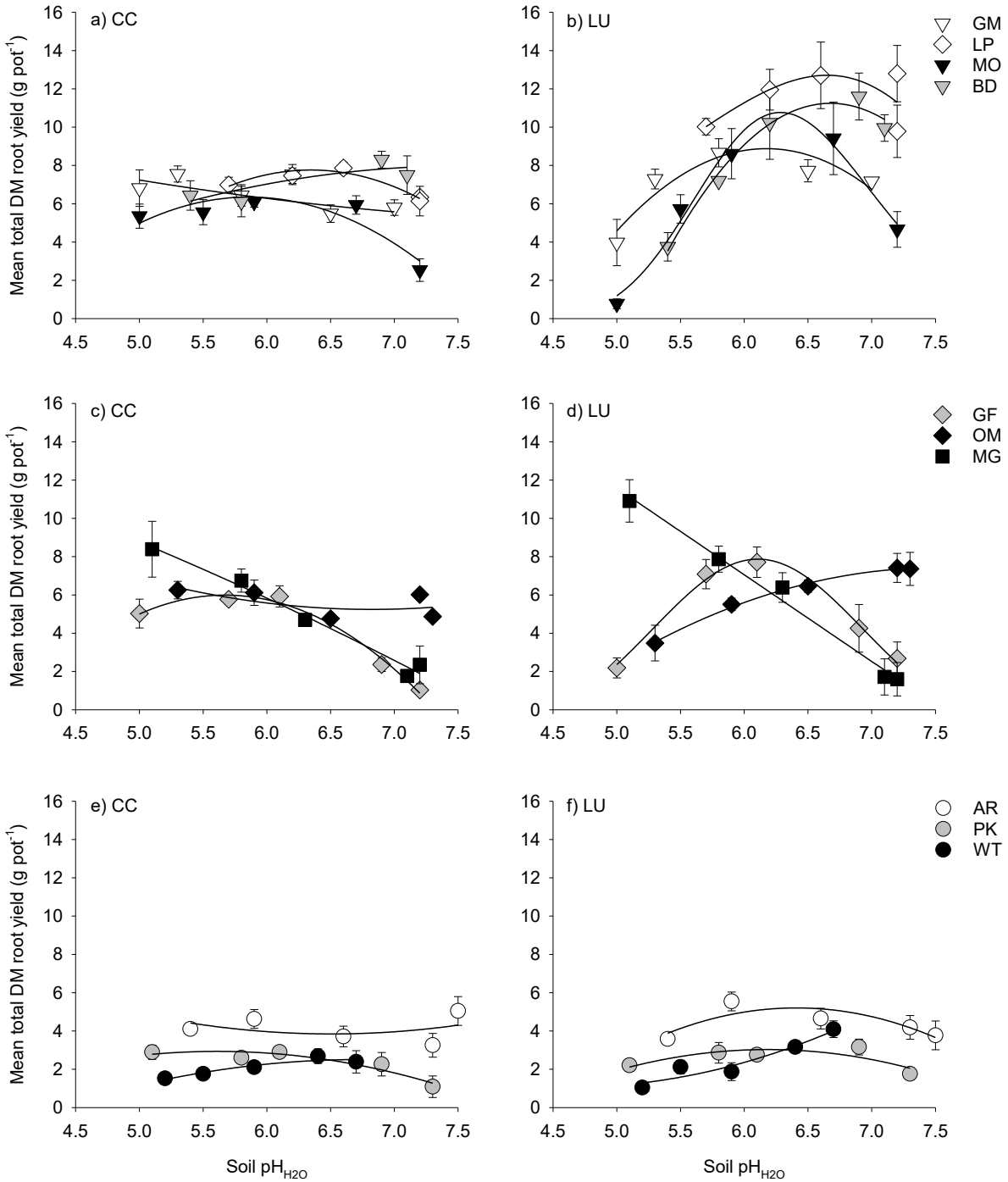

Figure 5.5 Mean total root dry matter yield (g pot^{-1}) for Caucasian clover (a, c, e) and lucerne (b, d, f) and soil pH_{H2O} obtained with the application of 0 (L0P0), 2 (L1P0), 4 (L2P0), 8 (L3P0) and 12 t lime ha^{-1} (L4P0) for the WT (●), AR (○), PK (●), MO(▼), GM (▽), BD (▼), OM (◆), LP (◇), GF (◆) and MG (■) soils. Soil acronyms are described in Table 5.1. The standard error bars indicate the standard error of the mean ± one SEM (n=4). The equations for fitted lines, R^2 and P value are presented in Appendix Tables 3.9 and 3.10.

5.3.1.5 Root nodules

The nodule score was affected (*P*<0.01) by the interaction of soil type by plant species (Appendix Table 3.13). Overall, although there were significant differences in nodule score, differences were small (Appendix Figure 3.3). The lowest nodule score was for lucerne on the PK soil at 8.9 and all other plant and soil combinations had nodule scores of ≥10. There was no effect (*P*>0.05) of lime on nodule score in this experiment.

5.3.1.6 Shoot yield and soil extractable Al_{CaCl2} concentration

The soil extractable Al_{CaCl2} concentration did not influence (*P*>0.05) the shoot yield of Caucasian clover (Table 5.10). In contrast, the soil extractable Al_{CaCl2} concentration influenced (*P*<0.05) the shoot yield of lucerne growing in the low lime treatments (0, 2 and 4 t lime ha^{-1}). The relationship between log soil Al_{CaCl2} and lucerne shoot yield is presented in Figure 5.6a.

Table 5.10 The effect of soil type and lime applied at 0 (L0P0), 2 (L1P0) and 4 t lime ha^{-1} (L2P0) on the Caucasian clover (CC) and lucerne (LU) total mean accumulated (six harvests) shoot yield and the influence of log soil Al_{CaCl2} concentration as a covariate.

		Total shoot yield (g pot^{-1}) CC	Total shoot yield (g pot^{-1}) LU
Main treatment effects			
Lime rate (t lime ha^{-1})	*P* value	*	***
Soil type	*P* value	***	***
Interaction	Soil x lime	***	***
covariates	Log soil Al_{CaCl2}	ns	*

Note: *** Significant at *P*<0.001 level, ** significant at *P*<0.01 level, * significant at *P*<0.05 level, ns- no significant difference.

5.3.1.7 The influence of other variables on plant measurements at the low lime treatments (L0P0, L1P0 and L2P0)

5.3.1.7.1 Shoot yield

In addition to log soil Al_{CaCl2} (*P*<0.01), shoot K% (*P*<0.001) and shoot B (mg kg^{-1}) were found to be associated with (*P*<0.05) the lucerne shoot yield in the low lime treatments (Table 5.11). On most soils, the higher lucerne shoot yields were associated with lower log soil Al_{CaCl2} concentrations. However, on the MG and PK soil there appeared to be no relationship between soil Al_{CaCl2} and lucerne yields (Figure 5.6a). A decrease in lucerne shoot yields was associated with an increase in shoot K% on most soils in the low lime treatments (Figure 5.6b). There was a decline in lucerne shoot yields which was associated with an increase in shoot B concentration on most soils (Figure 5.6c).

Table 5.11 The effect of soil type and lime applied at 0 (L0P0), 2 (L1P0) or 4 t lime ha^{-1} (L2P0) on the total mean accumulated (six harvests) lucerne shoot yield, root yield and root: shoot ratio and the influence of covariates including soil measurements (log soil Al_{CaCl2} and log soil Olsen P) and shoot nutrient concentrations (K, log S, B, log Mn, log Zn, P, Mo, N and log Al).

		Total shoot yield (g pot^{-1})	Total root yield (g pot^{-1})	Root: shoot ratio
Main treatment effects				
Lime rate (t lime ha^{-1})	*P* value	***	**	ns
Soil type	*P* value	***	***	ns
Interaction	Soil x lime	***	***	*
covariates	Log soil Al_{CaCl2}	**	*	ns
	Shoot K%	***	ns	ns
	Log shoot S (mg kg^{-1})	ns	ns	ns
	Shoot B (mg kg^{-1})	*	ns	*
	Log shoot Mn (mg kg^{-1})	ns	ns	ns
	Log shoot Zn (mg kg^{-1})	ns	ns	ns
	Shoot P%	ns	*	ns
	Shoot Mo (mg kg^{-1})	ns	ns	ns
	Log soil Olsen P	ns	ns	ns
	Shoot N%	ns	*	ns
	Log shoot Al (mg kg^{-1})	ns	ns	ns

Note: *** Significant at *P*<0.001 level, ** significant at *P*<0.01 level, * significant at *P*<0.05 level, ns- no significant difference.

5.3.1.7.2 Root yield

Log soil Al_{CaCl2}, shoot P% and shoot N% was associated with (*P*<0.05) the lucerne root yield in the low lime treatments (Table 5.11). Lower root biomasses were associated with higher log soil Al_{CaCl2} concentrations on most soils (Figure 5.7a). However, on the MG soil the opposite effect was found. On most soils, higher root yields were associated with a higher P% in the shoots (Figure 5.7c). A similar trend was shown for N% in the shoots, however, on the MG soil a lower root yield was associated with an an increase in shoot N% (Figure 5.7b).

5.3.1.7.3 Root: shoot ratio

Shoot Boron concentration was associated with (*P*<0.05) the root: shoot ratio of lucerne in the low lime treatments (Table 5.11). Data are shown in Appendix Figure 3.5 and fitted linear equations in Appendix Table 3.21.

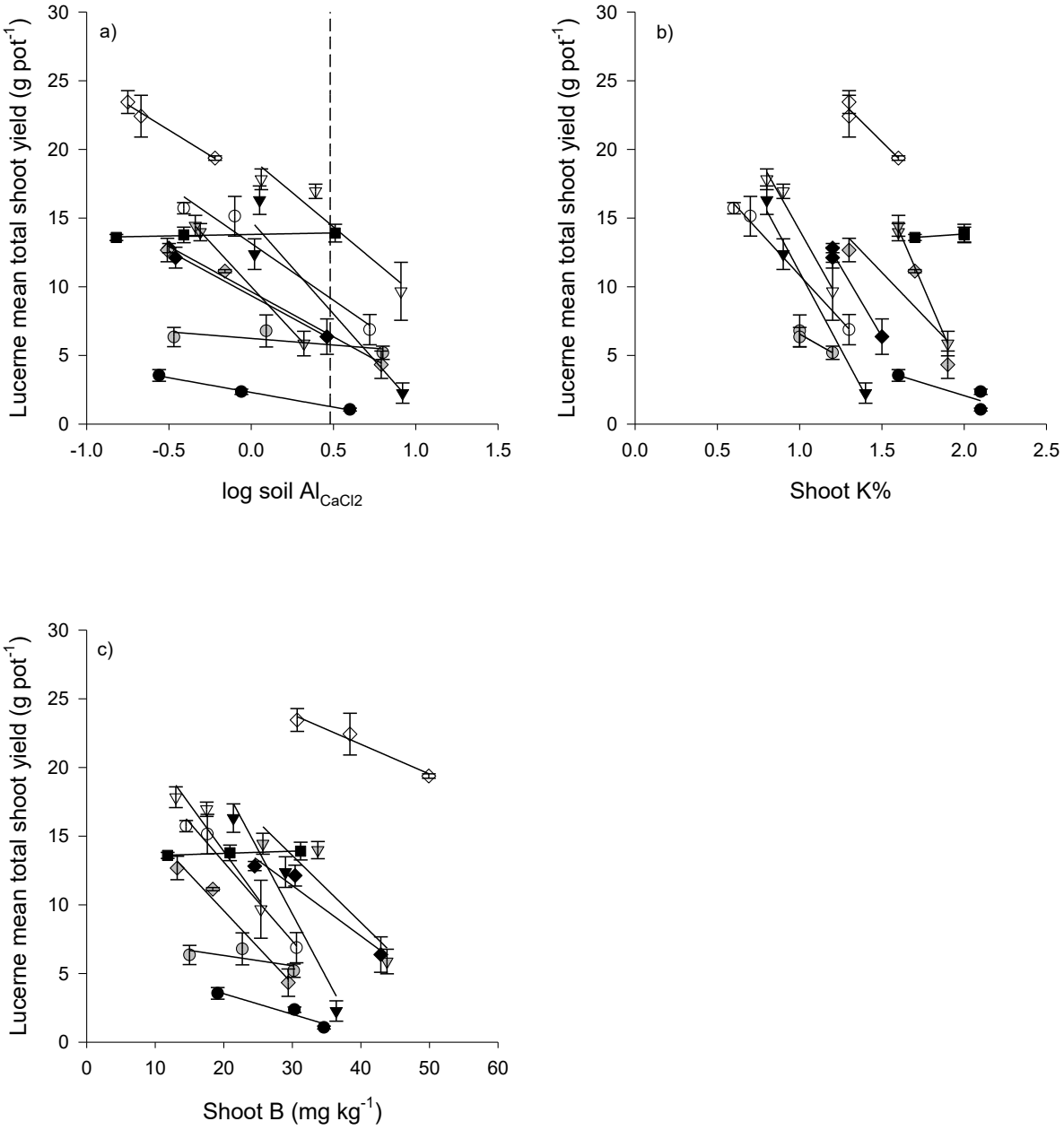

Figure 5.6 Mean total accumulated (six harvests) lucerne shoot yield and a) log soil Al_{CaCl2}, b) shoot K% and c) shoot B concentration (mg kg^{-1}) with 0 (L0P0), 2 (L1P0) and 4 t lime ha^{-1} (L2P0) applied for WT (●), AR (○), PK (●), MO(▼), GM (▽), BD (▼), OM (◆), LP (◇), GF (◆) and MG (■) soils. Soil acronyms are described in Table 5.1. Linear trendlines have been fitted and equations are presented in Tables 3.15-3.17 the Appendix. The fragmented vertical line (a) indicates the log soil Al that is equivalent to the 3 mg kg^{-1} threshold.

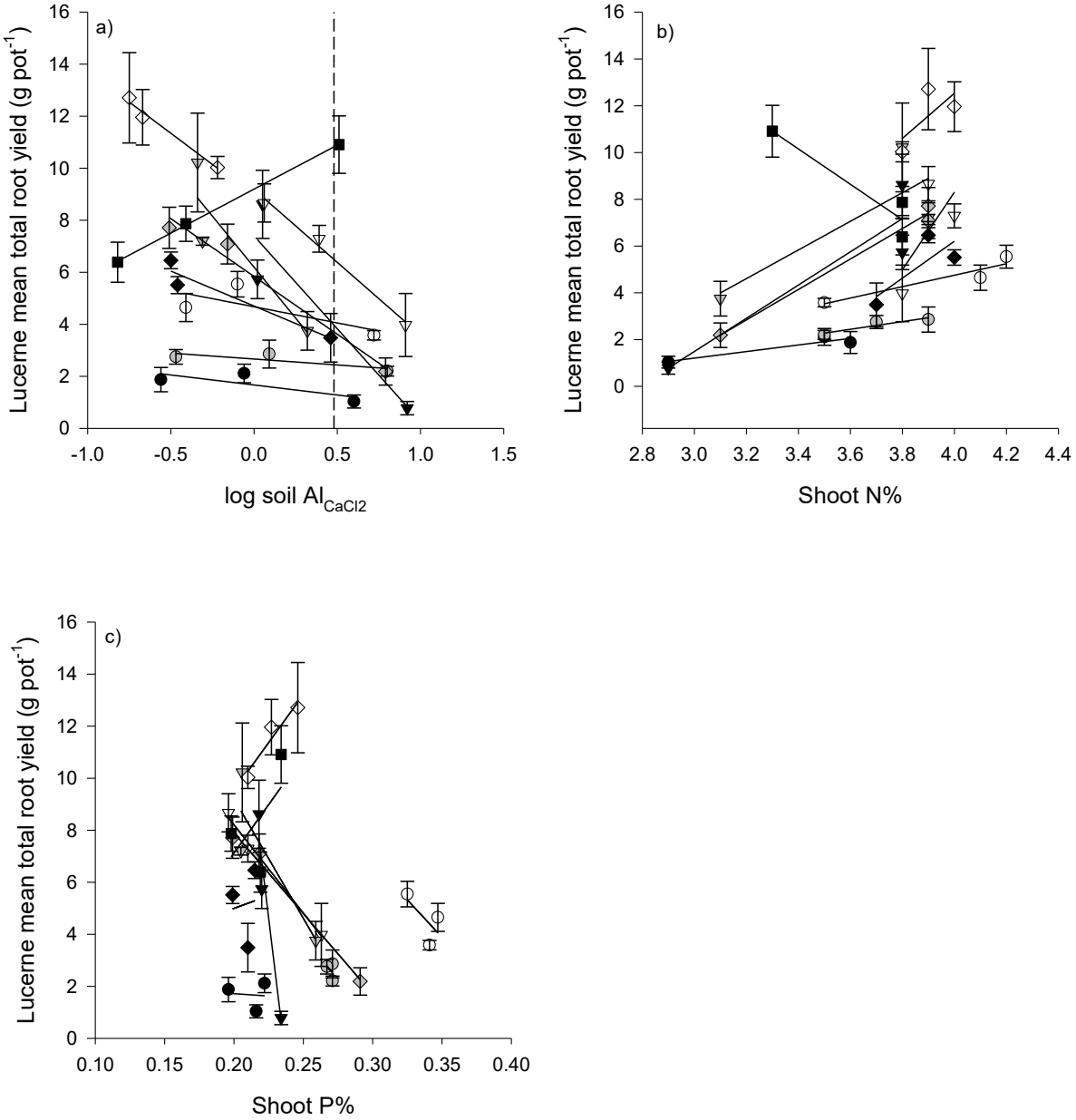

Figure 5.7 Mean total lucerne root yield and a) log soil Al_{CaCl2}, b) shoot N% and c) shoot P% with 0 (L0P0), 2 (L1P0) and 4 t lime ha^{-1} (L2P0) applied for the WT (●), AR (○), PK (●), MO(▼), GM (▽), BD (▼), OM (◆), LP (◇), GF (◆) and MG (■) soils. Soil acronyms are described in Table 5.1. Linear trendlines have been fitted and equations are presented in Table 3.18-3.20 in the Appendix. The fragmented vertical line (a) indicates the log soil Al_{CaCl2} that is equivalent to the 3 mg kg^{-1} threshold.

5.3.2 P treated soils

This set of data looked at different rates of P applied to the soils and the effects on shoot yield, soil pH_{H2O}, extractable Al_{CaCl2} concentration and Olsen P at zero lime application. This dataset is used to support the main lime dataset from this glasshouse experiment.

5.3.2.1 Soil analyses

Soil pH_{H2O} was affected (P<0.001) by the interaction of soil type and plant species (Appendix Table 3.32) and soil type and P application (Appendix Table 3.33; Table 5.12). The soil by plant species interaction means ranged from a pH_{H2O} of 4.8 in the GM soil growing Caucasian clover to pH_{H2O} 5.6 for the LP soil growing both species. The mean pH_{H2O} of the soil by lime rate interaction ranged from 4.8 for the GM soil (30 mg P L^{-1} soil and 150 mg P L^{-1} soil) to 5.7 for the LP soil (L0P0). However, all soils remained within 0.1-0.2 pH units of the L0P0 soil with P applied.

Soil Al_{CaCl2} concentration was affected (P<0.001) by the interaction of soil type and P rate by species applied (Table 5.12). Soil Al_{CaCl2} concentrations ranged from 0.5 mg kg^{-1} in the LP L0P0 and 30 mg P L^{-1} soil growing Caucasian clover plants to 13.8 mg kg^{-1} on the MO soil, with 30 mg P L^{-1} soil applied also growing Caucasian clover (Appendix Table 3.34).

There was an overall increase in soil Olsen P (P<0.001) with P applied (Table 5.12 and Appendix Table 3.35). As expected, the magnitude of the change in Olsen P differed among the 10 soils and ranged from 0.08 to 0.26 Olsen P units per mg P applied.

Table 5.12 Mean soil pH_{H2O}, extractable Al_{CaCl2} concentration (mg kg^{-1}), Olsen P (µg mL^{-1}) and shoot yield (g pot^{-1}) for legumes grown on 10 New Zealand high and hill country soils supplied with 0 (L0P0), 30 (L0P1) or 150 mg P kg^{-1} (L0P2).

		Soil pH_{H2O}	Log soil Al (0.02 M $CaCl_2$)	Log Olsen P	Total shoot yield (g pot^{-1})
P (mg P L^{-1} soil)	*P* value	*	***	$***_i$	***
Soil type	*P* value	***	***	***	***
Plant species	*P* value	ns	ns	***	ns
Interactions	Soil x Species	$***_i$	***	***	***
	Soil x P	$*_i$	***	***	***
	Species x P	ns	**	***	***
	Soil x P x Species	ns	$**_i$	$***_d$	$***_i$

Note: *** Significant at P<0.001 level, ** significant at P<0.01 level, * significant at P<0.05 level, ns- no significant difference. The i symbol refers to important effects (high F value) and the d refers to discounted effects. The Olsen P is back-transformed for presentation in Figure 1.11 and tables in the appendix, as is the soil extractable Al.

5.3.2.2 Shoot yield

Shoot dry matter (DM) yield was affected (P<0.001) by the interaction of soil type, P rate applied and plant species (Table 5.12). Caucasian clover yields appeared to be unresponsive to P additions on most soils. However, on the WT, MO, BD, OM and GF soils there was a yield response to P (Figure 5.8a, c, e). Lucerne yield response to P differed among the 10 soils. There was an increase in lucerne yield with P

applied on several soils (WT, AR, GM, BD, OM, LP, GF and MG), but P application on other soils (PK and MO) appeared to have little effect on yield (Figure 5.8b, d, f).

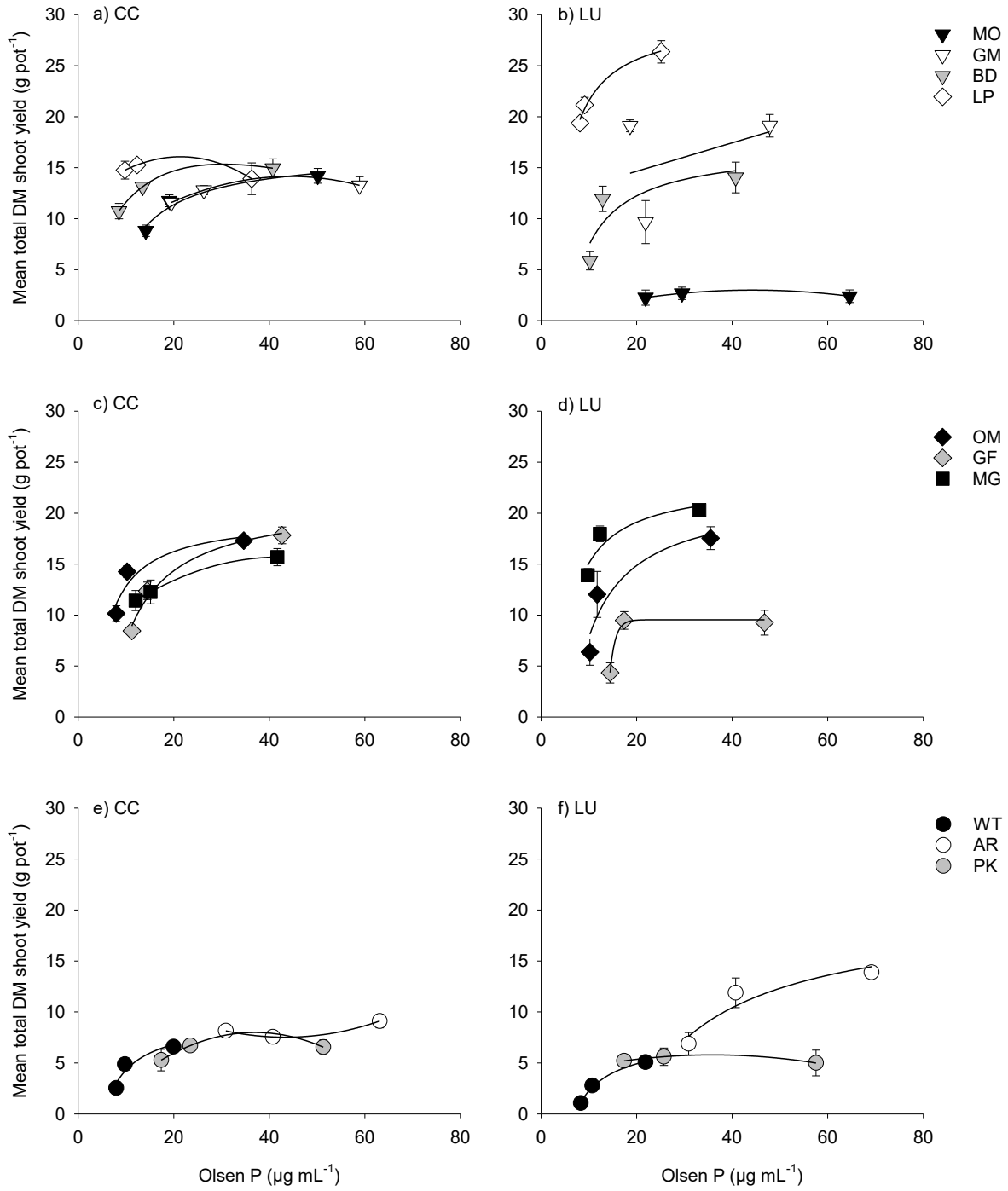

Figure 5.8 Mean total accumulated (six harvests) shoot dry matter (DM) yield (g pot^{-1}) for Caucasian clover (a, c, e) and lucerne (b, d, f) across six harvests at the back-transformed mean Olsen P (µg mL^{-1}) achieved by the application of 0 (L0P0), 30 (L0P1) and 150 mg P L^{-1} soil (L0P2) soils for the WT (●), AR (○), PK (●), MO(▼), GM (▽), BD (▼), OM (◆), LP (◇), GF (◆) and MG (■) soils and with no lime. Soil acronyms are described in Table 5.1.The standard error bars indicate ± one SEM (n=4). The equations for fitted lines, R^2 and P value are presented in Appendix Tables 3.36 and 3.37.

5.3.2.3 The influence of other variables on lucerne shoot yield in the P treatments

5.3.2.3.1 Shoot yield

Log soil Al_{CaCl2} and shoot K% had a strong influence (*P*<0.001) on lucerne shoot yield in the P treatments (Table 5.13). Also B mg kg^{-1} (*P*<0.01) and pH_{H2O} (*P*<0.05) were associated with lucerne shoot yield for the P dataset. In contrast to the lime dataset, an increase in lucerne shoot yield was associated with higher log soil Al_{CaCl2} concentrations on most soils when P was applied to the soil and without lime (Figure 5.9a). A decline in lucerne shoot yield was associated with an increase in shoot K% (Figure 5.9b) and an increase in shoot B concentration on most soils (Figure 5.9c). There appeared to be a general decrease in shoot yield associated with an increase in soil pH_{H2O} on most soils (Figure 5.9d).

Table 5.13 The effect of soil type and P applied at 0 (L0P0), 30 (L0P1) or 150 mg P L^{-1} soil (L0P2) on the mean total accumulated (six harvests) lucerne shoot yield and the influence of covariates including soil measurements (log Al and pH_{H2O}) and shoot nutrient concentrations (K, log S, B, Mn, Zn, P, Mo, N and log Al).

		Total shoot yield (g pot^{-1})
Main treatment effects		
P rate (mg P L^{-1} soil)	*P* value	**
Soil type	*P* value	***
Interaction	Soil x P	***
covariates	Log Soil Al_{CaCl2}	***
	Shoot K%	***
	Log shoot S (mg kg^{-1})	ns
	Shoot B (mg kg^{-1})	**
	Log shoot Mn (mg kg^{-1})	ns
	Log shoot Zn (mg kg^{-1})	ns
	Shoot P%	ns
	Shoot Mo (mg kg^{-1})	ns
	Soil pH_{H2O}	*
	Shoot N%	ns
	Log shoot Al (mg kg^{-1})	ns

Note: *** Significant at *P*<0.001 level, ** significant at *P*<0.01 level, * significant at *P*<0.05 level, ns- no significant difference.

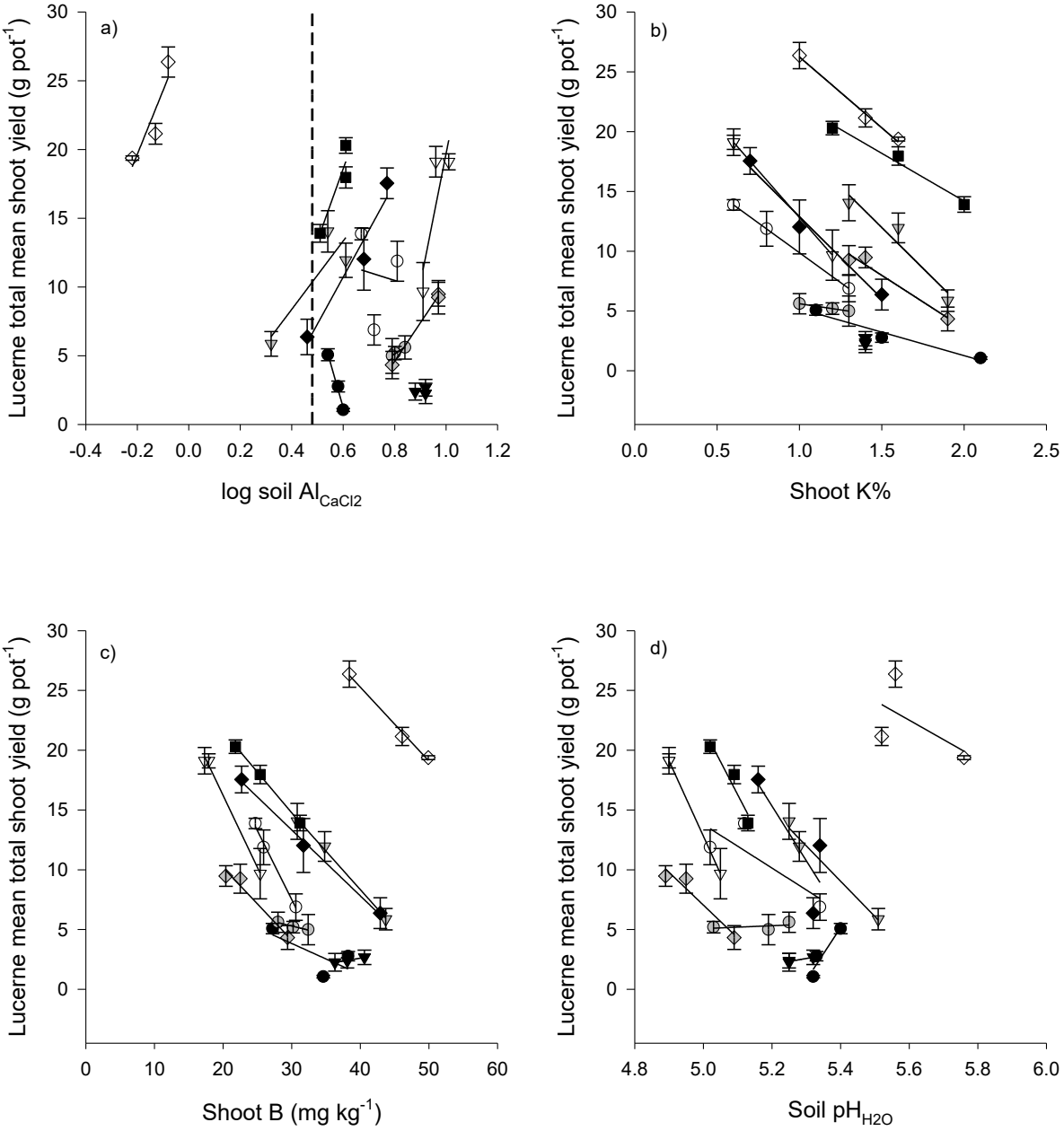

Figure 5.9 Mean total accumulated (six harvests) lucerne shoot yield and a) log soil Al_{CaCl2}, b) shoot K%, c) shoot B concentration (mg kg^{-1}) and soil pH_{H2O} with 0 (L0P0), 30 (L0P1) and 150 mg P L^{-1} soil (L0P2) applied for the WT (●), AR (○), PK (●), MO(▼), GM (▽), BD (▼), OM (◆), LP (◇), GF (◆) and MG (■) soils. Soil acronyms are described in Table 5.1. Linear trendlines have been fitted and equations are presented in Tables 3.38-3.41 in the Appendix. The fragmented vertical line (a) indicates the log soil Al_{CaCl2} that is equivalent to the 3 mg kg^{-1} threshold.

5.3.2.4 Lucerne yield response to lime compared with P additions

Lucerne shoot yield responded more to lime in terms of growth on the AR, PK, WT, MO, BD,GF and MG soils (Figure 5.10a) ,while on the OM and LP soils, the yield response was greater to P. The lucerne yields on the GM soil were similar for both.

The highest maximum lucerne yield was observed in the lime addition series for the WT, AR, GF, MO and BD soils compared to the P (Figure 5.10b). However, it is important to clarify that the difference on the MG soil was due to a decline in lucerne yield with lime applied (Figure 5.4b). On the OM, LP and MG soils the greatest yield difference was achieved with P applied. On the PK and GM soils the highest maximum lucerne yields were similar with lime and P applied.

The maximum shoot yield of lucerne plants for the lime dataset occurred at a log soil Al_{CaCl2} concentration below the equivalent of 3 mg kg^{-1} (0.48), except for lucerne growing in the MG soil (Figure 5.11). In contrast, for the P dataset, the maximum lucerne shoot yields occurred on most soils with a soil Al_{CaCl2} concentration above this threshold.

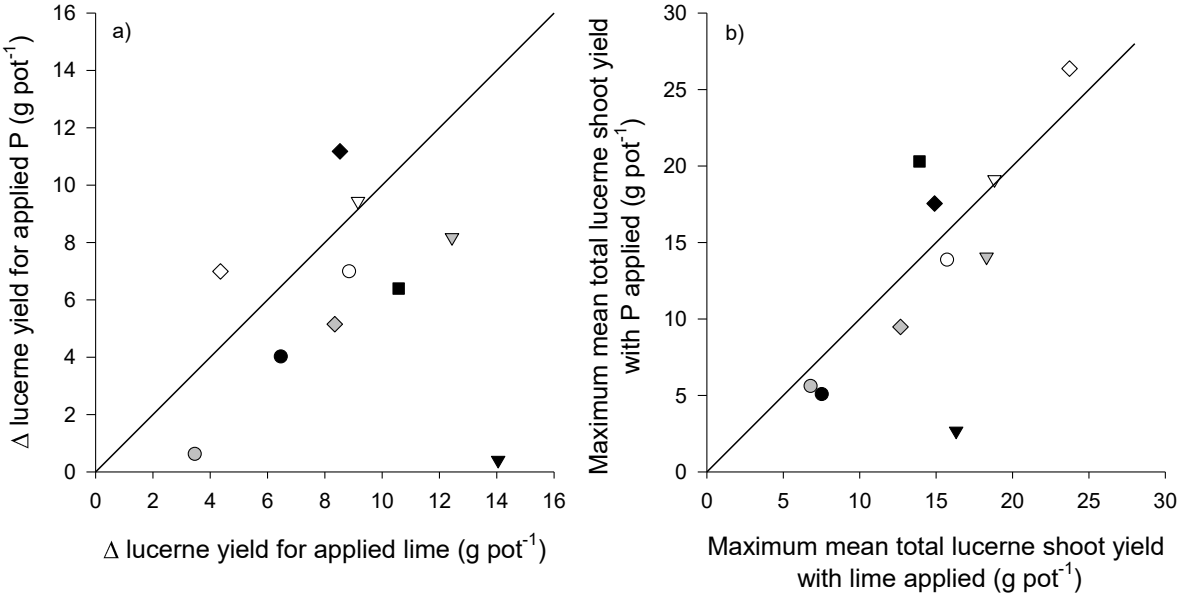

Figure 5.10 a) The difference (Δ) between the highest and lowest mean total accumulated (six harvests) lucerne shoot yield for the lime dataset with 0 (L0P0), 2 (L1P0), 4 (L2P0), 8 (L3P0) and 12 t lime ha^{-1} (L4P0) applied and the difference (Δ) between the highest and lowest mean total lucerne shoot yield for the P dataset with 0 (L0P0), 30 (L0P1) and 150 mg P L^{-1} soil (L0P2) applied and b) The maximum mean total lucerne shoot yield for the lime dataset with 0 (L0P0), 2 (L1P0), 4 (L2P0), 8 (L3P0) and 12 t lime ha^{-1} (L4P0) applied and the P dataset with 0 (L0P0), 30 (L0P1) and 150 mg P L^{-1} soil (L0P2) for the WT (●), AR (○), PK (●), MO(▼), GM (▽), BD (▼), OM (◆), LP (◇), GF (◆) and MG (■) soils. Soil acronyms are described in Table 5.1. A linear 1:1 line has been drawn on a and b.

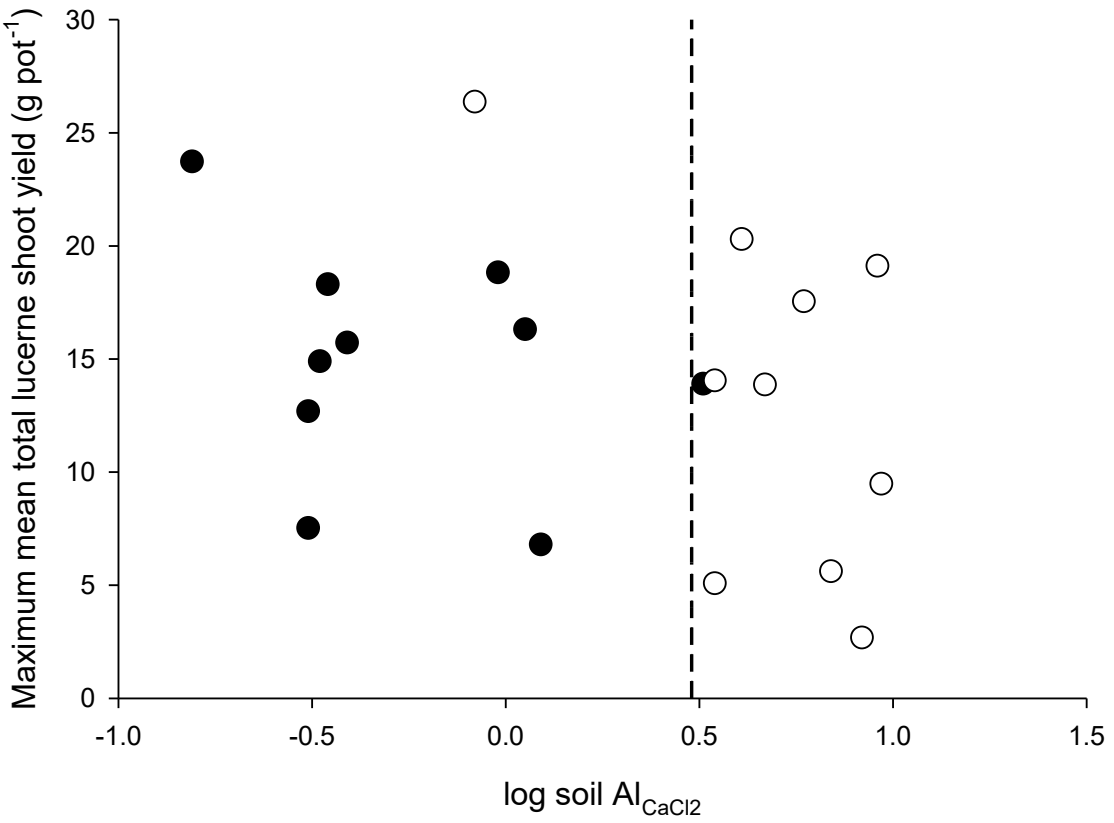

Figure 5.11 Relation between total maximum lucerne yield and log soil Al_{CaCl2} concentration for the lime dataset (●)at 0 (L0P0), 2 (L1P0), 4 (L2P0), 8 (L3P0) and 12 t lime ha^{-1} (L4P0) and the P dataset (○)at 0 (L0P0), 30 (L0P1) and 150 mg P L^{-1} soil (L0P2) on all soils. The fragmented vertical line indicates the log soil Al concentration that is equivalent to 3 mg kg^{-1}.

5.4 Discussion

5.4.1 Legumes as bioindicators of soil extractable aluminium

5.4.1.1 Lime response of lucerne shoot yield

Soil extractable Al_{CaCl2} concentration was strongly associated with (P<0.01) lucerne shoot yield in this experiment. The investigation of the relationship between soil *extractable* Al_{CaCl2} concentration and lucerne yield was restricted to the low lime treatments (0, 2 and 4 t lime ha^{-1}; Figure 5.6a). This was because many studies have shown that an increase in the lime rate applied affects the plant availability of nutrients such as Zn and B (Keenan, 2014; Moir *et al.*, 2016; Schwass, 2013; Whitley, 2013) and this is a likely explanation for the decline in yield in some soils at higher lime rates. The low lime treatments also had the largest shift in soil Al_{CaCl2} concentration and lucerne shoot yield (Figure 5.3 and Figure 5.4b, d and f). The full range of lime application rates were used to determine at which soil pH_{H2O} to split the data and focus on a pH_{H2O} zone where there was a large change in soil Al concentration. Lucerne shoot

yields followed the expected trend of an increase in shoot yield with an increase in soil pH_{H2O} and an associated decrease in soil extractable Al_{CaCl2} concentration on most soils (Figure 5.6a), as reported in other studies on acidic and high Al high country soils (Berenji, 2015; Moir *et al.*, 2016; Schwass, 2013; Whitley, 2013). Lime is the most effective means of controlling Al toxicity on acid soils (Haynes, 1982). Lucerne yields ranged from 840-17939 kg DM ha^{-1} equivalent in 10 months in the low lime treatments. The largest increase in lucerne shoot yield occurred between the L0P0 and 2 t lime ha^{-1} application on most soils. The associated increase in soil pH_{H2O} reduced the extractable Al_{CaCl2} concentrations to below the suggested toxicity thresholds for lucerne at ≤2.5 mg kg^{-1} on all soils (Moir *et al.*, 2016).

On some soils, particularly on the GM soil, lucerne had a reasonable yield in the L0P0 soil (Figure 5.6a and Figure 5.4b). Effects of toxic soil Al_{CaCl2} concentrations on legumes have been discussed in Section 2.7.1. Lucerne plants survived in the L0P0 treatment of the WT, AR, PK, MO, GM, GF and MG soils, even when Al concentrations were at levels considered toxic to legumes (4.0 mg kg^{-1} to 8.3 mg kg^{-1}) (Moir *et al.*, 2016). However, in the field it is likely that negative effects such as horizontal root growth would be observed, that were otherwise restricted by the pot. Berenji (2015) found horizontal root growth and abnormal branching of lucerne growing in response to high soil Al concentrations (>8.9 mg kg^{-1}) in field trials at two high country sites on Acidic Orthic Brown Soils. These field trials, other glasshouse studies and this experiment show that, while lucerne can grow at high Al concentrations (above the toxicity thresholds reported in the literature), the growth is restricted which is evidenced by the increase in shoot yield on most soils with lime applied (Figure 5.4b, d and f). The BD, LP and MG soils had extractable Al concentrations <3 mg kg^{-1} and, therefore, the application of lime was not required to alleviate Al toxicity for legumes. However, a lucerne yield response to lime on these soils indicated an increase in nutrient availability to the plant, possibly P and Mo (Figure 5.4b,d and f).

5.4.1.2 Lime response of Caucasian clover shoot yield

In contrast, soil extractable Al_{CaCl2} did not have a major influence on Caucasian clover shoot yield in this experiment. Caucasian clover shoot growth was more consistent with lime rates applied (across an extensive pH_{H2O} range of 5.9-7.5) compared with lucerne (Figure 5.4a, c and e). Caucasian clover yields responded to lime application on several soils, including the BD, MO and GF soils. The Caucasian clover yields on the BD soil increased with lime applied and peaked at the higher lime rates of 8 t lime ha^{-1} and 12 t lime ha^{-1} (pH_{H2O} 6.8 and 7.1). In contrast, the Caucasian clover shoot yields on the MO and GF soils peaked at 4 t lime ha^{-1} (a shift from pH_{H2O} 5.0- 5.9 and 5.0-6.1 respectively) and declined with further lime applied (Figure 5.4a and c). These results on the MO and GF soils are similar to findings by Moir *et al.* (2016), who observed an increase in Caucasian clover yield with lime applied to a peak at 2

and 5 t lime ha^{-1} before declining (pH_{H2O} 5.3 and 6.0). Whitley (2013) measured an increase in Caucasian clover yield with a shift in pH_{H2O} from 4.8-6.1 with 4 t lime ha^{-1} applied to an acidic and high Al Immature Pallic Soil. However, on many soils the yields were unresponsive to lime additions (WT, AR, PK, GM, OM and LP). Caucasian clover shoot yields ranged from 2.0 g pot^{-1} (PK with 12 t lime ha^{-1}) to 16.8 g pot^{-1} (BD with 12 t lime ha^{-1}), which is equivalent to 1527-12824 kg DM ha^{-1}, with a mean yield of 7099 kg DM ha^{-1} (9.3 g pot^{-1}).

Caucasian clover generally produced higher yields in the L0P0 soil compared with lucerne. Shoot yields were not reduced, even when the extractable Al concentration was >5 mg kg^{-1} (AR, MO, GM and GF), above the threshold for toxicity, on particular soils (Moir *et al.*, 2016). Caucasian clover has shown promise as a productive species that is tolerant of acidic, high Al and low fertility conditions (Berenji, 2015; Caradus *et al.*, 2001). This is likely to be partly a result of the Caucasian clover and rhizobia being better adapted to low pH, high Al and low P environments (control soil: pH_{H2O} 5.2, Al 3.8 mg kg^{-1}). Moreover, Caucasian clover roots grew normally and no adverse effects were observed in soils that caused lucerne roots to grow horizontally as a response to high extractable Al concentrations (Berenji, 2015). These results show Caucasian clover can grow and persist in acidic and low fertility soils, but it does respond to lime additions on some soils, and poor performance has been reported in acidic soils related to deficiencies in Mo and Ca uptake by plants (Barnard & Folscher, 1988).

5.4.1.2.1 Lucerne shoot Potassium

Shoot K% was identified as a covariate (P<0.001) associated with lucerne shoot yield in the low lime treatments. There was a decline in lucerne shoot yield which was associated with an increase in shoot K%, particularly above 1.3-1.5 K% (Figure 5.6b). Lucerne plants with shoot K% of <1.3% are considered to be deficient in K (McLaren and Cameron, 1996). However, on the AR, GM, MO and OM soils with various lime rates applied, the highest yielding plants had shoot K% below this threshold. Lucerne yields ranged from 9.7 g pot^{-1} to 17.8 g pot^{-1}, which were equivalent to 7405 and 13588 kg DM ha^{-1} in 10 months. Therefore, shoot K concentration does not appear to be limiting lucerne yields in this experiment. The reason for the low K% in high yielding plants could be a result of harvesting technique, as both the leaflets and stems were harvested together. This could be diluting the K% in the shoots, due to more stem being present in the higher yielding plants, whereas the small plants have more leaves and less stem. This suggests that other factors such as soil Al_{CaCl2} are more important in driving lucerne shoot yields.

5.4.1.2.2 Lucerne shoot Boron

Shoot Boron was identified as a covariate (P<0.05) associated with lucerne shoot yield in the low lime treatments. There was a decrease in the shoot yield which was associated with an increase in the B concentration on most soils, except for the MG soil (Figure 5.6c). Shoot B concentrations of legumes declined with lime applied, as has been reported in other studies on acidic soils (Hayes *et al.*, 2008; Keenan, 2014; Maxwell *et al.*, 2012; Moir *et al.*, 2016; Schwass, 2013; Whitley, 2013). Boron is an essential plant nutrient. It is involved in cell division and the development of growing regions and maintains the cell wall and membrane. It also supports metabolic reactions (Bolaños *et al.*, 2004). At 4 t lime ha^{-1} and above, the shoot B concentration dropped below the critical level for B deficiency, <13-18 mg kg^{-1} for lucerne (Dear & Weir, 2004; Morton *et al.*, 1999). However, the plants with potentially deficient B concentrations in the shoots were on the AR, GF, GM, MG and PK soils with 4 t lime ha^{-1} applied (Figure 5.6c) and generally yielded the highest, 4840 and 13611 kg DM ha^{-1} in 10 months (with lime applied). The lower shoot yields at higher shoot B concentration are in the L0P0 treatment and plant growth was likely limited by other factors. This finding indicates that in this experiment, B was not limiting the lucerne shoot yields at this lime treatment (4 t lime ha^{-1}). However, B deficiency due to reduced bioavailability could account for the observed decline in lucerne yields on many soils with further lime applied (Figure 5.4b, d and f).

5.4.1.2.3 Lucerne shoot macro and micronutrients

Shoot S, P, N, Zn, Mn and Mo concentrations were not identified (P>0.05) as covariates for lucerne shoot yield in the low lime treatments. Lucerne plants had adequate shoot S (0.18-0.22%), P (≥0.2%), N (>2.8%), Zn (>15 mg kg^{-1}) and Mn (>24 mg kg^{-1}) concentrations across all soils and lime rates and were therefore, non-limiting in the experiment (Appendix Tables 3.26-3.31)(Craighead & Metherell, 2006; McLaren & Cameron, 1996b; Mengel & Kirkby, 2001; Morton *et al.*, 1999).

The shoot N%, although not below the deficiency threshold mentioned in the previous paragraph, was lower in the L0P0 treatment of all soils and increased with lime applied, particularly between the L0P0 and 2 t lime ha^{-1} treatment (Appendix Tables 3.22 and 3.26). This was likely a result of an increase in pH_{H2O}, a decline in soil extractable Al_{CaCl2} and the conditions becoming more favourable for N fixation for both the lucerne plant and the sensitive rhizobia specific to lucerne (*S. meliloti*) (Berenji *et al.*, 2017; Graham & Vance, 2000; Merbach *et al.*, 1990). However, the N% in the shoots that was attributed specifically to N fixation and the N from soil N uptake were not able to be differentiated in this

experiment. Moreover, the rhizobia numbers were not measured and therefore this effect was unable to be confirmed.

Manganese concentrations in the herbage of lucerne growing in the PK L0P0 soil, indicated potential toxicity, with values > 340 mg kg^{-1} (Smith *et al.*, 1983). However, shoot Mn concentrations near toxic levels were not observed in any other soil and lime treatment combinations growing lucerne.

As expected, Mo concentrations in the herbage increased with lime additions, as reported in many other studies (Keenan, 2014; Maxwell *et al.*, 2012; Moir *et al.*, 2016; Rayner, 2015; Schwass, 2013; Wheeler & O'Connor, 1998; Whitley, 2013). Plants growing in many of the soils had shoot Mo concentrations <0.1 mg kg^{-1} (Morton *et al.*, 1999), particularly in all L0P0 soils and the lower lime rates of all soils (pH_{H2O} ranging from 5.0-6.3), indicating deficiency (Appendix Table 3.30). Legumes rely on the Mo as an essential part of the plant enzymes involved in biological N fixation (Davies, 1956; Kaiser *et al.*, 2005; Neyra, 1993). However, in the 2 t lime ha^{-1} and 4 t lime ha^{-1} treatments, many plants had concentrations <0.1 mg kg^{-1}, but shoot yields were high. This indicates that Mo may not be limiting and reasons for the low concentrations may be either, the Mo is diluted due to harvest technique (as previously mentioned) or is being utilised in the roots for N fixation.

5.4.1.2.4 Lucerne root yield

Lucerne root yields generally responded to lime additions and showed similar trends to the shoot yields (Figure 5.5d). The soil extractable Al concentration, the shoot P% and N% were identified as covariates (P<0.05) for lucerne shoot yield in the low lime treatments (Table 5.11). As expected, an increase in the lucerne root yield was associated with a decrease in soil Al_{CaCl2} (with lime additions), as was reported for the shoot yields (Figure 5.7a). This was similar to findings by Berenji (2015), who found that lucerne root yields were higher with 2 t lime ha^{-1} applied (pH_{H2O} 4.9-5.4) compared to the L0P0, however, yields then plateaued with further lime applied. In contrast to our findings, lucerne did not survive in their control treatment (pH_{H2O} 5.0 and extractable Al_{CaCl2} 13.9 mg kg^{-1}). On all soils in this study the lucerne survived and generally increased in root growth with lime applied (to a critical point) except for the MG soil, which declined with lime additions. This trend for lucerne growth (shoot and root yields) indicates that something else was limiting the growth of lucerne in the MG soil. An increase in root yield was associated with an increase in the shoot P% and N% on most soils (Figure 5.7 b and c), indicating that lucerne roots grew more with higher nutrient availability. While there appeared to be a trend of a decrease in lucerne shoot P% associated with an increase in soil Al_{CaCl2} (Appendix Figure 3.4a), there was no clear trend of N% and soil Al_{CaCl2} concentration (Appendix Figure 3.4b).

The root yield in this experiment was probably affected and restricted by the size of the plant pot. At the completion of the experiment (10 months of growth), many of the pots were root bound, had exhausted the soil of resources and could not grow any further. However, there were some treatments which yielded less root mass and consequently there was space for the roots to explore the pot. This is a limitation of a glasshouse experiment. The plant roots are the primary site of Al toxicity, however, it was difficult to attribute low root yields to extractable Al_{CaCl2} (although it was a covariate), or assess the specific effects of Al on the roots, based on restricted space of root growth as a confounding factor. This assessment would need to be conducted for plants grown under field conditions.

5.4.1.3 P response of lucerne shoot yield

Soil extractable Al_{CaCl2} concentration was strongly associated with (P<0.001) lucerne shoot yield on soils with P applied and no lime inputs. In contrast to the result on the limed soils, there was an increase in lucerne yield associated with an increase in soil extractable Al_{CaCl2} concentration on many soils (Figure 5.9a). On eight of ten of the soils the soil extractable Al concentration was highest in the 30 mg P L^{-1} soil or 150 mg P L^{-1} soil treatments, which corresponded to the highest shoot yields (Figure 5.9a and Figure 5.8b, d and f). Moreover, nearly all the soil and P treatment combinations were above the recommended threshold for Al toxicity, ranging from 2.1 to 10.2 mg kg^{-1}, except for the LP soil. The P applied did not appear to be binding the Al, as the soil extractable Al_{CaCl2} concentrations were still high. This was in contrast to several other studies that have reported in addition to improving soil fertility, the application of P decreased the soil solution Al, due to the formation of insoluble $Al(OH)_2H_2PO_4$ and reduce phytotoxicity (Adams, 1981; Manoharan, 1997; Zheng *et al.*, 2005). Coleman *et al.* (1960) found that for 60 acidic subsoils, the amount of P converted to $Al(OH)_2H_2PO_4$ was highly correlated to the exchangeable Al concentration. Sloan *et al.* (1995) reported a decrease in both the soil solution Al^{3+}, and at higher P rates, the exchangeable Al with the addition of P ($NaH_2PO_4.2H_2O$) due to Al-orthophosphate on an acid soil. Iqbal (2014) found that after 13 days in an acidic soil (pH_{CaCl2} 4.5), the soil extractable Al concentration declined with P applied, particularly in the highest Al treatment (150 mg $AlCl_3$ kg^{-1}). A P rate of 80 mg P kg^{-1} (highest rate applied) was most effective at reducing soil extractable Al concentrations, suggesting a detoxification effect through the formation of Al-P. If P application was locking up Al, then the soil extractable Al concentration would be expected to decline with an increase in P applied, as was reported in the previous studies, however, this has not occurred in this experiment (Figure 5.9a). Coupled with the high lucerne shoot yield, this suggests that the soil extractable Al concentration measured by the standard Al test may be a result of the test measuring the Al-phosphates. Many Al-phosphates are insoluble Ortho and Meta Al-phosphates and therefore not extractable by the $CaCl_2$ test. However, if the precipitate is at molecular size, it may pass through

the filter paper and be detected by the ICP instrument. However, it might not have a detrimental effect on plant growth. This is a highly speculative theory and requires further investigation to elucidate this unexpected trend and the mechanism behind it.

The response of lucerne shoot yield to P applied differed (P<0.001) among the soils (Figure 5.8b, d and f, Table 5.12). In particular, there was a sharp increase in the lucerne shoot yield between the L0P0 and 30 mg P L^{-1} soil application rate on several soils. There was a large range in yields and on several soils the lucerne yields plateaued at the higher rates of P, exhibiting a 'rise to maximum' curve at an Olsen P of 20 µg mL^{-1} (GM, BD and GF). This indicates that these soils responded to P when it was limited, however, with further P applied, the plants had either reached their biological optimum or something else was limiting growth. The lucerne yields on other soils (OM, LP and MG) increased at the higher rate of 150 mg P L^{-1} soil, showing a slightly higher second threshold. This lucerne growth response to higher P applied may be partly attributed to the lower P retention (P sorption capacity) of these (OM, LP, MG) soils (Table 5.2). Soils with a lower P retention (high P use efficiency) are likely to have more P become plant available than on high P sorption (low P use efficiency) soils due to sorption processes of the fertiliser P applied (McLaren *et al.*, 2016). Many studies have reported an increase in legume shoot yields and P uptake with P application, which becomes a non-limiting factor (Berenji, 2015; Jordan, 2011; Maxwell *et al.*, 2013). Schwass (2013) and Whitley (2013) found that lucerne yields increased by 1.5 times with 500 mg P kg^{-1} applied compared with the control soil, on an acidic (pH_{H2O} 4.8) Immature Pallic Soil with a starting Olsen P of 24 µg mL^{-1}.

In addition to soil extractable Al concentration and pH_{H2O}, shoot K% and Boron concentration were identified as covariates (P<0.05) for lucerne shoot yield for the P applied treatments (Table 5.13). The relationships were consistent with those outlined in the lime experiment (Figure 5.6b and c), the shoot K% was <1.3% on most soils, however, shoot yields were highest (Figure 5.9b). Boron concentrations were all above deficient due to the low soil pH_{H2O} (no lime applied; Figure 5.9c). Soil pH_{H2O} trends differed among P treatments (Figure 5.9d, Appendix Table 3.33).

5.4.1.4 P response of Caucasian clover shoot yield

Caucasian clover shoot yield showed little response above an Olsen P of 20 µg mL^{-1} with P applied on most soils (Figure 5.8a, e and c). Caucasian clover was able to maintain reasonable yields at low P levels relative to their potential yield at unlimited P availability on most soils (Figure 5.4a,c and e and Figure 5.8a, c and e). This result indicates that this species is well adapted to acidic and low fertility (P) environments, which is why it has been recommended as a potential species for the high and hill

country of New Zealand (Bryant, 1974; White, 1995; Wurst, 2004). This supports findings by Whitley (2013), who found that in an acidic Immature Pallic Soil in the glasshouse, that there was no difference in the total shoot yield of Caucasian clover with P additions of 0, 50, 150 and 500 mg P kg^{-1} soil applied.

5.4.2 Comparison of lucerne yield response to lime or P

There were substantial shoot yield increases with applied lime on many soils between the L0P0 and 2 t lime ha^{-1} treatment, particularly for lucerne. Lime increases soil pH, reduces Al toxicity and increases P availability, enhancing plant growth (Edmeades *et al.*, 1983; Haynes, 1982). However, it remains uncertain as to whether this increase in yield is a result of the reduction of soil Al concentrations to below toxic levels, or an increase in the P availability to the plant. On the high Al soils, the yield increase with 2 t lime ha^{-1} ranged from 0.99 – 5.4 times higher for lucerne than the L0P0 soil. With 2 t lime ha^{-1} applied, the P% in the herbage generally declined or was similar to the L0P0 (Appendix Table 3.27). The only plants to increase in P% were those in the LP soil, however, extractable Al concentration was not an issue on this soil. Phosphorus uptake between the L0P0 and 2 t lime ha^{-1} treatment generally increased, but the magnitude of the increase differed among soils. Plant P uptake is partly driven by increased yields, where soil labile P is sufficient. However, the small changes in P% between these treatments implies that there was not a large increase in P release from the Al-P and Fe-P complexes at this rate of lime applied. Moreover, P in the soil is most bioavailable within the pH range of 6.0 to 7.0, and is most available at pH 6.5 (McLaren & Cameron, 1996c). Most soils were not within this soil pH zone with 2 t lime ha^{-1} applied, except the LP soils, which did not have an Al issue in the L0P0 soil.

For the soil with the highest Olsen P (AR soil), the addition of 2 t lime ha^{-1} resulted in a higher increase in lucerne shoot yield compared with the addition of 30 mg P L^{-1} soil (2.2 times vs. 1.7 times). The greater response to lime indicates that the yield response was related to the decrease in soil extractable Al_{CaCl2} concentration, as P was not a limiting factor on this soil Figure 5.4f, Figure 5.8f and Figure 5.10 a and b). However, plants growing in the MO soil, and all other L0P0 soils were Mo deficient, and the response to lime could have been related to Mo availability, which became non-limiting at 2 t lime ha^{-1} applied. However, the application of 2 t lime ha^{-1} did not increase the Mo in the shoots to above deficient on all soils. This was seen with the WT, BD, MO LP, GF, MG soils and yet many of these soils demonstrated a large increase in yield, indicating that it was not a yield response to Mo availability. Manganese was not limiting or toxic in those soils, therefore the increase in yield is not in response to Mn availability. These findings suggest that the increase in yield was most likely related to the decline in soil extractable Al concentrations, which supports the hypothesis of this experiment.

In contrast, the yield response to P applied on those same high Al soils was 1.0- 2.5 times. The increase in yield above the L0P0 was higher for the soils with 2 t lime ha^{-1} applied compared to the 30 mg P L^{-1} soil treatment (maintenance P equivalent). The 2 t lime ha^{-1} application reduced the soil extractable Al concentrations to ≤2.5 mg kg^{-1} on all soils, whilst the soil Al concentrations differed (P<0.001) across P rates (Table 5.12). Phosphorus additions did not appear to reduce soil extractable Al concentrations without the presence of lime, as has been reported in the literature (Iqbal, 2014). The large differences in yields among soils are likely due to something other than P, which could be Al. Phosphorus additions improved plant P nutrition and yields on some soils, however, it appears that the lime additions and the reduction in extractable Al concentrations led to greater yield responses on these high Al soils. Jordan (2011) showed slightly higher increases in lucerne yields with 2 t lime ha^{-1} applied compared to the 30 mg P kg^{-1} rate with basal lime (5 t lime ha^{-1}), particularly for the lucerne in an acidic high country soil. This suggests that the response to lime without P was attributed to the increase in pH_{H2O} and reduction in Al_{CaCl2} concentrations, which were high in the control soil. This was a similar finding to our study. Lucerne yields on most soils responded more to lime applied compared to P, in terms of maximum yields (across all P and lime rates) and yield differences between the highest and lowest yields for each dataset. Those soils in which lucerne responded more to P applied, had the lower soil Al_{CaCl2} concentrations or did not respond to lime additions, due to other factors limiting growth (MG soil) (Figure 5.10 a and b).

Caucasian clover and lucerne shoot yields were low across all lime treatments for the PK and WT soil (Figure 5.4a-f). The PK soil is a Pumice Soil and had a lower Olsen P than the other Pumice Soil (AR), however, it did not respond to P applied. Therefore, something else must be limiting shoot growth on this soil, as yields did not respond to lime additions. The WT soil was the only soil derived from Allophanic ash and properties that distinguish it from the other soils include a high P retention, CEC, total carbon and organic matter content (Table 5.2). Yields on this soil did respond to lime and P but overall yields were much lower than on other soils. The reason for this was unable to be determined in this experiment. Further studies on Allophanic Soils are required to determine the relationship between soil Al and legume growth.

If the same result was found under field conditions (natural) with reasonable P and S, it would support the idea that it is actually soil pH_{H2O} and soil extractable Al driving the yields of a sensitive species, such as lucerne and not the P or S applied.

5.5 Conclusions

- In this experiment soil extractable Al_{CaCl2} concentration was a covariate of lucerne shoot yield. Shoot yield increased with lime applied, particularly between the L0P0 and 2 t lime ha^{-1} treatment, which was associated with a decline in the extractable Al_{CaCl2} concentration.
- Caucasian clover shoot yields were generally not affected by soil Al concentration and yields were more consistent across the range of pH_{H2O} achieved by liming. Compared to lucerne, Caucasian clover showed a minimal response to P applied. This supports other studies and highlights the potential of this species in the high and hill country of New Zealand.
- Both lucerne and Caucasian clover plants yielded consistently low on the Allophanic Soil (WT) and the lower Olsen P Pumice Soil (PK) across all lime and P treatments. The reason for this was unclear and further studies need to be conducted on these North Island volcanic soils to determine why legumes yielded much lower on these soils.
- The relationship between soil extractable Al_{CaCl2} and shoot yield differed on the low lime soils compared to the soils with P applied. This may be related to the soil Al test measuring a form of Al-P that was not detrimental to the plant, which could explain the reasonably high yields on soils with potentially toxic soil Al_{CaCl2} concentrations. There was no apparent reduction in soil extractable Al with applied P, which contrasts with other studies in the literature.

6 A rhizobox experiment investigating the effects of legumes and pH on Al at the root-soil interface

6.1 Introduction

In acidic soils, the Al^{3+} in solution increases and above a threshold concentration, the ions become toxic to plants (Kinraide, 1991). The bioavailability of Al is affected by soil pH and, therefore, factors such as liming, which alter the soil pH, affect the concentrations of extractable Al in the soil and relative toxicity to plants. Liming is a common method for improving soil pH and nutrient availability and is recommended for New Zealand grassland soils with a pH <5.8 (Edmeades *et al.*, 1983; McLaren & Cameron, 1996c). The manipulation of soil pH_{H2O} and extractable Al_{CaCl2} through lime additions was assessed on ten New Zealand soils (Chapter 5). This chapter leads on from that work and focusses on the effects of pH_{H2O} at the root-soil interface. It would be beneficial if the effect of pH_{H2O} on extractable Al concentrations could be examined in and around the root, through different measurements of Al at the root scale i.e. 100-1000 µm.

Plant roots influence the chemistry in the surrounding soil. The rhizosphere is the soil environment surrounding the plant root, which is directly influenced by the living root (Hiltner, 1904). It is an important site for both nutrient acquisition and the release of root exudates by the plant. This is also a zone of importance in relation to Al toxicity, as Al affects plant roots which become shorter and thicker with reduced branching (Ryan & Delhaize, 2012; Schroth *et al.*, 2003). The damage to plant roots compromises the ability of the plant to acquire nutrients and water from the soil through reduced root to soil contact and reduced shoot yield. Negative impacts are not restricted to nutrient uptake. Decreased water uptake by the plant roots can induce drought stress on the plant. Edmeades and Ridley (2003) reported the link between Al toxicity and drought stress due to the reduced ability of roots to grow downwards to acquire water stored deeper in the soil profile. The effects of Al on the plant roots can lead to a reduction in plant growth.

Mechanisms of plant tolerance to Al are either by exclusion of Al from plant tissues (external) or by increasing the tolerance of Al absorbed through the roots (internal detoxification) (Arunava & Khriedinuo, 2013; Barcelo & Poschenrieder, 2002; Ryan & Delhaize, 2012). Exclusion is by the release of root exudates, in particular Al-chelating organic acids from the growing tip into the rhizosphere (Liu *et al.*, 2014). The excreted organic acids chelate with Al^{3+} ions, preventing them from entering the root tip.

The ionic strength of the soil, which is affected by soil moisture, has an inverse relationship with pH (Edmeades *et al.*, 1985b). Therefore, soil moisture could have an effect on the soil extractable Al concentrations. Rayner (2015) in a glasshouse experiment, found a significant difference in the soil extractable Al_{CaCl2} between different moisture treatments. However, this was only at extractable Al_{CaCl2} concentrations >4 mg kg^{-1} and the effect of moisture was not as strong as anticipated. A better understanding of soil moisture effects on extractable Al_{CaCl2} may provide insight into factors causing Al toxicity.

In order to measure the supply of cations from the soil, and in particular Al, in this experiment, the diffusive gradient in thin-films (DGT) passive sampling technique was used (Lehto *et al.*, 2012). Diffusive gradient in thin-films (DGT) is used widely to measure solutes in water, sediments and soils (Panther *et al.*, 2012; Santner *et al.*, 2012; Zhang & Davison, 1995; Zhang *et al.*, 2001). In this method, a DGT gel is imbedded with a chelating resin, which binds dissolved trace metals from the soil pore water and acts as a sink for dissolved metals in the soil (Gao & Lehto, 2012; Hooda *et al.*, 1999; Zhang *et al.*, 2001). This removal of metals from the soil solution creates a diffusive flux from the soil environment adjacent to the resin gel. If the rate of uptake by the DGT exceeds the rate at which the metal can be re-supplied from the soil, the concentration at the resin-soil interface and, therefore the flux onto the DGT, will decrease. In soils, the supply of metals to plants via diffusion, is often by a tortuous pathway through the soil pores. A localized decrease in dissolved metals can induce the desorption of sorbed metals from solid phases, thus buffering the solution concentration. When the uptake rate by the DGT is greater, the mass taken up by the resin reflects the extent to which the dissolved metal concentrations are buffered. In view of this, the DGT results are expressed as an average flux over the deployment time. This is acknowledgement of the fact that we cannot be certain about the extent of soil resupply at any given location. The diffusive gels are analysed by Laser Ablation Inductively Coupled Plasma Mass Spectrometry (LA-ICP-MS), which gives high-resolution (100-1000 µm) measurements across the gel of selected metals and can be used to produce an image of the spatial variation of the sample (Gao & Lehto, 2012). The localised measurements of DGT mass (measured using the LA-ICP-MS) reflect the soil's capacity to sustain a diffusive flux of metal to the DGT resin at that location.

Solute (anion or cation) mobilisation in proximity to plant roots, using DGT, has been successfully studied by many authors (Hoefer *et al.*, 2015; Santner *et al.*, 2012; Williams *et al.*, 2014). DGT gels can mimic the diffusive supply of metal ions to the plant from the soil (Degryse *et al.*, 2009). The DGT- labile solute fraction in the soil has been reported in several studies to best correlate with plant

uptake, compared to that measured by chemical extractants (Degryse *et al.*, 2009; Zhang *et al.*, 2001). The chemical extractants can be harsh and may often substantially disturb the natural equilibrium between dissolved and sorbed metal (Lehto, 2016), whereas this method binds dissolved metals from soil porewater without adding chemicals to the system. The DGT technique has been used for 2D measurements to give an indication of the relative magnitude of mobilisation of different metals including P, Fe, Mn, Cu, Zn, Ni, K, Mg, Cd, As, Pb around the roots of species such as white lupin, oil seed rape, grass, lettuce, willow and rice (Koster *et al.*, 2005; Santner *et al.*, 2012; Valentinuzzi *et al.*, 2015; Williams *et al.*, 2014). All of these studies used rhizoboxes similar to those used by Wenzel *et al.* (2001) to grow plant roots adjacent to a transparent window, thus allowing observation of the roots and measurement of soils in proximity to the roots, without disrupting the soil or the plant during the growth cycle.

While DGT has been used to measure Al in aqueous systems (Panther *et al.*, 2012), it has not been used to measure Al in soils. This is the first time DGT has been used to measure the mobilisation of Al in the soil. Nevertheless, its DGT uptake dynamic, as a cationic metal species in soils, should not be any different to others (Fe, Cu and Zn) at these pHs.

In order to gain added insight into the results of the glasshouse study in the previous chapter, this rhizobox experiment was designed to observe the plant effects on Al mobilisation and immobilisation at the plant root scale. A rhizobox experiment was conducted growing Russell lupin and lucerne in an acidic high country soil containing elevated levels of Al_{CaCl2}. The objective was to examine the effect of pH_{H2O} on the extractable Al concentrations at the rhizosphere scale. The hypothesis tested, was that plants influence the soil extractable Al and pH_{H2O} at the root-soil interface and that the degree of influence differs between Russell lupin and lucerne.

6.2 Materials and methods

6.2.1 Soil selection and preparation

The soil used in this experiment was an Allophanic Brown Soil from Molesworth Station, Marlborough; from now on, it will be referred to simply as “MO soil”. This is an acidic soil with a pH_{H2O} of 5.2 and the highest extractable Al_{CaCl2} of the ten glasshouse soils; 13.3 mg kg^{-1}. It was important to select an acidic soil with high Al content in order to examine the change in extractable Al_{CaCl2} and pH_{H2O} in terms of nutrient mobilisation and plant uptake in response to increased lime application. Information on the collection of the soil, soil chemistry and site details are given in Chapter 5, Materials and Methods (Section 5.2). The soil was air dried at 30 °C and 2 mm sieved in preparation for packing the rhizoboxes.

6.2.2 Experimental design and project management

6.2.2.1 Trial design and set up

Five lime rates ($CaCO_3$) were applied based on the shoot yields of lucerne in the glasshouse experiment in the previous chapter (0.5, 1.0, 2.0, 4.0 and 8.0 t lime ha^{-1}, labelled 0.5-8). All rhizoboxes had basal P ($H_4CaO_8P_2.H_2O$) and S ($CaSO_4 \cdot 2H_2O$) (30 mg S kg^{-1} and 50 mg P kg^{-1}). A parallel treatment had 4.0 t lime ha^{-1}, but no basal S or P fertiliser (4-). This treatment was included to compare the differences in both plant growth and soil Al at 4 t lime ha^{-1} with and without basal nutrients applied. This combination was selected at the 4 t lime ha^{-1} rate, as this was predicted to be the treatment that would produce the highest yield, based on the lucerne yields growing in the MO soil in the glasshouse experiment.

Vials of pre-weighed fertiliser were added to a plastic bag filled with 2 mm sieved, air-dried MO soil (1068.8 g); one for each rhizobox. The appropriate rate of lime, P and S was added to the bag and mixed thoroughly.

The rhizobox dimensions were: volume 213.8 cm^3, length 28.5 cm, width 15 cm and depth 2.5 cm. The experimental soils were packed in five layers (depth per layer of soil was 0.5 cm) into the rhizoboxes to ensure a constant bulk density of 1.00 g cm^{-3} across the depth of the rhizobox (homogeneous). This bulk density was chosen because it is close to a field bulk density and allows the growth of the roots in the rhizobox. This bulk density has been used successfully in recent rhizobox studies with white lupin (Valentinuzzi *et al.*, 2015). Some rhizoboxes had moisture and water potential sensors placed in them. These were packed carefully within the layers of soil and were slotted into holes at depths of 5-10 cm. The soil surface was 1.5 cm below the top of the rhizobox.

Two legumes species were grown: a species more tolerant of acidic soils Russell lupin (*Lupinus polyphyllus L.*) and lucerne (*Medicago sativa L.,* 'Grasslands Kaituna'), a more sensitive species. Lupin (LP 0.5-8) and lucerne (LU 0.5-8) were grown and an optimal moisture treatment aimed for approximately -20 kpa. There was another set of six rhizoboxes with lucerne which had a drier moisture regime (aimed for -100 kpa) to compare to the lucerne with optimal moisture applied (DLU 0.5-8). A total of 18 rhizoboxes were installed.

Three wooden stands were set up, upon which the rhizoboxes sat at a 45° angle with the cover plate facing downwards, to ensure that plant roots would grow up against the cover plate. The rhizoboxes were set up across three wooden stands to ensure that the treatments were randomized (Plate 6.1).

The rhizoboxes were then wrapped around the soil line in black polythene to stop the light getting into the soil thereby reducing the growth of algae.

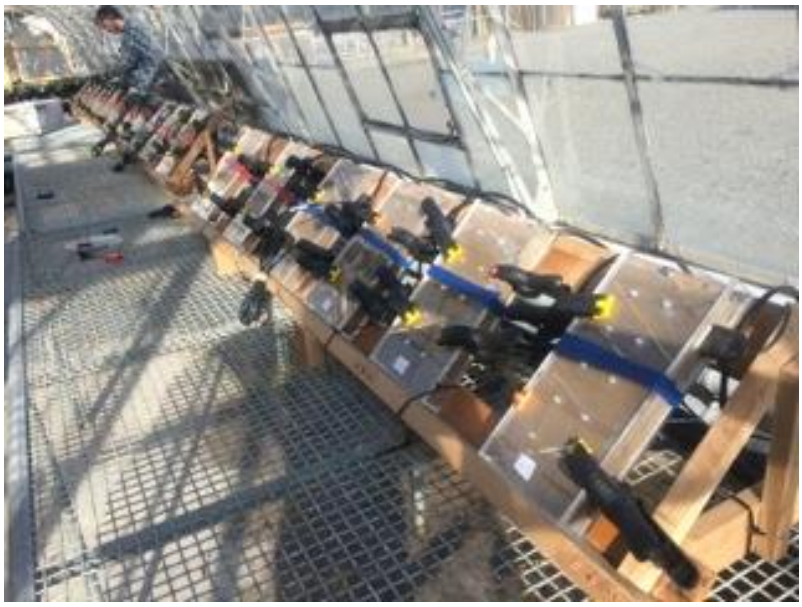

Plate 6.1 The set-up of the rhizoboxes on wooden stands before they were wrapped in black plastic wrap to reduce algal growth.

6.2.2.2 Glasshouse conditions

This rhizobox experiment was conducted in the Forrester Glasshouse, at Lincoln University. The glasshouse has computer controlled heating and ventilation. It is heated with hot water pipes running the length of the glasshouse and ventilated with fans, in conjunction with wet pads. The glasshouse temperature is logged every two hours and recorded by a computer. The mean daily temperature during the experimental period (23rd July- 22nd September 2015) was 17.9 °C, with mean daily temperatures ranging from 15.8 °C to 22.0°C. There were four days during the experiment on which the temperature measured was greater than 27°C (between 2pm and 4pm), however, these were exceptional glasshouse conditions.

6.2.2.3 Plant establishment and inoculation

Germination testing was conducted to determine the number of seeds to sow. Lupin had a germination rate of 96% and lucerne had a germination rate of 80%. The soils were pre-incubated with the treatments prior to seeding. Seeds were sown on the 23rd July directly into the soil; three lupin seeds and four lucerne seeds per rhizobox. They were then thinned out to one plant per rhizobox. This process started five days after germination and the timing was determined by the growth of the plants. Healthy plants which had grown a well-developed root, which did not grow near the edge of the rhizobox, were selected; the remaining plants/seeds in each rhizobox were removed during thinning. This caused minimal disturbance to the rhizobox soil.

Liquid inoculants were used for both lupin and lucerne to encourage nodulation and nitrogen fixation. The Rhizobium strain used for lupin was *Bradirhizobia* and for lucerne was RRI 123. The inoculant was applied to each species on the 7th August; 10 mL to each rhizobox. This is equivalent to 10^6 bacteria cells per mL and was delivered in a 0.85% saline solution (NaCl). Another seed was sown on the 3rd August in the LP-4-treatment, as there were few roots and one seed was struggling to germinate in this soil.

6.2.2.4 Soil moisture and water management

Soil moisture sensors (Decagon 5TM, Decagon Devices LTD, USA) were deployed in rhizoboxes LU-4, LP-4 and DLU-4, and water potential sensors (Decagon MPS-6, Decagon Devices LTD, USA) in rhizoboxes LU-0.5 and 4-, LP-0.5 and 4-, DLU 0.5 and 4-. The water potential sensors have an in-built six- point calibration and range from -9 kpa - -100,000 kpa. Both sensors simultaneously measure temperature using a thermistor. The moisture sensors were calibrated in the MO soil prior to the experiment using standard procedures, after which the water potential and moisture sensors were placed 5 cm below the soil surface. This provided the most representative measurement of soil moisture near the roots during the entire growth period of the plants. The length of the waveguides of the moistures sensors were 5.2 cm (measuring approximately 2 mm around the waveguide) and the length of the water potential sensors were 9.5 cm (the ceramic disk is 3.5 cm in diameter). The water potential sensors were imbedded in the soil during the packing of the rhizobox, compared to the moisture sensors, which were inserted after set up.

This experiment was set up with a dripper irrigation system. The LP and LU rhizoboxes received optimal water application (approximately -20 kpa), while the DLU rhizoboxes received less water. There were two drippers (Antelco Murray Bridge South Australia) per rhizobox, at a water application rate of 2 L hr^{-1} for each dripper. Water was applied in 16 second intervals, resulting in application of 18 mL of water per interval per rhizobox. The water was applied from the top to mimic the water application by either rainfall or irrigation in a natural system (the field).

Due to experimental difficulties, a significant difference in soil moisture between the nominally ‘optimal’ and ‘dry’ treatments could not be achieved consistently throughout the experiment. The moisture results for the ‘dry’ treatment were highly variable and gave cause to exclude the DLU data from further consideration.

6.2.2.5 Trial management

Photographs and observations of the plant roots and shoots were taken regularly to create a visual record of development during the growth period (Appendix Plates 4.3 a, b and c). The root length was monitored to ensure that the DGT deployments took place just before the roots reached the bottom of the box. The soil moisture and soil water potential were monitored throughout the experiment. The experiment was conducted for nine weeks to ensure that the root tips could be analysed using DGT thin films and the roots did not form a mat at the edges of the rhizobox.

6.2.3 Measurements and analysis

6.2.3.1 DGT gel deployment

The diffusive gradient in thin-films (DGT) technique was used to measure the two-dimensional distribution of metal fluxes in the lupin and lucerne rhizosphere in 18 rhizoboxes. Eight of the gels were analysed using laser ablation ICP-MS. The cation- binding DGT gels were prepared following the procedure used by Lehto *et al.* (2012). Briefly, 2 mL of SPR-IDA resin suspension 10 % (w/v; CETAC Technologies Inc., USA) was mixed with 2 mL acrylamide (40%, Fisher Scientific, USA). To this, DGT cross-linker (DGT Research, Ltd., UK) was added in a ratio of 4:1, followed by 28 µL of Ammonium Persulfate (Merck, Germany) and 8 µL of *N*,*N*,*N*′,*N*′-Tetramethylethylenediamine (TEMED, ~99%, Sigma–Aldrich, USA) were added. The gel mixture was then immediately pipetted between two glass plates, where a 0.25 mm spacer was used to ensure that the solution polymerized into a gel sheet with a uniform thickness. The glass plate assembly was then placed in an oven at 45 °C for 1 h, after which the glass plates were separated and the resin gel was placed into 0.5 L of high-purity deionised (DI) water (18.2 MΩ resistivity, Barnstead™ GenPure™ Pro Water Purification System, ThermoFisher Scientific, NZ) and allowed to fully hydrate for a minimum of 24 h. The hydrating solution was changed four times in the subsequent 72 h, after which, the resin gel was stored in 0.5 L of 0.01 M $NaNO_3$ solution. Prior to deployment, the resin gel was cut to size using PTFE-coated razor blades and mounted between a 10 µm-thick polycarbonate filter membrane (Nucleopore, Whatman, 0.4 µm pore size) on one side and a sheet of cellulose acetate (OfficeMax New Zealand Ltd.) on the other, to exclude solid particles from the soil. The filter and resin gel were fastened to the underlying acetate sheet using white vinyl tape (3M^{TM}, New Zealand) on four sides. To minimize contamination, all preparation and processing of gels was conducted using ultraclean trace metal techniques, ensuring that all equipment was acid-washed and rinsed in DI water.

Near the completion of the experiment (three weeks from sowing for lupin and eight weeks from sowing for lucerne), the DGT were placed against the plant roots in each rhizobox for 24 hours (square of around 5 cm by 5 cm) and the removal of metals from the soil solution created a diffusive flux from the soil environment adjacent to the resin gel (Plate 6.2). In the 24 hours prior to DGT deployment, the soil moisture content in the rhizoboxes was carefully and uniformly raised using a syphoning system with hoses attached to the back of each rhizobox, allowing a controlled ingress of Reverse Osmosis (RO) water until the soil was saturated. The rhizoboxes were then left to equilibrate for 24 h under that moisture content. The DGT gels were first deployed on the lupin roots (17/08/15), followed by lucerne (17/09/15, 12 rhizoboxes). All rhizoboxes had one gel deployed, except for LU-1, which had two sites of deployment. The different times of deployment were determined by the growth stage of the plant species. The site of deployment was selected to obtain measurements of Al resupply from the rhizosphere soil near root tips as well as the soil away from the plant roots (background soil). The exact deployment time was recorded for each DGT and used to calculate the time-integrated metal fluxes into the gel. Glasshouse temperature ranged between 16.1°C and 29.4°C during the deployments. After the deployment, the DGT gel was gently peeled off the rhizosphere interface and any soil particles were carefully removed by thoroughly rinsing with DI water. The exposed areas of the deployed DGT gels were then cut away using PTFE-coated razor blades, separated from the filter paper and stored horizontally in acid washed, zip-lock, polyethylene bags. The gels were then dried following previously established procedures (Stockdale *et al.*, 2009), including 8 h at 60 °C in a gel drier (Thermo Savant SGD210D Speedgel System). The dried gels were then mounted flat onto ~ 8 × 6 cm glass plates using double-sided tape, in preparation for analysis using LA-ICP-MS.

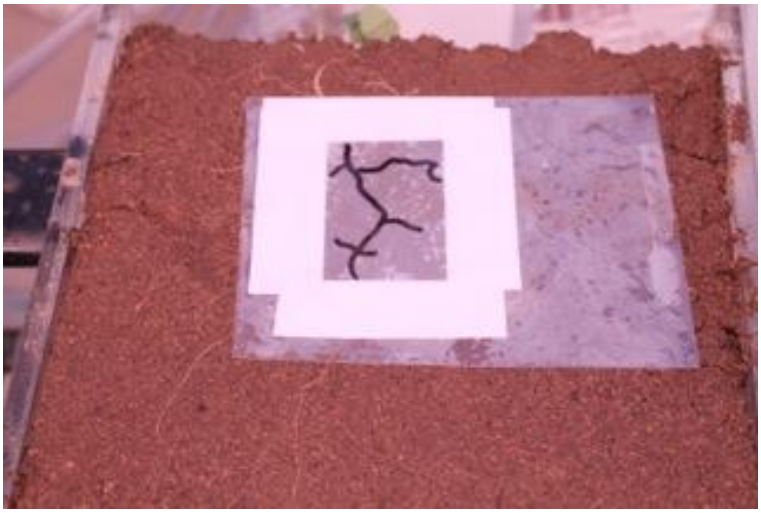

Plate 6.2 A DGT gel at the root tip. The white features seen within the sampling window are pockets of air trapped between the acetate supporting membrane and the gel. The root that is shown within the DGT gel was drawn on using a marker pen to help locate the root on the DGT gel later.

Six calibration standards were prepared for the LA-ICP-MS analysis using the methods described by Lehto *et al.* (2012). Briefly, four replicate SPR-IDA resins, identical to those used in the rhizobox experiment, were loaded with increasing concentrations of Al and Mn. This was achieved by deploying replicate DGT piston probes for different periods of time in solutions containing 0.1 mM hydroxyl ammonium chloride (BDH Laboratory Supplies, Poole, U.K.), 0.01 M $NaNO_3$ (BDH Laboratory Supplies, Poole, U.K.) and adjusted to pH 5. The deployment solutions were spiked with Al and Mn to concentrations of 150 µg L^{-1} (Al) and 800 µg L^{-1} (Mn) prior to deployment. The aim was to achieve a range of 0 - 1650 ng Al cm^{-2} and 0 – 12000 ng Mn cm^{-2} evenly bound across one side of the resin gel standards. After deployment, the probes were rinsed in high purity water and disassembled. The metal bound by three replicate resin gels was eluted in 1.8 mL of 1 M HNO_3 (Trace Analysis Grade, Fisher Scientific UK, Loughborough, U.K.) for 24 hours. The eluent was then analysed using Inductively Coupled Plasma-Optical Emission Spectrometer (ICP-OES) (Varian 720-ES ICP-OES; Varian Pty Ltd, Melbourne, Australia) to determine the average mass of metal bound to the replicate resin gels. The remaining resin gel was dried using identical methods previously described for the sample gels. Glass slides were mounted with the hydrogel samples and fixed in place by double sided tape, awaiting LA-ICP-MS analysis.

6.2.3.2 Soil sample collection

The experiment was completed on September 23rd and 24th 2015. Photographs were taken of the plant in each rhizobox before the sampling took place. Both rhizosphere and bulk soil samples were taken from each rhizobox (treatment combination). Plants were gently removed from the rhizoboxes and the 'rhizosphere soil', the soil directly in contact with the roots, was obtained by gently shaking the soil from the plant roots (Plate 6.3). As before, 'bulk soil' was defined as the soil in the rhizobox that had no observable contact with the plant roots and was collected after the removal of the plant. The roots and shoots were then separated and washed. The plant roots were stored in the refrigerator at 4°C, prior to nodule scoring and root scanning.

Plate 6.3 The rhizosphere soil, which is adhered to the roots, collected for LP-4-.

6.2.3.3 Nodule scoring and root scanning

Roots were nodule scored based on the nodule colour, number, position on the roots and the size, according to criteria adapted from Rice *et al.* (1977) (Appendix Table 4.1). Plant root morphology including root surface area, length and volume were measured by scanning the roots using WinRHIZO software (Regent Instruments Inc, Quebec, Canada; appendix Plates 4.1a-d and 4.2a-f). Bouma *et al.* (2000) considered root length, diameter, distribution and volume as important characteristics when describing and comparing root systems. The specific root length (SRL), specific root area (SRA) and specific root volume (SRV) were calculated, as a ratio of the length, surface area and volume of the root system to its dry weight (Merkl *et al.*, 2005; Paula & Pausas, 2011).

6.2.3.4 Shoot and root yield

After conducting the WinRhizo root sample scans, the root and shoot samples were oven dried at 70 °C for 48 hours. The weight of the herbage samples was recorded to within ± 0.01 g, to obtain the dry weight for each sample. The weight represented the shoot and root yield (g DM pot^{-1}) for the given growth period.

6.2.3.5 Plant tissue analysis

The dried herbage samples were ground and digested (Chapter 5; Section 5.2.5.1 and 5.2.5.2) before nutrient analysis on the ICP-OES to determine the composition of the herbage and the roots. Total

shoot and root Al concentrations (mg) were calculated based on the dry matter yield and the mg kg^{-1} of Al measured in the plant tissue. We assume that the Al deposited on plant leaves was negligible.

6.2.3.6 Soil analysis

Final chemical analysis was conducted at Lincoln University for each rhizobox on the bulk and rhizosphere soil samples at the harvest (62 days from sowing), including final soil pH at 1:2.5 air-dried soil: water ratio (standard soil test in New Zealand). The extractable Al_{CaCl2} (0.02 M $CaCl_2$) was measured using methods described in Chapter 5. The extractable organic carbon was obtained using the hot-water soluble C method outlined by Sparling *et al.* (1998). Briefly, 2 g of air-dried soil with 10 mL water was incubated in a capped falcon tube at 70 °C for 18 hours. Samples were then filtered through Advantec 5C filter paper and the filtrate was measured on the Vario TOC cube (Elementar, Hanau, Germany).

6.2.3.7 Laser Ablation Inductively Coupled Plasma Mass Spectrometry (LA-ICP-MS) analysis.

Specific treatments were identified, which covered a range of lime rates, to show differences in Al mobilisation at the root: soil interface. A total of seven of the deployed gels were analysed using LA-ICP-MS analysis: four lupin and three lucerne. To manage the high cost of the LA-ICP-MS analysis, a pragmatic decision was made to favour the lupin gels as the larger plants and roots were believed to induce greater changes in the rhizosphere than the lucerne. The laser ablation analysis was conducted at Waikato University, New Zealand. The laser ablation unit was an SE-series RESOlution Laser ablation System (Resonetics Ltd., Boston, MA, USA). This was attached to an Elan DRC II ICP-MS (Perkin Elmer Sciex, Waltham, MA, USA). Each sample was setup up in the sample holder to ensure the sample slide was held firmly in place. At the start of each day, NIST standards 612 and 610 (NIST, Gaithersburg, MD) were run to confirm optimal performance of the laser ablation unit and the ICP-MS. Before each sample was analysed, the previously prepared set of calibration standards was assessed.

The LA-ICP-MS analysis was conducted in line scan mode with a laser beam diameter of 150 µm, scanning speed of 150 µm s^{-1}, laser pulse frequency of 20 Hz, a laser energy of 2 MJ and an attenuation factor of 50%. These settings are specific to the Laser Ablation unit and were determined during specific testing prior to analysis. A large proportion of the metal bound by the resin gel is fixed within a thin layer closest to the resin gel-filter (viz. soil) interface (Lehto *et al.*, 2006). The established settings allowed the targeted ablation of a small proportion of the total gel thickness, while minimizing the unnecessary vaporisation of extraneous gel matrix, which could interfere with the ICP-MS analysis. The

mass spectrometer was set to detect the following analytes: ^{13}C, ^{27}Al and ^{55}Mn. There was an analysis time of 0.276 s per reading and line spacing was 500 µm. ^{13}C occurs naturally in the gel matrix and was used as the internal standard for the analysis (Gao & Lehto, 2012; Lehto *et al.*, 2012).

Three 10 mm-long lines were ablated on each standard to provide a total of ~720 measurements for each analyte on each standard. The counts for each metal from the mass spectrometer detector were normalized against the counts for the internal standard (IS: ^{13}C) to obtain the metal/internal standard (M/IS) ratio. The average M/IS for each standard was used, along with the previously determined mass of metal bound on the resin gels, to determine the relationship between M/IS and mass of metal bound by a unit area of resin gel. Upon LA-ICP-MS analysis of the standards, it was found that four of the six standards (standards II, III, IV and V) did not show a signal for any of the metals, while two (the blank, I, and V) did. This is despite the eluted replicates (analysed using ICP-OES) showing a consistent, progressively increasing, metal content across the series. Therefore, the calibration of M/IS to mass of metal bound by the resin gel is based only on two standards (I and VI). While this can strictly be considered only semi-quantitative, it allows for the comparison between masses of metal bound by gels analysed at different times.

The deployed gel samples were analysed using LA-ICP-MS, using the same procedure as the standards, following procedures described by Gao and Lehto (2012). This analysis provides a spatial representation of metal bound by the DGT resin, where each unit of measurement ('pixel') represents the total metal bound by 150 µm × 41 µm of gel. Because the distance between the lines was 500 µm, there was a 350 µm space in between the lines that was not analysed. An assumption was made that the 150 µm wide pixel, actually represented 500 µm, thus extrapolating the result (and the effective size of the pixel) to 500 µm × 41 µm. This approach was taken to optimize the amount of data that could be obtained within the allocated time and budget for this analysis and is in line with previous analyses (Hoefer *et al.*, 2015; Williams *et al.*, 2014).

Gels deployed in the LP and LU treatments were analysed to compare different plants under similar moisture regimes. Gels deployed in the DLU treatments were not selected because of the different moisture regime and, also, the plants had very small roots, which may not have shown clear results using this analysis. This was a practical decision, made to maximize the return from expensive analyses. Eight deployed gels were analysed in two batches: five gels were analysed in April 2016 (LP-0.5, LP-1, LP-2, LP-4-and LU-1) and three more (LP-4, LU-0.5 and LU-4) were analysed in July 2016. The LP-4- gel

was the only gel not to have a calibration factor, as the results from the standards were not good enough, indicating that the instrument was not analysing the gel optimally at the time this gel was run.

6.2.4 Data Analysis

The calibrated data were then processed using Microsoft Excel to provide a matrix where the data from the ablated lines were aligned and used to create a two-dimensional (2D) image in ImageJ (version 1.47v; National Institute of Health, Bethesda, MD). The Al and Mn images were analysed in ImageJ using a method defined as the 'Areal method' in which two types of features were targeted: metal depletion along the root axis and soil showing above-background metal mobilisation. Metal depletion along the root axis was determined by calculating the average solute fluxes within a rectangular area defined by the root diameter and 0.5 mm either side of the root (in most cases this was a total width of 2 mm) and the length of the root within the area of analysis. Different widths were considered, however, 0.5 mm allowed for the best comparison between soil treatments.

To systematically define areas of soil from which the metal fluxes were above or below background levels, the average and standard deviation (SD) of the fluxes across the entire area of analysis were calculated. Areas of soil where the metal flux was more than 0.25 × SD above the average were defined as 'hotspots', whereas areas of soil where the flux was less than 0.25 × SD below the average were defined as 'depletion zones'.

The sites of Al analysis were first chosen for Al on each gel and the same areas were analysed on the Mn gels, to enable the comparison of the flux of these toxic metals. In addition to the main analysis, three transect lines were drawn on the LP-0.5 and LU-1 gel images, perpendicular to the root and at different positions across the root axis, to show how the mobilisation of Al changes away from the root at key locations (Figure 6.6 and Figure 6.11).

6.3 Results

6.3.1 Initial soil chemistry (pre-treatment)

The Molesworth (MO) soil is a high country Allophanic Brown Soil. This soil was selected because it was acidic and had the highest Al_{CaCl2} concentration of the 10 high and hill country soils used in the glasshouse experiment in the previous chapter. This suggests that more Al was available to the plants. Under the New Zealand soil classification (NZSC), this is a Brown Soil, which is the most extensive soil order in New Zealand, has low fertility and is therefore a representative soil of high and hill country

areas. The soil chemical properties and total metals are presented in Table 6.1 for the native soil, before the addition of lime, S and P treatments.

Table 6.1 Initial soil chemical analyses for the Molesworth 'native' soil used in this experiment (as described in Table 5.1), before the addition of treatments.

	Chemical properties
pH	5.2
Extractable Al_{CaCl2} (mg kg^{-1})*	13.3
Olsen P (µg mL^{-1})	13
Sulphate S (µg g^{-1})	9
Organic matter (% w/w)	8.5
Total C (% w/w)	4.91
Total N (% w/w)	0.38
Base saturation (%)	12.9
CEC ($cmol_c$/kg)	14
Total Al (mg kg^{-1})	33,081
Total P (mg kg^{-1})	737
Total S (mg kg^{-1})	302
Total Mn (mg kg^{-1})	304
Total Fe (mg kg^{-1})	22,933

Note that * is 0.02 M $CaCl_2$ extractable Al.

6.3.2 Soil moisture during the experiment

The daily average soil moisture content ranged between 39.5% and 42.6%, which equated to a daily average water potential of -47.6 kpa. The LP-0.5 soil was consistently drier for part of the experiment than the mean of the other lupin and lucerne rhizobox soils, owing to the plant's greater biomass and higher evapotranspiration (ET) rate. It was therefore difficult to establish a realistic estimate of the soil moisture, as the plant grew so rapidly thereby consuming the available water, which required continual application to replenish.

6.3.3 Soil testing at the completion of the experiment.

The soil pH_{H2O} increased with increasing lime rate (Figure 6.1). The soil pH_{H2O} in the lupin soils increased from pH_{H2O} 5.1 in the 0.5 t lime ha^{-1} lime treatment (bulk soil) to 7.2 in the 8 t lime ha^{-1} treatment. This is the equivalent of an increase in pH_{H2O} by 0.26 pH units with every tonne of lime applied. The bulk soil pH_{H2O} for the rhizoboxes containing lucerne, ranged from 4.8 in the 0.5 t lime ha^{-1} treatment to 7.1 in the 8 t lime ha^{-1} treatment. This represents an increase of 2.3 pH units across the lime sequence. For lupin plants, generally the rhizosphere soil was between 0.1 and 0.3 pH units lower than the bulk soil across all treatments. For lucerne, across all treatments, the soil pH_{H2O} between the bulk and rhizosphere was similar. Treatments LU-1, LU-2 and LU- 4- measured the same pH_{H2O} in both the bulk

and rhizosphere soils. LU-0.5 and LU-8 rhizosphere soils were slightly higher compared to the bulk soil, but only differed by 0.1 pH units.

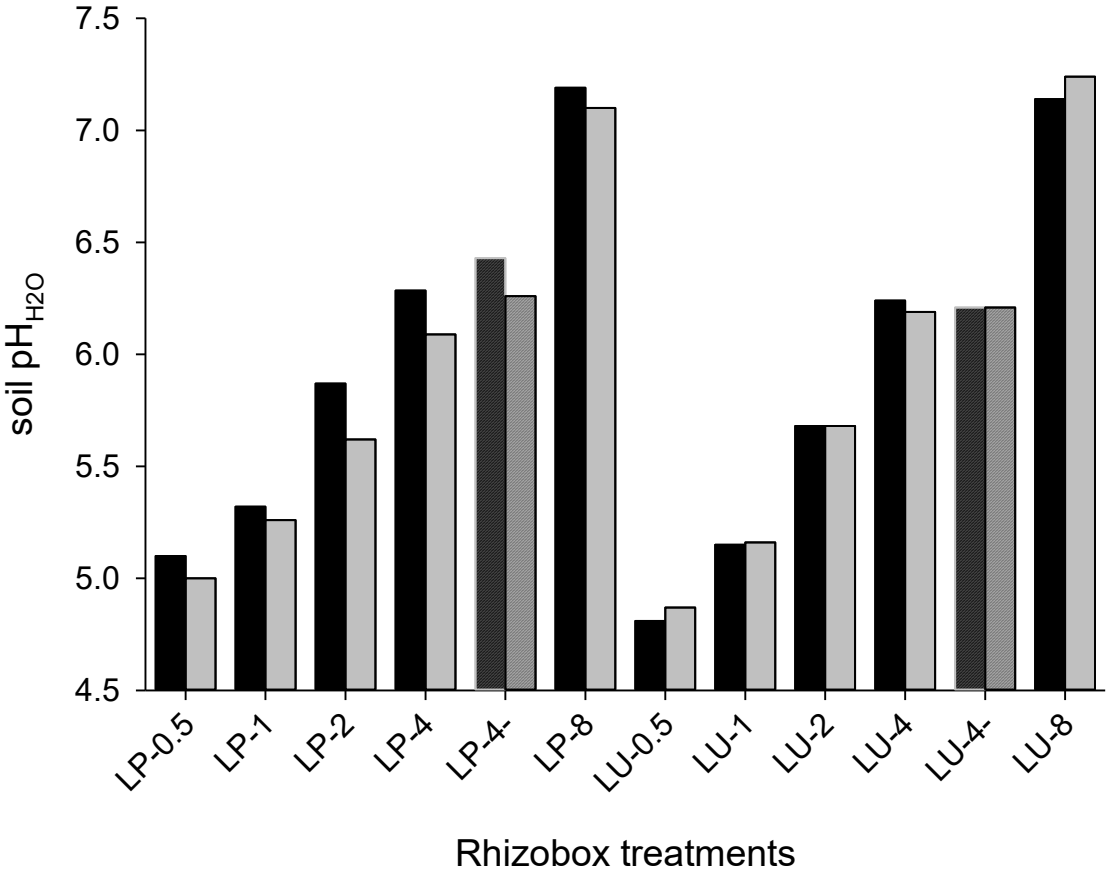

Figure 6.1 The soil pH measured in the bulk (■) and rhizosphere soil (▒) samples for the 12 rhizobox treatments for lupin (LP) and lucerne (LU) across the five lime rates of 0.5, 1, 2, 4 and 8 t lime ha^{-1}. The textured bars for LP-4- and LU-4- show that these treatments had no basal P or S.

The extractable Al_{CaCl2} showed a general decrease with increasing lime rate up to a certain rate and then showed no further decline (Figure 6.2). In the lupin soils, there was a 35 % decrease in Al_{CaCl2} between the 0.5 t lime ha^{-1} (bulk soil) treatment (5.1 mg kg^{-1}) and 2 t lime ha^{-1} (3.3 mg kg^{-1}). Above 2 t lime ha^{-1} applied there was no further decrease in the Al_{CaCl2} measured despite the increase in lime rate. The general trends were similar in the lucerne bulk soils. The Al_{CaCl2} in the lucerne soils (bulk) decreased from 7.6 mg kg^{-1} in the 0.5 t lime ha^{-1} treatment to 1.1 mg kg^{-1} in the 2 t lime ha treatments. Further lime application did not decrease bulk soil extractable Al_{CaCl2} further.

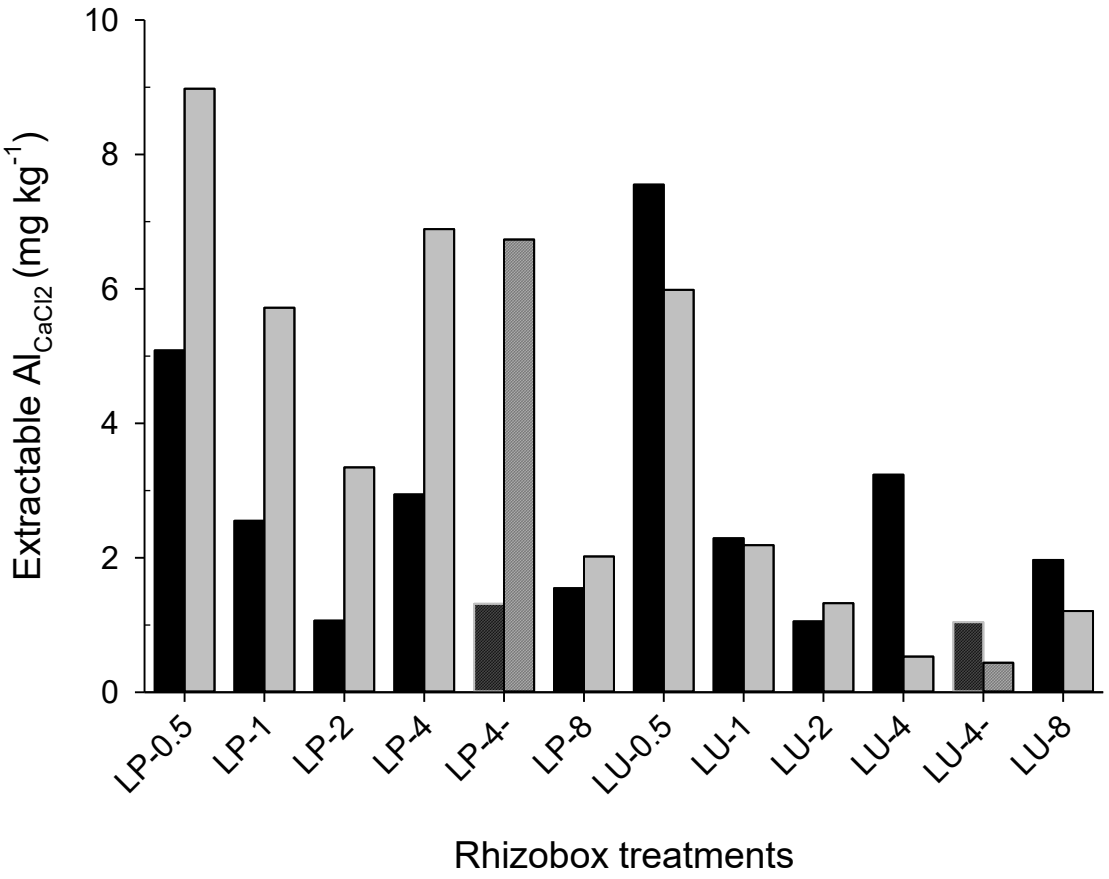

Figure 6.2 The extractable Al_{CaCl2} (mg kg^{-1}) measured in the bulk (■) and rhizosphere soil (■) samples for the 12 rhizobox treatments for lupin (LP) and lucerne (LU) across the five lime rates of 0.5, 1, 2, 4 and 8 t lime ha^{-1}. The textured bars for LP-4- and LU-4- show that these treatments had no basal P or S.

For lupin plants, the rhizosphere Al_{CaCl2} was between 0.5 mg kg^{-1} and 5.4 mg kg^{-1} higher than the bulk soil across all treatments. These large differences in the Al_{CaCl2} concentration between the rhizosphere and bulk soil for the lupin plants justified the decision to analyse more lupin gels LA-ICP-MS than lucerne gels. Laser ablation is an expensive analysis technique and this approach increased the likelihood of measuring observable differences in the Al flux across the gel. For the lucerne plants, there were generally higher Al_{CaCl2} concentrations in the bulk soil, between 0.1 mg kg^{-1} and 2.7 mg kg^{-1} compared to the rhizosphere soil. Treatment LU-2 measured a slightly lower Al_{CaCl2} concentration in the bulk soil of 1.1 mg kg^{-1} compared to the rhizosphere Al_{CaCl2} of 1.3 mg kg^{-1}.

The hot water extractable carbon (HWEC) over the first four lime rates appeared to be similar in the lupin soils and increased by 1.5 times at the highest lime rate (Figure 6.3).The HWEC in the bulk and rhizosphere soil growing lupins ranged from 904.7 to 1631.4 µg C g^{-1} soil and the lucerne ranged from 845.0 to 1186.8 µg C g^{-1} soil. It cannot be said, however, that the concentration of HWEC was significantly higher in the rhizosphere compared to the bulk soil due to a lack of replicates. However,

the HWEC was apparently higher in the rhizosphere soil (Figure 6.3) and there appeared to be a repeatable difference, suggesting a consistent effect.

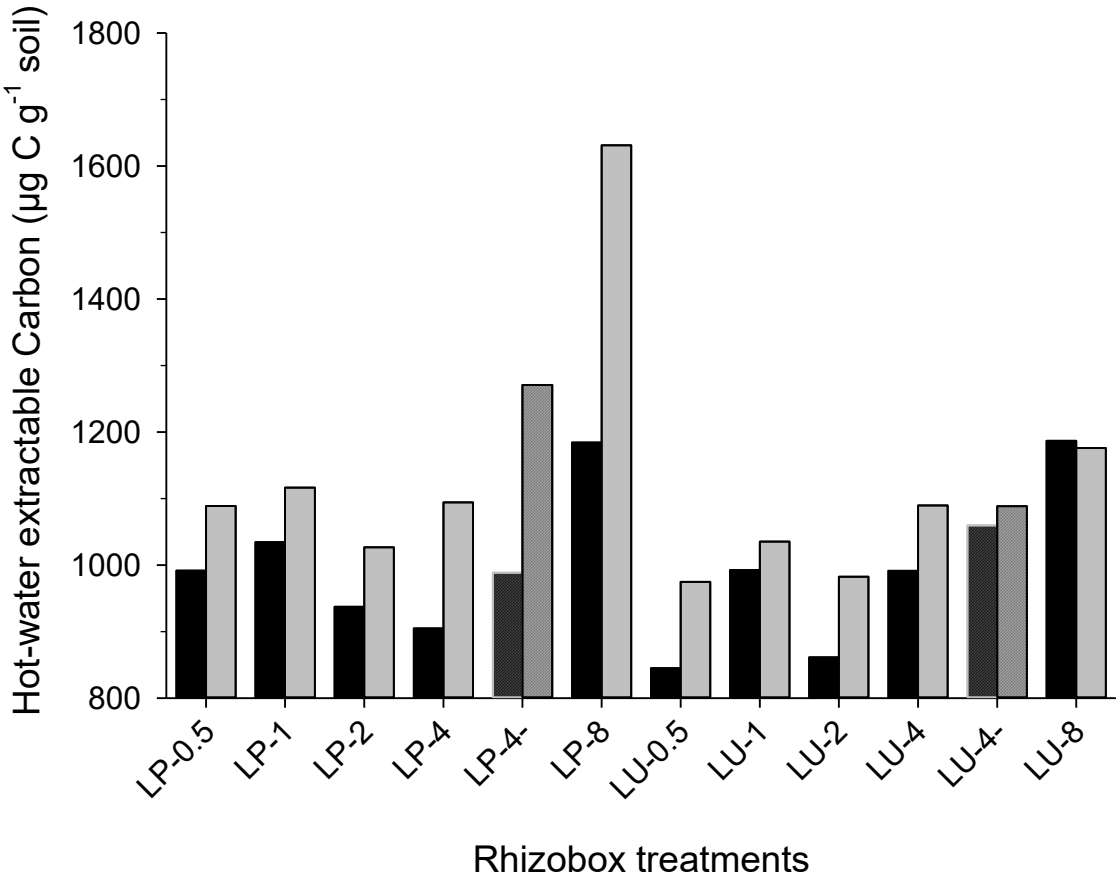

Figure 6.3 Extractable organic Carbon (µg C g^{-1} soil) measured in the bulk (■) and rhizosphere soil (■) for the 12 rhizobox treatments for lupin (LP) and lucerne (LU) across the five lime rates of 0.5, 1, 2, 4 and 8 t lime ha^{-1}. The textured bars for LP-4- and LU- 4- show that these treatments had no basal P or S.

6.3.4 Plant Physiology

6.3.4.1 Biomass Production

The shoot yields for lupins were consistently high across all treatments and higher than for lucerne (Figure 6.4). The lupin yielded highest at the lowest lime treatment of 0.5 t lime ha^{-1} (2.40 g) and lowest in the 8 t/lime treatment (0.33 g). The LP- 4- shoot yield was lower (1.08 g) than that of the corresponding lime treatment (LP-4) 4 t lime ha^{-1}, which had basal fertilizer applied (1.70 g). For all lupin plants except LP-4-, the shoot yield was higher than the root biomass by between 0.12 g and 0.69 g. The lucerne shoot yield was highest at 0.41 g and 0.73 g in the 1 and 2 t lime ha^{-1} lime treatments (Figure 6.4). The LU yield declined with further lime application.

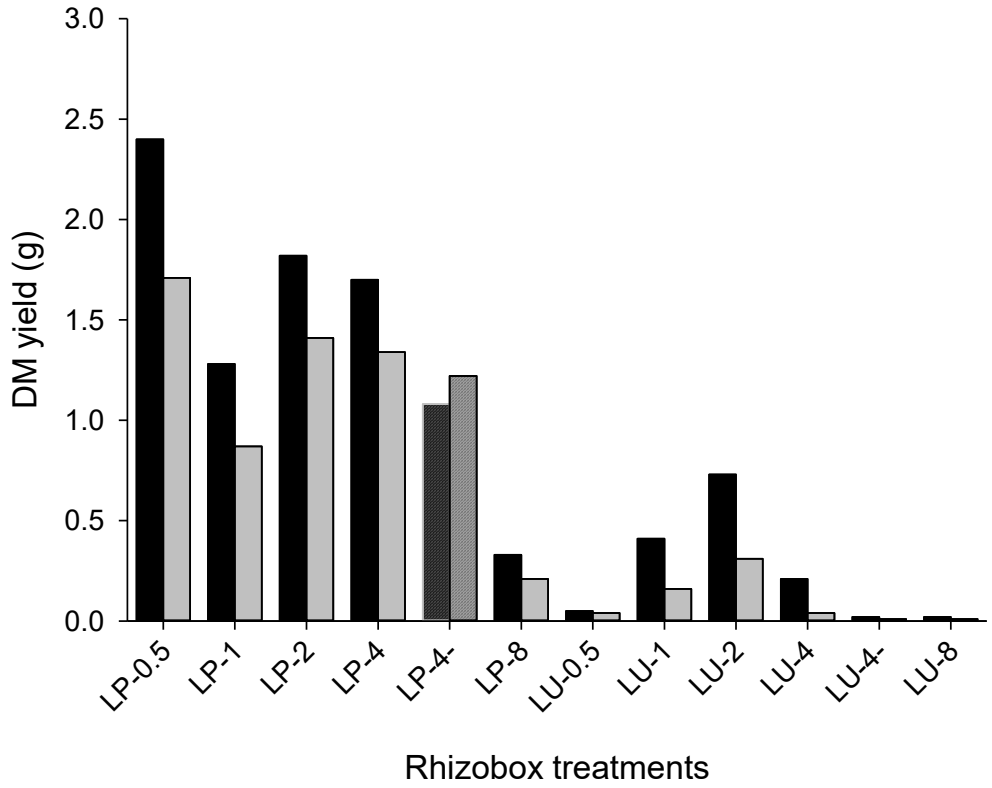

Figure 6.4 Biomass production, shoots (■) and roots (■) in grams per rhizobox for the 12 rhizobox treatments for lupin (LP) and lucerne (LU) across the five lime rates of 0.5, 1, 2, 4 and 8 t lime ha^{-1}. The textured bars for LP- 4- and LU- 4- show that these treatments had no basal P or S.

6.3.5 Root Physiology

The root: shoot ratio varied across lime treatments for both lupin and lucerne (Table 6.2). The specific root length (SRL) was constant across the lupin treatments and was highest in the 8 t lime ha^{-1} at 19 m g^{-1}. The SRL ranged from 16 to 338 m g^{-1} and the SRL of lucerne plants were generally higher than the lupin. The root volume was highest in the LP-0.5 treatment at 10.1 cm^3 and was lowest in the highest lime treatment (LP-8) at 2.7 cm^3 (Plate 6.4 and Plate 6.5). The lucerne root volume varied across the treatments and was highest in the LU-1 and LU-2 treatments at 1.3 cm^3 and 3.8 cm^3. The total root length and total surface area appeared relatively constant for lupins and lucerne plants across the lime sequence. Both characteristics reduced in the highest lime treatments of the LP and LU plants. At the completion of the experiment, fine hair-like roots were observed on the lupin roots, which may have been the early development of cluster roots. The 8 t lime ha^{-1} lupin plant did not appear to have these hairs/early development cluster roots.

Table 6.2 Root characteristics of lupin (LP) and lucerne (LU) plants measured by the Winrhizo software at the completion of the experiment including Root: shoot ratio, Total root length, Total surface area, root volume, calculated Specific root length (SRL), Specific root area (SRA) and Specific root volume (SRV).

rhizobox treatment	Root: shoot	SRL m g^{-1}	Total root length (m)	SRA m^2 kg^{-1}	Total Surface area (cm^2)	SRV mm^3g^{-1}	Root volume (cm^3)
LP-0.5	0.7	3	5.2	168	28.7	0.59	10.1
LP-1	0.7	6	4.8	321	27.9	0.64	5.6
LP-2	0.8	3	4.9	191	26.9	0.61	8.6
LP-4	0.8	3	4.7	200	26.8	0.67	9.0
LP-4-	1.1	4	4.5	214	26.1	0.60	7.3
LP-8	0.6	19	4.1	1119	23.5	1.26	2.7
LU-0.5	0.8	105	4.2	6325	25.3	0.95	0.4
LU-1	0.4	29	4.6	1638	26.2	0.79	1.3
LU-2	0.4	16	5.1	906	28.1	1.23	3.8
LU-4	0.2	101	4.1	6175	24.7	1.93	0.8
LU-4-	0.5	338	3.4	20600	20.6	2.30	0.2
LU-8	0.5	172	1.7	14400	14.4	1.40	0.1

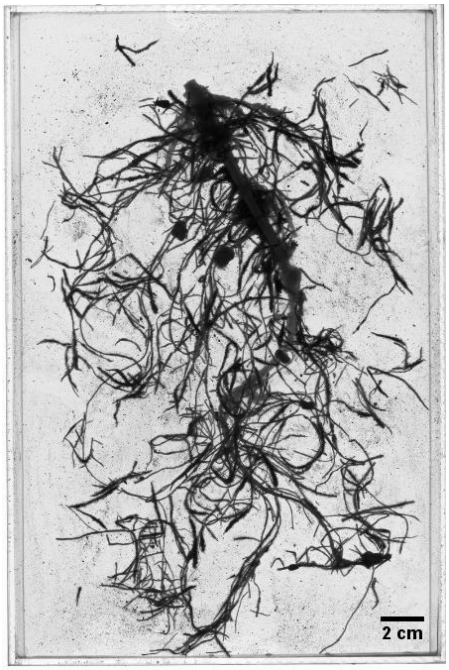

Plate 6.4 LP- 0.5 root scan

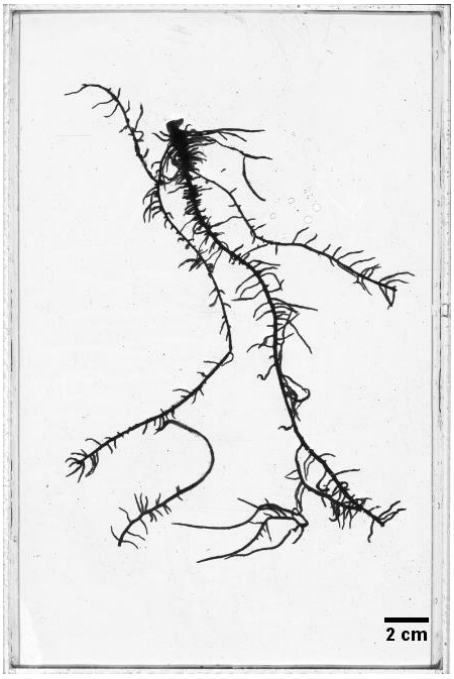

Plate 6.5 LP- 8 root scan

6.3.5.1 Root nodules

All lupin plants formed root nodules, while only the LU-2 and LU-4 lucerne plants grew nodules. These data were inconclusive and therefore will not be discussed further.

6.3.6 Plant chemistry

6.3.6.1 Shoots

The lupin shoot Al concentration decreased with increased lime applied from 81 mg kg^{-1} to 38 mg kg^{-1}. The lupin total Al shoot uptake declined with increased lime applied along the sequence from 0.19 mg to 0.01 mg (Table 6.3). The Al concentrations in the shoots of the LU plants were variable across the lime treatments. Lucerne total Al shoot uptake was highest in the 1 t lime ha^{-1} and 2 t lime ha^{-1} treatments and declined with further lime application. The shoot Boron concentration generally declined with increasing lime applied. Lupin shoot B concentration declined from 23 mg kg^{-1} to 8 mg kg^{-1} across the lime sequence. The B in the LU herbage was much higher than in the lupins and ranged from 37 mg kg^{-1} (LU-0.5) to 19 mg kg^{-1} (LU-4). The shoot Mo increased with lime applied for both species, however, there were plants with Mo concentrations below the detection limit (<0.001 mg kg^{-1}). The lowest P and S concentrations were generally in the 4- and 8 treatments, however, the S concentration in lupin shoots in the LP-8 treatment was higher than the LP-2 and LP-4 treatments, at 7109 mg kg^{-1}. The Mn and Fe concentrations generally declined with an increase in lime application for both lupin and lucerne plants. In both sequences, there was an increase between the treatment with lime and basal nutrients and the 4- treatment. However, in the highest lime treatment, the Fe in the lucerne shoots was higher than in all other treatments.

Table 6.3 The shoot Al, B, Mo, P, S, Mn and Fe concentration (mg kg^{-1}) and Al uptake (mg) for lupin (LP) and lucerne (LU) plants measured at the completion of the experiment for legumes grown in the Molesworth soil with 0.5, 1, 2, 4 and 8 t lime/ha applied (+ basal P and S) and 4- (no basal P or S applied).

Treatment	Al (mg kg^{-1})	Total Al uptake (mg)	B (mg kg^{-1})	Mo (mg kg^{-1})	P (mg kg^{-1})	S (mg kg^{-1})	Mn (mg kg^{-1})	Fe (mg kg^{-1})
LP-0.5	81	0.19	23	0.03	1886	7958	373	147
LP-1	77	0.10	19	0.28	2626	9726	229	153
LP-2	52	0.09	16	<0.001	1816	6705	178	122
LP-4	45	0.08	10	<0.001	1409	6736	100	109
LP-4-	86	0.09	12	0.48	1694	5553	171	213
LP-8	38	0.01	8	1.16	695	7109	25	45
LU-0.5	272	0.01	37	<0.001	1323	2689	197	206
LU-1	268	0.11	34	0.11	3176	2991	92	210
LU-2	205	0.15	31	0.29	2511	2638	56	195
LU-4	142	0.03	19	1.02	2025	2742	39	167
LU-4-	940	0.02	26	<0.001	945	1644	71	196
LU-8	1175	0.02	29	<0.001	1075	2334	52	805

6.3.6.2 Roots

The total root Al (mg) for lupins was highest in the 0.5 t lime ha^{-1} treatment at 29.55 mg kg^{-1} (Table 6.4) and varied across the liming sequence. The Al concentrations were higher in the lupin roots across all treatments compared to the lucerne. Total root Al (mg) for lucerne peaked at 0.75 mg kg^{-1} (2 t lime $ha^{-}1$) and declined with further lime application. The B concentrations remained consistent in the lupin roots and was lowest in the LP-8 treatment at 15 mg kg^{-1}. Boron concentrations were generally lower in the lucerne plants compared to the lupins. B root concentrations were similar across the lime sequence for the LU roots. Mo was lowest in the roots of the lupin under the no basal fertilizer treatment, LP- 4-, (0.06 mg kg^{-1}) and was highest in the LP-0.5 treatment (1.47 mg kg^{-1}). Mo was below the detection limit of <0.001 mg kg^{-1} in most of the roots of the lucerne plants, except for LU-1 and LU-2 at 0.10 mg kg^{-1} and 0.15 mg kg^{-1} respectively. For P and S, root concentrations were fairly similar across the liming sequence for LP and LU. The lowest concentrations in P and S were generally in the 4- and 8 treatments. There was uncertainty around the root nutrient data and therefore it will not be considered further in this experiment.

Table 6.4 The root Al, B, Mo, P, S, Mn and Fe concentration (mg kg^{-1}) and total Al (mg) for lupin (LP) and lucerne (LU) plants measured at the completion of the experiment for legumes grown in the Molesworth soil with 0.5, 1, 2, 4 and 8 t lime/ha applied (+ basal P and S) and 4- (no basal P or S applied).

Treatment	Al (mg kg^{-1})	Total root Al (mg)	B (mg kg^{-1})	Mo (mg kg^{-1})	P (mg kg^{-1})	S (mg kg^{-1})	Mn (mg kg^{-1})	Fe (mg kg^{-1})
LP-0.5	17281	29.55	29	1.47	1656	3509	203	10419
LP-1	20277	17.64	31	0.59	1277	1718	206	11735
LP-2	18203	25.67	29	0.54	1392	2513	180	10606
LP-4	20861	27.95	31	0.62	1215	2335	196	12337
LP-4-	24960	30.45	36	0.06	826	1153	231	14342
LP-8	5705	1.20	15	0.76	599	1072	57	3082
LU-0.5	743	0.03	18	<0.001	1097	1187	78	1882
LU-1	2304	0.37	15	0.10	2652	2751	100	1246
LU-2	2411	0.75	14	0.15	2234	3023	44	1349
LU-4	646	0.03	14	<0.001	1527	1697	33	1501
LU-4-	2498	0.02	23	<0.001	760	574	75	3957
LU-8	4274	0.04	26	<0.001	1020	382	61	2146

Note: total root Al incorporates the yield of each plant.

6.3.7 Root-soil interface

Of the gels analysed by LA-ICP-MS, as outlined in the Materials and Methods, the LP-4 gel produced unusable data due to an apparent instrument malfunction during the analysis. The seven gels, which produced the high-resolution images for Al that are required for interpretation, are presented and

include LP-0.5, LP-1, LP-2, LP- 4-, LU-0.5, LU-1 and LU-4. Only the Mn flux for the: LP-0.5, LP-1, LP-2, LP- 4- and LU-1 gels are presented. No large differences in Mn flux were observed for the other lucerne gels, therefore these gels were not presented. The images and sites of analysis are presented both in the results section (Figure 6.5-Figure 6.13) and the appendix (Figures 4.1 and 4.2). As a result of the prolonged dry conditions in the laser ablation chamber, several of the gels became desiccated and cracked, shown by black lines on the LP-2 (Figure 6.8) and LP-4- (Appendix Figure 4.2) gel images.

6.3.7.1 Differences in the Al and Mn flux across the gel

Areal analysis was conducted for which the mean flux over a 24 hour period along the root axis and the bulk soil were determined for Al and Mn across all gels (Table 6.5 and Table 6.6).

Table 6.5 The distribution of Al (flux over the 24 hour deployment period) as taken up by the DGT gel for lupin (LP) and lucerne (LU) plants; mean ng cm^{-2} day^{-1} ± SD in the Molesworth soil with 0.5, 1, 2, 4 and 8 t lime/ha applied (+ basal P and S) and 4- (no basal P or S applied).

	Root axis	Bulk Soil	Hotspot
LP-0.5	1490 ±410	1764±2935	2510±521
LP-1	1231 ±336	1405±444	1881±189
LP-2[@]	780±345	395±287	-
LP-4-	0.61±0.38	0.34±0.31	0.59±0.22
LU-0.5[@]	6234±3174	4842 ± 3012	-
LU-1	212±282	389 ± 414	981±1096
LU-4[@]	1409±1005	691±1770	-

[@]Hotspots could not be identified in these soils.

Table 6.6 The distribution of Mn (flux over the 24 hour deployment period) as taken up by the DGT gel for lupin (LP) and lucerne (LU) plants; mean ng cm^{-2} day^{-1} ± SD in the Molesworth soil with 0.5, 1, 2, 4 and 8 t lime/ha applied (+ basal P and S) and 4- (no basal P or S applied).

	Root axis	Bulk Soil	Hotspot
LP-0.5	70±17	66±26	76±15
LP-1	43±7	41±7	48±6
LP-2[@]	17±3	19±5	-
LP-4-	0.10±0.03	0.09±0.03	0.16±0.02
LU-1	8±3	7±8	11±7

[@]Hotspots could not be identified in these soils.

6.3.7.2 Lupin

The average flux of Al into the DGT gel across the analysed area in the LP-0.5 treatment was 1764 ng cm^{-2} day^{-1} (Table 6.5). Along the lupin root axis, the average flux of Al bound was approximately 250 ng cm^{-2} day^{-1} lower than from the bulk soil. There was a distinct area 'hotspot' where the flux of Al to the DGT was 1000 ng cm^{-2} day^{-1}, higher than along the root axis (Figure 6.5a). Along one root axis the average flux of Mn bound to the LP-0.5 DGT gel was approximately 28 ng $cm^{-2}day^{-1}$ lower than the background, while along the other root axis the flux of Mn into the DGT gel was greater than the

background (Figure 6.5b). There was a distinct area where the flux of Mn to the DGT was 6 ng $cm^{-2}day^{-1}$ higher than along the root axis.

High resolution DGT analyses showed evidence of depletion along the axis of the lupin root, with a hotspot of mobilisation identified near the root tip at the lowest lime treatment (Figure 6.6). There was a distinct area where the flux of Al to the DGT was approximately 2000 ng cm^{-2} day^{-1} greater than the centre of the root (Figure 6.7A). The average flux of Al bound to the DGT along the lupin root axis was approximately 1000 ng cm^{-2} day^{-1} and as the distance from the root increased, the average flux of Al bound to the gel increased to approximately 2000 ng cm^{-2} day^{-1} (Figure 6.7B).

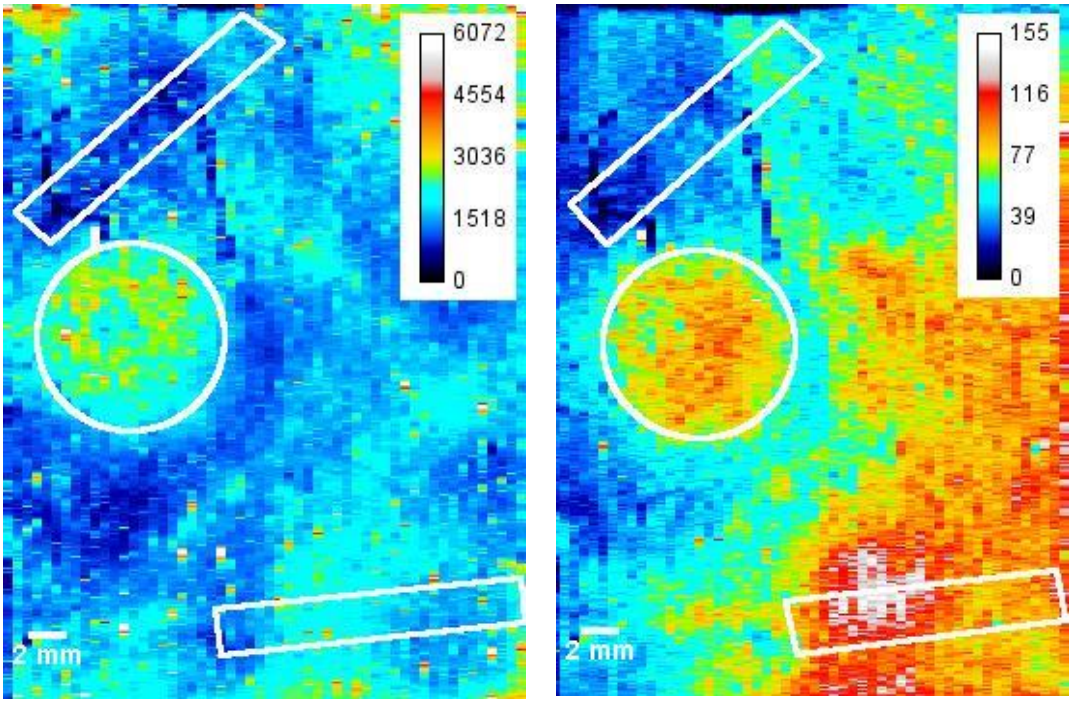

Figure 6.5 Al (a) and Mn (b) fluxes (ng cm^{-2} day^{-1}) from the LP-0.5 soil to the DGT gels. White rectangles are aligned along the root axis and indicate the locations used for root analysis. The white circle denotes the general location where an Al and Mn hotspot was identified.

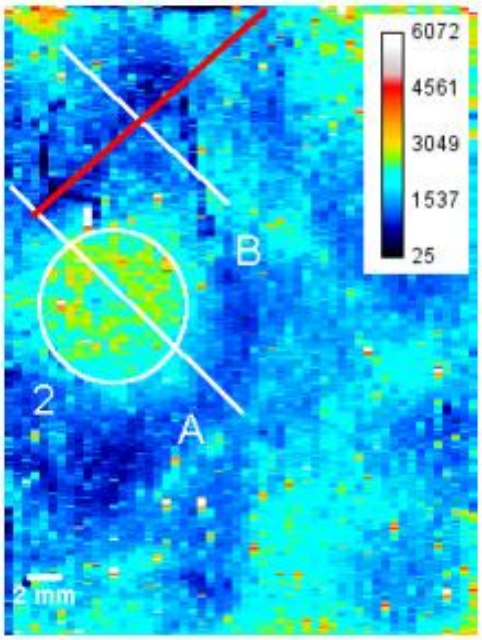

Figure 6.6 LP-0.5 Al relative metal mobilisation (ng cm^{-2} day^{-1}) along transect lines (A and B) and a hot spot (2) onto the DGT gels. The red line shows the approximate location of the plant root.

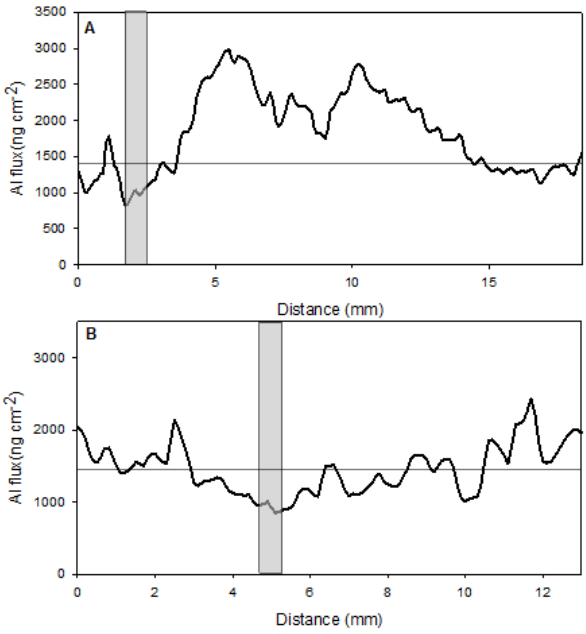

Figure 6.7 Two profiles of Al supply on the DGT gel (ng cm^{-2} day^{-1}) in proximity to the root for two transect lines, A and B for LP-0.5. The grey rectangles show the approximate location of the plant root. The grey horizontal line indicates the bulk average Al flux. The hotspot in profile A is located between 4 and 14 mm.

The average flux of Al from the soil onto the DGT in the LP-1 treatment was 1405 ng cm^{-2} day^{-1} (Appendix Figure 4.1a). There was a distinct area in the centre of the gel, located along the root, in which the flux of Al bound was approximately 1125 ng $cm^{-2}day^{-1}$ lower than along the root axis. There was a distinct area where the flux of Al to the DGT was 400 ng $cm^{-2}day^{-1}$ higher than the bulk soil. The average flux of Mn over the 24 hour deployment onto the DGT (LP-1) across the analysed area was 41 ng $cm^{-2}day^{-1}$ (Appendix Figure 4.1b). However, at the location on the gel that the average flux of Al bound was lower than the background, the average flux of Mn bound was at background levels and showed no depletion. There was a distinct area where the flux of Mn to the DGT was 9 ng cm^{-2} day^{-1} higher than the background.

The average flux of Al onto the DGT gel across the analysed area in the LP-2 treatment was 395 ng cm^{-2} day^{-1}. Along the lupin root axis the average flux of Al bound was approximately 780 ng $cm^{-2}day^{-1}$ (Figure 6.8a). The mass of Mn bound by the DGT resin gel across the analysed area in the LP-2 treatment was 19 ng $cm^{-2}day^{-1}$ (Figure 6.8b).

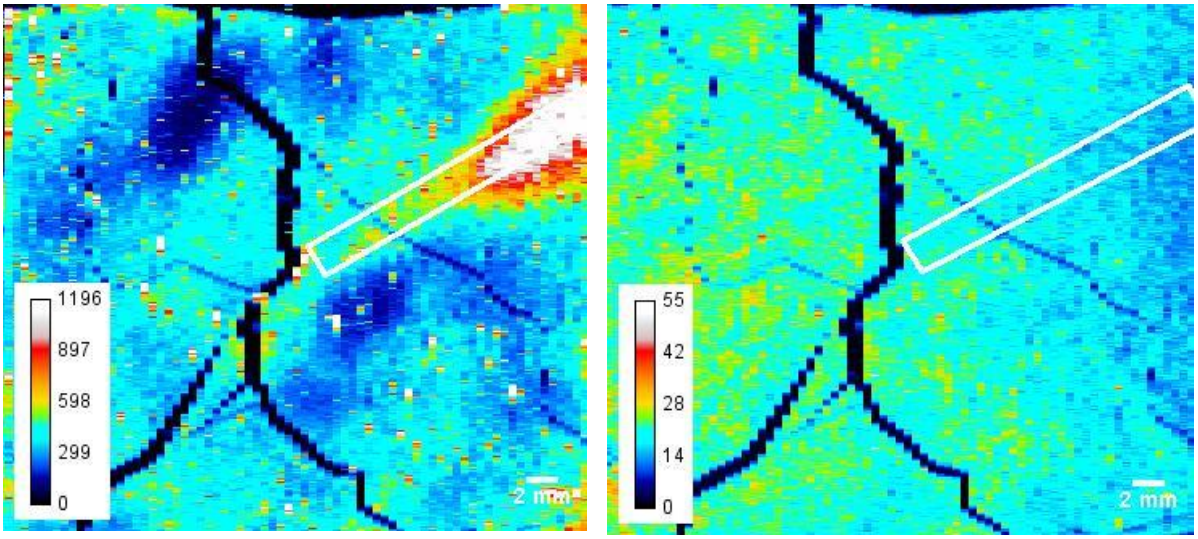

Figure 6.8 Al (a) and Mn (b) fluxes (ng cm^{-2} day^{-1}) from the LP-2 soil to the DGT gels. White rectangles are aligned along the root axis and indicate the locations used for root analysis. The black line running through the image is a crack in the gel caused by the prolonged dry conditions in the laser ablation chamber.

The average flux of Al into the LP- 4- DGT gel along the root axis was 0.34 ng cm^{-2} day^{-1} which was 0.61 ng cm^{-2} day^{-1} lower than the background Al. There was a distinct area where the flux of Al to the DGT was 0.25 ng cm^{-2} day^{-1} higher than the background soil. However, the average flux of Al into the DGT in that area was similar to the flux of Al bound along the root axis (Appendix Figure 4.2a). There was a distinct area where the flux of Mn to the LP- 4- DGT was 0.07 ng cm^{-2} day^{-1} higher than the background soil (Appendix Figure 4.2b).

6.3.7.3 Lucerne

Along the root axis, there was an increase in the average flux of Al bound to the LU-0.5 DGT gel; 1000 ng cm^{-2} day^{-1} higher than the bulk soil (Figure 6.9). There was a decrease in the average flux of Al bound with an increase in the distance from the plant root. There was uncertainty around the variability of the flux and range of Al concentration, given the errors (Table 6.5).

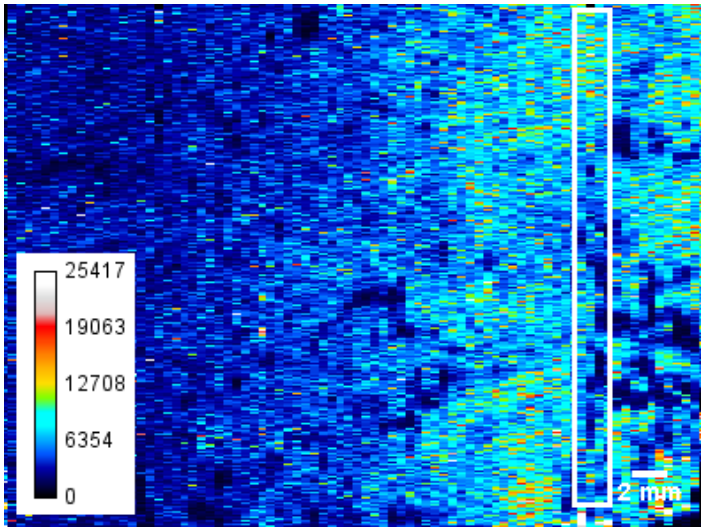

Figure 6.9 Al mobilisation (ng cm^{-2} day^{-1}) from the LU-0.5 soil to the DGT gel. White rectangles are aligned along the root axis and indicate the locations used for root analysis.

The average flux of Al into the DGT gel in the LU-1 treatment along the lucerne root axis was approximately 100 ng cm^{-2} day^{-1} lower than from the bulk soil 389 ng cm^{-2} day^{-1} (Figure 6.10a). The average flux of Mn into the DGT across the area analysed in the LU-1 treatment was similar to the Mn bound along the root axis (Figure 6.10b, Table 6.6).

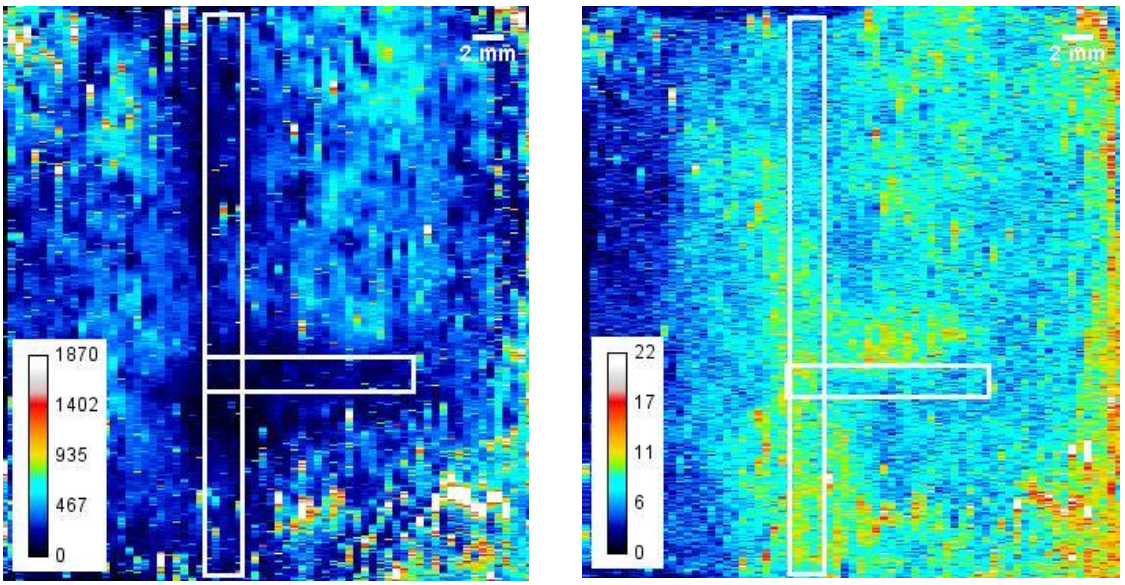

Figure 6.10 Al (a) and Mn (b) mobilisation (ng cm^{-2} day^{-1}) from the LU-1 soil to the DGT gel. White rectangles are aligned along the root axis and indicate the locations used for root analysis.

The average flux of Al bound to the DGT gel, for the LU-1 treatment, along the root axis was approximately 200 ng cm^{-2} day^{-1} (Figure 6.11 and Figure 6.12A). Close to the plant root, the average Al flux bound was approximately 100 ng cm^{-2} day^{-1} and the average flux of Al bound increased to

600 ng cm^{-2} day^{-1} with distance from the root axis (an increase of 500 ng cm^{-2} day^{-1} (Figure 6.12B). The average flux of Al close to the root tip was higher, an increase to 600 ng cm^{-2} day^{-1} away from the root axis, indicating mobilisation of Al (Figure 6.12C). However, the data for this treatment was much noisier than for the LP-0.5 gel and therefore is subject to more uncertainty.

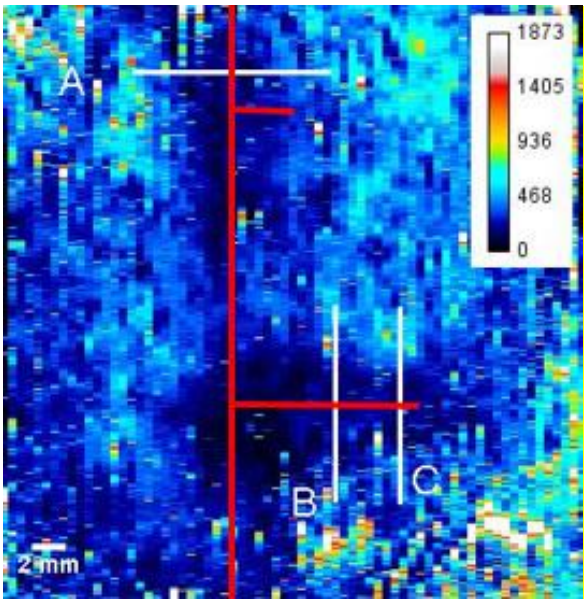

Figure 6.11 LU-1 Al relative metal mobilisation (ng cm^{-2} day^{-1}) along transect lines (A, B and C) onto the DGT gels. The red line shows the approximate location of the plant root.

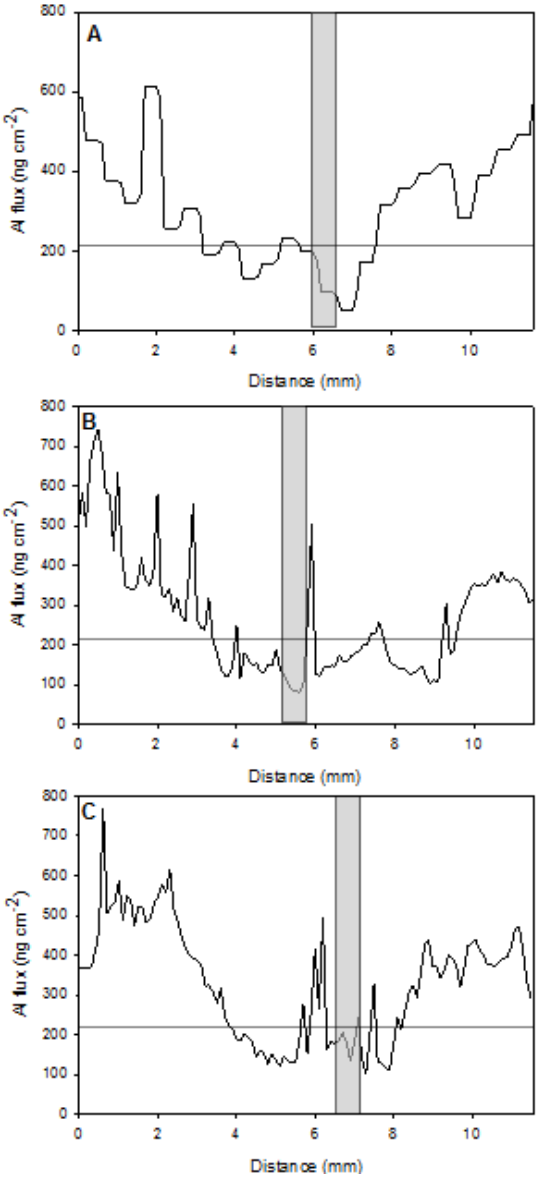

Figure 6.12 Three profiles of Al supply on the DGT gel (ng cm^{-2} day^{-1}) in proximity to the root for three transect lines, A, B and C for LU-1. The grey rectangles show the approximate location of the plant root. The grey horizontal line indicates the bulk average Al flux.

The average flux of Al into the DGT gel across the analysed area in the LU-4 gel treatment was approximately 691 ng $cm^{-2}day^{-1}$. Along the lucerne root axis, the average flux of Al bound was

approximately 1409 ng $cm^{-2}day^{-1}$, indicating an accumulation of Al at the roots and increased mobilisation (Figure 6.13).

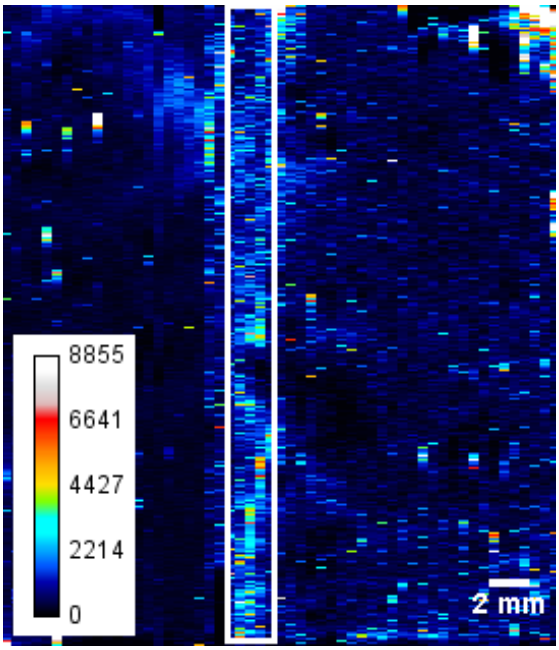

Figure 6.13 LU-4 Al relative metal mobilisation (ng cm^{-2} day^{-1}) onto the DGT gel. White rectangles are aligned along the root axis and indicate the locations used for root analysis

6.4 Discussion

6.4.1 Al biogeochemistry at the root-soil interface

The release of organic acids from plant roots is a well characterised method of exclusion by many plant species, as a tolerance mechanism for Al toxicity. Organic acids are involved in nutrient acquisition, in particular, P (Raghothama & Karthikeyan, 2005) and the alleviation of Al toxicity through chelating metals. Root exudates, including malate and citrate, chelate Al^{3+} in the rhizosphere, forming non-toxic compounds that are unable to enter the plant root (Kochian *et al.*, 2015). High concentrations of Al in the soil trigger plant root exudation, which can also solubilise P for plant uptake. Concentrations of extractable Al_{CaCl2} measured in this study were high, particularly in the rhizosphere of lupin (Figure 6.2), and could have triggered organic acid release from the roots.

The H^+ ion concentration and the hot water extractable carbon appear to play a central role in determining the extractable Al_{CaCl2} concentrations in the lupin and lucerne rhizospheres. However, the general trends between the two plant species were different. While both plants appear to increase the

amount of soil HWEC next to their roots across the different lime rates (small differences), in the lupin rhizosphere there was a lower pH_{H2O} and an increase in the extractable Al_{CaCl2} concentration, irrespective of the lime rate. In the lucerne rhizosphere, the pH_{H2O} was similar or slightly higher than the bulk soil and the extractable Al_{CaCl2} concentration was lower. It has been well recognised that plants actively influence the surrounding soil, altering the pH in the rhizosphere, which is often significantly different from that of bulk soil (Marschner & Römheld, 1983)**.** Plant roots release protons H^+ or OH^- into the rhizosphere to account for an unbalanced cation-anion uptake at the root- soil interface during nutrient uptake (Riley & Barber, 1969). An increase in H^+ ions during nutrient uptake causes a reduction in soil pH and this is the likely cause of the more acidic rhizosphere soils in close proximity to the plant roots, compared to the overall bulk soil**.** Soil respiration contributes to acidification and the proton budget through the cation-anion balance. Acidification rates are greater in neutral or alkaline soils compared to acidic soils (Van Breemen *et al.*, 1983, 1984). The plant species, variation in the cation: anion uptake, soil N source (NH_4^+ or NO_3^-), N fixation by legumes and diffusion processes determine the extent of differences between the rhizosphere soil and soil further away from the plant roots (Bolan *et al.*, 1991; Kim & Silk, 1999; Marschner, 1995; Marschner & Römheld, 1983; Riley & Barber, 1971).

White lupin (*Lupinus albus L.*) was reported to acidify the rhizosphere soil by 0.2 pH units to pH_{H2O} 4.9 compared to the bulk soil, after 56 days growing in an acidic African soil in rhizoboxes (George *et al.*, 2002). Li *et al.* (1997) also found the rhizosphere soil of lupin plants was more acidic than the bulk soil, however, the difference in soil pH_{H2O} was greater than the previously mentioned study at 2.4 pH units in a Brown forest soil. A similar difference in pH (measured by a pH indicator and an antimony microelectrode) was reported by Dinkelaker *et al.* (1989) of 2.7 pH units in a Calcareous soil (20% $CaCO_3$), however, this difference was only measured in the rhizosphere of cluster roots and the authors found no decrease in the pH where cluster roots were not present. Blossfeld *et al.* (2010) also reported acidification by maize roots (up to 0.7 pH units) isolated to specific areas along the roots. Acidification was restricted to the elongation zone of the plant measured by a pH optode, and thus was likely temporary in their experiment. Petersen and Böttger (1991) found that the secretion of protons from maize roots was the main cause of rhizosphere acidification, while the efflux of organic acids only contributed to 0.2-0.3% of acidification. In contrast, many plant species have been reported to increase the soil pH in the rhizosphere. Barley roots increased the soil pH_{H2O} by 2.0 units in both a clay loam and sandy soil, regardless of the application of basal fertiliser with or without additional sewage sludge (Youssef & Chino, 1989). However, in the same rhizobox experiment it was found that both barley and soybean increase their rhizosphere pH in acidic soils and reduce the pH when grown in alkaline conditions. A similar increase in pH (up to 1.7 and 1.5 units) was reported in the rhizosphere of alpine

penny grass and ryegrass in three soils at pH 6.2, 6.3 and 8.1 (Blossfeld *et al.*, 2010). Unlike the maize in the same soils, the alkalinisation was permanent and not restricted to specific root areas. Van Breemen *et al.* (1983) reported that the acidification rate by oak trees was three times higher in an alkaline soil (Calcareous soil) with a pH of 7-8 compared to an acidic soil with a pH of 3.7-3.9. They noted that the large buffering capacity of Calcareous soils masks the high acidification by the consumption of protons by $CaCO_3$ dissolution. George *et al.* (2002) reported a smaller shift in pH_{H2O}, an increase of 0.4 pH units by Tephrosia plants in an acidic African soil. The acidification of the rhizosphere by lupin roots in this study was less (0.1-0.3) than several of the other studies. However, Dinkelaker *et al.* (1989) and Blossfeld *et al.* (2010) reported that acidification was isolated to specific zones, including the elongation zone and surrounding cluster roots. The measurement of pH in this experiment was an average of the entire rhizosphere, and it is likely that specific zones on the plant roots had a greater effect on the surrounding soil, which was masked by the measure used in this study. Moreover, although roots which appeared to be the early stages of cluster roots were observed, this was uncertain. In particular, the small change in pH_{H2O} measured in the rhizosphere soil (Figure 6.1), has likely contributed to a substantial increase in the extractable Al_{CaCl2} concentration close to the plant roots (Figure 6.2).

A higher concentration of solution Al^{3+} was measured in the rhizosphere of growing oak tree roots compared to the bulk soil in an acidic forest soil grown in rhizoboxes (Göttlein *et al.*, 1999). Aluminium concentrations in soil solution declined with distance from the root. Dissolved organic carbon levels were elevated, but were not able to decrease the Al in the root zone. The authors attributed the increase in Al^{3+} in the rhizosphere to a release when protons exuded by the roots (during nutrient uptake) were buffered by the soil. The uptake of base cations and buffering of root exuded protons or organic acids was reported to affect the solid phase, with an increase in the Al concentration near the plant root (< 6 mm) compared to the bulk soil (20 mm). Göttlein *et al.* (1999) proposed that the common soil analysis of Al^{3+}, which doesn't include rhizosphere processes, may greatly underestimate the Al^{3+} concentration in proximity to plant roots. In contrast, a study by Collignon *et al.* (2012) reported the decrease of Al^{3+} in the rhizosphere of Norway spruce trees in an acidic and Al toxic soil, by complexation with organic compounds released from the roots. In different studies, the ability of organic acids released by the roots to bind Al and reduce toxicity varied. However, it seems that the release of H^+ during nutrient uptake, has the greatest effect on Al concentrations in the rhizosphere through a reduction in pH. Differences in plant species affect the toxicity of Al in the rhizosphere of soils, as some species are able to detoxify Al^{3+}, whilst other species mobilise Al^{3+}. The soil next to the lupin root had a lower pH_{H2O} and higher extractable Al_{CaCl2} concentration. Yields in the lower lime

treatments, however, appeared to be unaffected by Al_{CaCl2}. This could be related to the release of extractable organic carbon in the rhizosphere, which may bind Al^{3+} through ligand associations (chelation), making it unavailable (non-toxic) to plants (Delhaize & Ryan, 1995; Kochian *et al.*, 2015; Ma, 2000; Ryan *et al.*, 2001).

There was a decrease in the soil extractable Al_{CaCl2} concentration close to the lucerne roots, the opposite trend to that observed in the lupin rhizosphere. Moreover, the differences in extractable Al_{CaCl2} concentration between the lucerne rhizosphere and bulk soil were generally smaller than for the lupins. There was a slightly higher pH_{H2O} in the rhizosphere of lucerne, in some treatments, compared to the bulk soil (Figure 6.1), which could be associated with the release of anions rather than cations, as has been reported for other species (Blossfeld *et al.*, 2010; Youssef & Chino, 1989). Studies have reported the release of anions from lucerne roots specifically, particularly under P deficient or acidic conditions (Cheng *et al.*, 2004; Richardson *et al.*, 2009). Another explanation could be that not all of the root is releasing H^+ ions, so the entirety of the rhizosphere soil is not affected by the roots when compared to the bulk. As such, differences in pH_{H2O} and Al_{caCl2} may be masked by the rhizosphere soil not affected by the roots.

The hot water extractable carbon (HWEC) appeared to be similar across the first four lime treatments and increased at the highest lime rate applied. In most cases, the HWEC seemed to consistently be slightly higher in the rhizosphere soil of lupin and lucerne plants. The extractable organic carbon in the rhizobox bulk soils ranged from 845-1187 µg C g^{-1} across the lime sequence. These results were generally within the range of 439-1068 µg C g^{-1} for a hill country Brown Soil, found in a study which measured hot water extractable carbon (organic carbon) for a wide range of New Zealand top soils (Sparling *et al.*, 1998). At the highest lime rate (8 t lime ha^{-1}), extractable organic carbon concentrations in the bulk soil were outside the range reported by Sparling *et al.* (1998). This is likely a result of increased extractable organic carbon solubility with an increase in soil pH through lime additions (Curtin *et al.*, 1998; Curtin *et al.*, 2016; Enrich & Trusty, 1997). Sparling *et al.* (1998) reported a linear relationship between the extractable organic carbon and the microbial biomass (R^2=0.79, P<0.001). In particular, they identified that top soils with <10% organic carbon were more closely related to microbial biomass than total carbon. The MO soil in this study has an organic matter content of 8.5% w/w and a total carbon content of 4.91 (%w/w). This falls within the extractable organic carbon threshold reported by Sparling *et al.* (1998) and, therefore, it could be implied that the soil extractable organic carbon measured in this rhizobox experiment is closely related to the microbial biomass. However, this was not tested and therefore needs to be confirmed. Ghani *et al.* (2003) also found that hot water extractable carbon was positively correlated with microbial biomass on pre-established trial

sites on Allophanic soils in both dairy and sheep/beef pastures. However, their ranges of extractable organic carbon measured for dairy farms (1786-4304 μg C g^{-1}) and sheep/beef (2139-5093 μg C g^{-1}) far exceeded the extractable organic carbon measured in the rhizobox experiment, both in the rhizosphere and bulk soil. This is likely attributed to different inputs into the system which affect the cycling of carbon and the different properties of an Allophanic soils.

Both lupin and lucerne plants, appeared to slightly increase the amount of extractable organic carbon next to their roots. In many treatments, the extractable organic carbon was higher than the maximum of the range reported for a similar soil (Sparling *et al.*, 1998), likely a result of the influence of both plant roots and lime application. Their study was conducted on bulk soils, whereas in the finer resolution rhizobox study, many processes occur in the rhizosphere which could affect extractable organic carbon, especially as rhizospheres are often characterised by high microbial activity. The higher concentration in the rhizosphere compared to bulk soil, generally with a lower soil pH, implies that processes in the rhizosphere have increased the extractable organic carbon present. Rhizodeposition is defined as the total amount of organic carbon released from plant roots and accounts for close to 17% of the carbon fixed by the plant during photosynthesis (Nguyen, 2003; Warembourg, 1997). The rhizodeposits include active exudates (e.g. enzymes and mucilage), passively leaked compounds from intact cells (e.g. sugars, organic acids) and the root debris released by cell and root decay (Rovira *et al.*, 1978; Shaw & Burns, 2005). Living roots release organic carbon into the rhizosphere, increasing microbial activity that stimulates the mineralisation of carbon and nitrogen, having a 'priming effect' on the soil (Zhu *et al.*, 2014)**.** Plants also have the ability to solubilise C already in the soil through root exudation, by disrupting organic compounds from associations with minerals in the soil e.g. Al/ Fe (Keiluweit *et al.*, 2015). A study by Egle *et al.* (2003) of two blue lupin cultivars grown in quartz sand, reported the release of organic acids (citric, malic and succinic acids) into the rhizosphere and increased rates of citrate and malate exudation by one cultivar under P deficient conditions. The authors reported the exudation rate of carboxylate anions (citrate and malate) were around ten times higher than white lupin (Egle *et al.*, 2003). Other studies have reported the release of organic acids, including malic and citric acids, from lupin cluster roots under P deficiency conditions, which contributes to acidification of the rhizosphere (Dinkelaker *et al.*, 1989; Li *et al.*, 1997; Neumann *et al.*, 1999). The release of organic acids from lupin roots are often from cluster roots, particularly for white lupin (Yan *et al.*, 2002). However, the study by Egle *et al.* (2003) found no cluster roots on blue lupin plants after 21 days grown in quartz sand and still measured organic acid exudation from the roots. This suggests that the lupin in our experiment, even if the roots observed were not in fact the early

stages of cluster roots, may have increased the extractable organic carbon measured in their rhizosphere through root exudation.

An increase in soil pH_{H2O} through lime additions can reduce the bioavailability of nutrients including P, Mn, Fe, Cu and Zn (Bolan *et al.*, 2003; Marschner, 1995; Naidu *et al.*, 1990). A decline in nutrient availability may have triggered the release of organic compounds from the roots to mobilise nutrients in the soil required for growth. A decline in P availability occurs above pH of 6.0, because of the formation of $CaPO_3^-$, due to P ions readily precipitating with metal cations, in the highest lime rates applied (Haynes, 1982; Larsen *et al.*, 1965; Lindsay *et al.*, 1989). In particular, the treatment with no basal P applied, would be most P limited. In the lupin rhizosphere, the extractable organic carbon was 15% higher in the 4 t lime ha^{-1} with no basal P and S (LP-4-) treatment compared to the treatment with the same lime rate and basal nutrients applied. It is possible that the Russell lupin plant has increased rhizodeposition (release of organic acids) as a strategy to mobilise P in the soil, under low P conditions, a mechanism that has been reported for white lupin (Neumann *et al.*, 2000; Yan *et al.*, 2002). This concept is supported by the higher shoot concentrations of Mo, P, Mn and Fe in the LP-4- plant (Table 6.3), which indicates that mobilisation and uptake has occurred through increased root exudation. Moreover, the higher Al concentration in the herbage of LP-4- plant compared to the LP-4 treatment, indicates that the plant is trying to actively mobilize P from the soil and in the process, the Al concentrations increased. However, the Al uptake was similar between the 4 and 4- treatments, as was the Al_{CaCl2} in the rhizosphere soil.

There was a different relationship between the extractable organic carbon and extractable Al_{CaCl2} for the lupin and lucerne soils. The higher extractable organic carbon levels in the rhizosphere soils of lupins seem to relate to the increase in $Al_{CaCl2,}$ however, there was an overall decrease in Al_{CaCl2} in the rhizosphere of lucerne plants. There are several possible reasons for this; i) that exudates from lucerne decrease Al_{CaCl2}, ii) Al uptake is taking place or iii) the effect is related to small plants. The lucerne plants produced smaller plant yields and root systems across the different treatments, therefore, they may have produced less extractable organic carbon from photosynthesis and nutrient acquisition.

One theory is that the high concentrations of extractable organic carbon released by the lupin roots (Figure 6.3), may include the release of organic ligands which may bind Al and make it unavailable to plants, however, it is still soluble. Therefore, the soluble Al^{3+} may be present at high concentrations in the rhizosphere (measured in the Al_{CaCl2}) but not toxic to lupin through organic ligand associations (Figure 6.2). However, organic ligands were not measured in this experiment. The second, is that lupin

plants are hardy and able to grow in higher Al environments. Lucerne plants are more sensitive to Al and had less exudation of extractable organic carbon, therefore the effects of toxicity are more pronounced (Berenji *et al.*, 2017; Moir & Moot, 2010).

6.4.2 Mobilisation of metals by plants at the root-soil interface

The results of this experiment show the ability of lupin and lucerne to influence the chemistry of the soil in their rhizosphere. In the lupin rhizosphere, the decreased pH_{H2O} is related to the mobilisation of Al in the soil in the roots' proximity (Figure 6.1 and Figure 6.2). The lupin plants seemed to have more of an effect on the rhizosphere soil compared to the lucerne; however, this may be partially linked to the larger biomass production and water transport to the lupin shoots across the different treatments. The difference in total yield (shoot and root) between LP and LU ranges from 0.5 g, at the highest lime rate, to 4.0 g at the lowest lime rate (Figure 6.4). The lupin plants had higher shoot and root yields and therefore, more root to soil contact (proportional effect), which affects nutrient acquisition and mobilisation. These results are supported by the high-resolution (DGT) analysis that showed an Al depletion zone along short segments of the root axis, indicative of previous removal of the metal by the plant at this location (Figure 6.5a). Conversely, there was a higher concentration of extractable Al_{CaCl2} in the rhizosphere soil of lupin plants compared to the bulk soil. However, these concentrations represent the rhizosphere for the whole root system, compared to the DGT measurement, which is a point measurement of a specific section of the root and rhizosphere. The hotspot at the root tip is indicative of Al mobilisation by the root at the location where most metal uptake occurs in growing plants. The Al flux profiles (Figure 6.7A and B) indicate that there has been mobilisation of Al near the root tip, shown by depletion zones near the root axis of A and B. This is supported by the visual record of the root tip travelling along the axis and the high Al uptake data by this plant (Table 6.3).

The form of Al that is measured by the DGT is the positively charged Al (cation binding resin) and is determined by the pH_{H2O} of the soil solution. At this pH_{H2O}, the most mobile Al species available will be preferentially bound (Figure 2.3). The soil pH_{H2O} of the rhizosphere and bulk soils ranged from 4.8 to 7.3. At a pH of <5.5, the Al^{3+} and $Al(OH)^{2+}$ ions are most active, with Al^{3+} the most dominant species (Kinraide, 1991), which is most likely bound. Panther *et al.* (2012) found that different dissolved species of Al in water samples dominated during Chelex-DGT measurement, which was determined by pH (pH 5.05, Al^{3+} and $Al(OH)^{2+}$ were dominant). At high pH (pH≥7.7) they found that $Al(OH)_4^-$ was most dominant and there was a decrease in the accumulated mass of Al over time, suggesting that Chelex-DGT is not suitable for Al measurement at high pH (Panther *et al.*, 2012).

There was both a hotspot of Al mobilisation (Figure 6.5a) and Mn mobilisation (Figure 6.5b) near the lupin root on the LP-0.5 gel at the lowest lime rate. Other studies have also reported hotspots of localised mobilisation of specific metals at different locations along the roots. Valentinuzzi *et al.* (2015) found hotspots of Mn mobilised near cluster roots of lupin and reduced mobilisation with increased lime application. Similar findings were observed in this rhizobox experiment. Hoefer *et al.* (2015) also reported Mn hot spots at different locations along willow roots and not consistently across the whole rhizosphere.

Clear zones of lower Al availability to the DGT were identified around the roots of lupin and lucerne compared to the bulk soil (Figure 6.6 and Figure 6.11). This may be indicative of removal by plants. The extent of depletion at the root axis, compared to the bulk soils, decreased with higher lime rates. Other studies have previously reported distinct zones of metal depletion around plant roots with increased uptake. Hoefer *et al.* (2015) reported a depletion zone of Mn surrounding willow roots, while Santner *et al.* (2012) found root zones of P depletion in the rhizosphere of *Brassica napus* roots compared to the bulk soil. An increase in P with increased distance away from the plant root was also reported. Similar findings were found in profiles in proximity to lupin and lucerne roots (Figure 6.7 A and B; and Figure 6.12 A, B and C). Al increased with distance away from the plant root. The absence of observed Al mobilisation at the higher lime rates in this experiment, may be due to suppression of the mobilisation mechanisms by the lime. The difference between the root zone and the bulk soil Al declined across the lime sequence and the overall Al supply reduced. It seems that the applied lime is buffering the effect that plants have on the Al in the rhizosphere, reducing the effect of depletion in Al by the root. This is supported by the reduced shoot Al uptake with increased lime applied (Table 6.3), which coincides with the suppression / immobilisation of Al in response to lime. Together with the soil pH_{H2O} and Al_{CaCl2} analyses, it indicates that it may be linked to localized acidification of the rhizosphere.

The soil Al and pH_{H2O} measurements for the rhizosphere are, in fact, an average of the entire rhizosphere, which includes areas with and without elongation zones. The rhizosphere soil tests are averages of the entire root system. Even though this measure is probably more representative of the root zone compared to the bulk soil, it is likely to mask the effects which occur at different locations along the root system. This is an effect that is able to be determined by DGT and laser ablation analysis (Figure 6.5 to Figure 6.13 and appendix Figures 4.1 and 4.2).

6.4.3 The effect of lime on plant biomass

Negative effects of lime application on the biomass of lupin were seen between the 0.5 t lime ha^{-1} and the 1 t lime ha^{-1}, as well as the 4 t lime ha^{-1} and the 8 t lime ha^{-1} treatments. The highest yielding lupin plant was in the lowest lime treatment, despite being deficient in Mo, having potentially toxic Mn concentrations in the shoots, P deficiency and high extractable Al_{CaCl2} concentrations in the rhizosphere. For part of the experiment the LP-0.5 soil was consistently drier than the mean of the other lupin and lucerne rhizobox soils. However, this did not seem to affect the shoot yield of this plant or the other soil conditions, pH_{H2O}, extractable Al_{CaCl2} and extractable organic carbon, which were consistent with other lupin plant treatments. The lupin roots seemed to be mobilising Al in the rhizosphere to concentrations higher than the bulk soil, while maintaining consistent yields. There have been no Al toxicity threshold values reported in the literature for lupin. This experiment supports other studies which have reported that lupin grow well in acidic soils and tolerate high concentrations of soluble Al (Davis, 1981; Scott, 1989; Scott & Covacevich, 1987; Scott *et al.*, 1995). Lime application increased the lucerne yields, which peaked at 1 t lime ha^{-1} and 2 t lime ha^{-1} and declined with further lime applied (Figure 6.4). The yield increase corresponded to a pH_{H2O} shift from 4.8 to 5.7, which reduced the rhizosphere Al_{CaCl2} to below toxic concentrations (Moir *et al.*, 2016). The DGT results for the 0.5 t lime ha^{-1} treatment showed increased mobilisation around the plant roots (Figure 6.9) compared to the bulk soil. Berenji (2015) found that lime application at 0.5 t lime ha^{-1} resulted in a 10 fold increase in lucerne yield above the control treatment, which was attributed to a reduction in soil Al_{CaCl2} concentration to below toxic levels. The success of increasing lucerne yields with lime application, which is a particularly sensitive legume to acidity and high extractable Al concentrations, has been reported in numerous studies in the literature (Caradus *et al.*, 2001; Grewal & Williams, 2003; Helyar & Anderson, 1971; Li *et al.*, 1997; Moir & Moot, 2010; Mullen *et al.*, 2006). Even though plants were not grown under a natural system, lucerne yields broadly agreed with those from glasshouse experiment (Chapter 5) on the MO soil. Lucerne yields increased with lime applied to a peak at 4 t lime ha^{-1} (seven times higher than the control) and declined at higher lime rates. However, the yields were higher in the glasshouse experiment and lucerne yields peaked at a higher lime rate than in the rhizobox experiment. The lucerne yields in the rhizobox experiment were relatively low, and although yields increased with lime applied, the increase was not as substantial as has been reported in other studies. This may be due to the use of young plants, single plants grown in each treatment, and restrictions to plant growth by the rhizobox apparatus.

6.5 Conclusions

This experiment shows the influence that actively growing lupin and lucerne have on the rhizosphere soil, compared to the bulk soil, over a lime application sequence.

- The pH_{H2O} was more acidic and Al_{Cacl2} concentrations were higher in the rhizosphere compared to the bulk soil for lupins only. This did not apply to lucerne.
- This is the first study that has shown, in high resolution, distinct patterns of soil Al mobilisation, induced by the roots of important pasture species.
- DGT data showed increased mobilisation of Al at the root tip and depletion along the root axis, indicative of the root progressively removing Al from the soil as the root grew. There was an increase in Al with increased distance from the plant root.
- Al flux can be effectively measured in proximity to plant roots using DGT and LA-ICP-MS analysis.
- The extractable organic carbon levels were consistently higher in the rhizosphere and seemed to increase at the highest lime application.
- A limitation of this study was that the specific organic acids released by plant roots were unable to be identified, which would have been a useful measure to relate to the extractable Al concentrations in the soil and potential for remediation of toxicity.
- The measurement of specific organic acids would be advised in future experiments.
- Due to lack of replication, these results need to be confirmed in further experiments.
- This experiment was unable to assess the effects of moisture in addition to soil pH_{H2O} on the rhizosphere, therefore this is an aspect which could be investigated in future studies.

7 A laboratory investigation of the soil aluminium test on contrasting New Zealand soils

7.1 Introduction

Al^{3+} has been recognised as the most plant-toxic Al form, with others being less toxic (Manoharan *et al.*, 1996a). The primary site of Al toxicity is the root zone of plants, and as a result, it can be difficult to diagnose Al toxicity by visual observation of plants. In order to determine soil Al concentrations, farmers sample their soils and send them for analysis at commercial laboratories. Managing extractable Al concentrations in the soil requires both the appropriate measure of Al and interpretation (Venter, 2016).

It is important to have a soil test that measures the bio-available form of Al, which is toxic to plants, as if other forms are measured, it can give a misrepresentative measure of the Al toxicity for the soil. Pioneering studies for soil Al testing conducted by Hoyt and Nyborg (1971; 1972) compared the amounts of Al extracted from a range of Canadian soils by $CaCl_2$ salt solutions and varied both the molarity and the extraction (shake) time. The soil Al measurements were correlated with the plant yield data to determine which test measured Al concentrations that best related to plant toxicity in the soil. They concluded that the 0.02 M $CaCl_2$ and 60 minute extraction time gave the best indication of plant available Al on the 40 Canadian acid soils, which correlated best to the yield and uptake response of barley, turnip rape and lucerne, compared to lower concentrations of $CaCl_2$. The 1:2 ratio was selected instead of wider ratios to give more concentrated extracts of Al and thus allow a better detection limit (Hoyt & Nyborg, 1972). The commercial test currently used in New Zealand for soil extractable Al is based on the findings of Hoyt and Nyborg (1972) on Canadian soils. The 0.02 M $CaCl_2$ extraction gives an indication of plant available Al (in soil solution) and is a favoured method for distinguishing Al toxicity (Edmeades *et al.*, 1983). The $CaCl_2$ test, which replaced the KCl method in New Zealand in 2010, has a lower ionic strength and only extracts a small amount (5%) of the Al compared to the KCl test (Venter, 2016, 2017a). KCl removes most of the exchangeable and soluble Al, while the $CaCl_2$ removes soluble and very little exchangeable Al (Manoharan, 1997). Several studies have successfully linked the $CaCl_2$ extractable Al to the plant growth response of barley, corn, white clover, sorghum, lucerne and turnip rape on a range of different topsoils and subsoils (Hoyt & Nyborg, 1972; Khalid & Silva, 1979; Shuman *et al.*, 1990; Webber *et al.*, 1982). In addition, the shift to the $CaCl_2$ method for Al extractions in New Zealand was likely due to the methodology of the test, which is simpler to conduct, compared to the KCl extraction method (Shuman, 1990).

The 1 M KCl extraction is an older method (1960s) that is associated with CEC measurements in the soil and was previously used by commercial laboratories in New Zealand (Blakemore *et al.*, 1987). This method is part of the effective cation exchange capacity (ECEC) measurement that determines the amount of exchangeable cations the soil is holding at field pH, which includes the acidic cations (H^+ and Al^{3+}) (Blakemore *et al.*, 1987). The KCl extraction is still used in many parts of the world, including Australia, China, South America, the USA and Canada as a measure of Al toxicity and as an index for lime requirement (Abreu Jr *et al.*, 2003; Amedee & Peech, 1976; Guo *et al.*, 2012; MacLeod & Jackson, 1967; Marques *et al.*, 2002; Schroder *et al.*, 2011; Shuman, 1990; Vendrame *et al.*, 2013; Wang *et al.*, 2016). Forthwith, the extractable Al determined by the two methods shall be referred to as Al_{CaCl2} and Al_{KCl} for the $CaCl_2$ and KCl extractions, respectively. However, the literature has raised questions over the reliability and uncertainty of extractable Al measured by both the $CaCl_2$ and KCl extraction methods. In particular, whether the Al measured by the KCl test is solely exchangeable Al, or if other forms (non-available) are also extracted (Amedee & Peech, 1976; Bache & Sharp, 1976; Lee 1988; Menzies, 2003; Percival *et al.*, 1996). Venter (2016) stated that the $CaCl_2$ method is affected by changes in the ionic strength of soil solution, including soils with a history of fertiliser application, while the KCl test (stronger salt) overwhelms any salts present, giving a more consistent measure of Al. There have been few tests around the uncertainty of the $CaCl_2$ and KCl tests. There is anecdotal evidence which suggests that the uncertainty is higher for the $CaCl_2$ test, (Venter, 2016), however, as yet there are no published studies on this in the literature. The KCl test has been reported in the literature to measure concentrations well above what is available to plants, resulting from the higher molarity (Marques *et al.*, 2002; Percival *et al.*, 1996). Exchangeable aluminium values decline to negligible amounts in soils with a pH_{H2O} above about 5.6 (Moir & Moot, 2010; Rayment & Lyons, 2011b). In the Ashburton Lakes catchment study (Chapter 4), Al_{CaCl2} concentrations have been measured at levels that could be toxic to plants at a soil pH_{H2O} above 5.6. For example, at a soil pH_{H2O} of 5.6-5.8, Al_{CaCl2} ranged from 1.2-16.5 mg kg^{-1}, with concentrations >8.1 mg kg^{-1} measured in the pH_{H2O} 5.6 soils. The Al_{CaCl2} concentrations generally declined with an increase in soil pH_{H2O}, however, Al_{CaCl2} was still measured at concentrations which could be toxic to plants at certain sites in the Ashburton Lakes catchment. Whitley *et al.* (2016) and Singleton *et al.* (1987) have also reported the measurement of concentrations of $CaCl_2$ extractable Al concentrations at potentially toxic levels for sensitive legumes (>3 mg kg^{-1}) at soil pH_{H2O} values of 5.7-5.9 (Moir *et al.,* 2016). These Al concentrations are being measured at soil pH values higher than those at which commercial laboratories recommend testing for potential Al toxicity (Hill Laboratories, 2014). Furthermore, this raises the question of whether the extractable Al concentrations at that soil pH_{H2O} are correct or whether they result as a consequence of the errors associated with the measurement of the current test as highlighted by Venter (2016). Venter (2017b)

suggested that the differences in the Al concentration extracted by the two test methods ($CaCl_2$ and KCl), implies that soil type differences related to weathering probably affect the Al concentrations measured and differences in relation to the critical Al concentrations with respect to pH_{H2O} for the two test methods. There may be specific soil properties which affect the measurement of Al by these two extraction methods. This needs to be investigated further, as this soil test is the current measure that farmers have available to inform them of potential soil Al toxicity on their farms and to assist in land-use decisions.

The objective of this experiment was to determine if changing the molarity and extraction time of the standard $CaCl_2$ and KCl soil Al tests altered the Al concentrations extracted. Results are discussed in terms of how specific soil properties may have affected the quantity of Al extracted. In order to carry out an assessment of the methodology of the standard soil Al tests, a detailed laboratory investigation was conducted on five of the glasshouse soils from Chapter 5. The hypothesis of this experiment was that the concentration of extractable Al from the soil varies with the type of extractant, molarity and extraction time.

7.2 Materials and methods

7.2.1 Soil selection and preparation

Five New Zealand soils were selected from the 10 soils collected for the Glasshouse experiment (Chapter 5). The native soils (no treatments applied) were WT, AR, MO, OM and GF. These soils have been selected for having similar low pH_{H2O} values and large variation in Al_{CaCl2} measured (Table 7.1). These soils include volcanic and sedimentary soils. A full list of chemical analyses and textures of these soils are presented in Tables 5.2 (Chapter 5) and Appendix Tables 3.1 and 3.2. The Ah horizon of a Temuka Gley soil was used as an internal quality control (QC) check for each run (treatment combination) of samples analysed. The soils were air dried and 2 mm sieved in preparation for analysis.

Table 7.1 The soil code, soil order, pH_{H2O} and Al_{CaCl2} concentration of the five soils used in this Laboratory Investigation.

Soil code	Soil order	pH_{H2O}	Al_{CaCl2} (mg kg^{-1})
WT	Allophanic	5.2	5.0
AR	Pumice	5.2	4.8
MO	Brown	5.2	13.1
OM	Recent	5.3	3.8
GF	Brown	5.3	7.1

Note the Al_{CaCl2} concentration is from the standard 0.02 M $CaCl_2$ extraction.

7.2.2 Experimental design

The experimental design is presented in Figure 7.1. The $CaCl_2$ test consisted of three different concentrations of $CaCl_2$: 0.01 M, 0.02 M and 0.05 M and three different extraction (shake) times of 20 minutes, 60 minutes and 180 minutes. There were two replicates of each treatment for each soil and a total of 90 samples for $CaCl_2$ extractions. The KCl test consisted of four concentrations: 0.2 M, 0.5 M, 1 M and 2 M and three different extraction times of 5 minutes, 30 minutes and 60 minutes. There were two replicates of each treatment for each soil and a total of 120 samples for KCl extractions. The concentrations were selected to encompass the standard tests for each extraction, and concentrations higher and lower. The standard test for $CaCl_2$ is 0.02 M and an extraction time of 60 minutes (Hoyt & Nyborg, 1972), while the standard test for KCl is 1 M and an extraction time of 30 minutes (Van Lierop, 1990). These tests are represented by an asterisk in Figure 7.1.

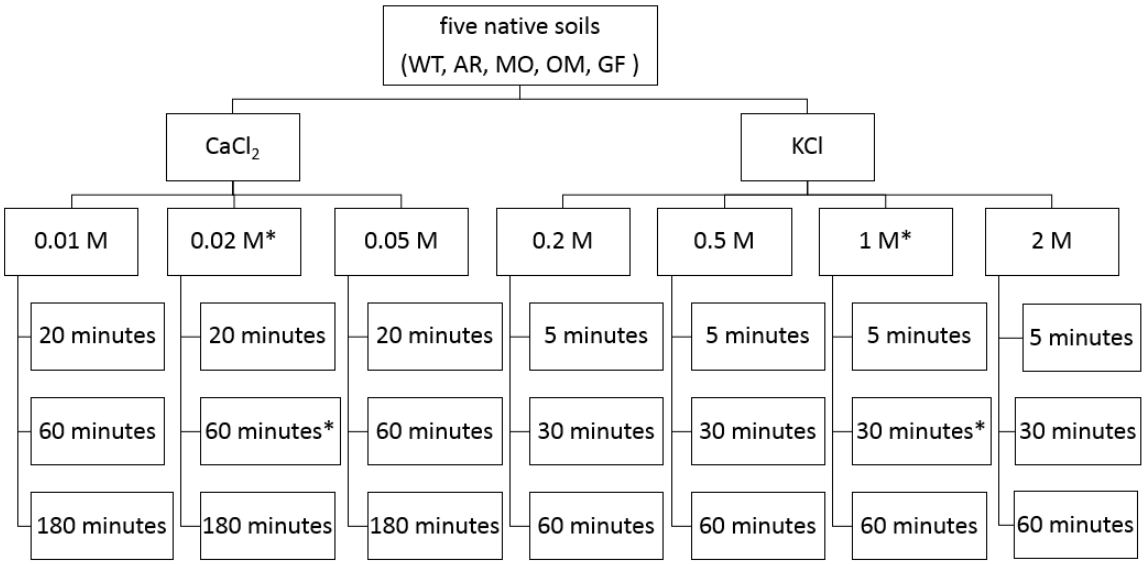

Figure 7.1 The design of the laboratory investigation of the soil aluminium test using both $CaCl_2$ and KCl as extractants. The standard test (reference) for each extractant are shown by an asterisk. Soil acronyms are described in Table 5.1.

7.2.3 Methods of extraction

7.2.3.1 $CaCl_2$ Extraction

Preliminary testing was conducted to compare the results of the standard soil Al test to a commercial laboratory and to establish the most suitable filter paper. Consistent results were achieved but were not essential to the objectives of this chapter and therefore are presented in the Appendix (Sections 5.1 and 5.2).

The $CaCl_2$ method was conducted using three different concentrations of calcium chloride dihydrate ($CaCl_2.2H_2O$); 0.01 M, 0.02 M and 0.05 M. These were selected to encompass the concentration of the current test of 0.02 M, the earlier $CaCl_2$ test, which used the 0.01 M $CaCl_2$ (Hoyt & Nyborg, 1971; Hoyt & Nyborg, 1972), and a molarity higher than the standard concentration. The 1:2 ratio for the $CaCl_2$ was implemented in this experiment, as this is the standard test ratio in the literature (Edmeades *et al.*, 1983; Hoyt & Nyborg, 1972).

10 g (±0.005 g) of soil was weighed into a 50 mL falcon tube and the exact weight of soil used was recorded as accurate to three decimal places. 20 mL of $CaCl_2$ extractant was added to the soil through a bottle top dispenser. The samples were placed in sample racks and shaken on an end- over- end shaker for 20 minutes, 60 minutes or 180 minutes. The standard extraction time for this soil test is 60 minutes (Hoyt & Nyborg, 1972). After shaking, samples were filtered into 30 mL vials which were placed in a stand with funnels in the top (Plate 7.1) holding Whatman 1 filter paper (110 mm; filter pore size 11 µm; Sigma Aldrich, St Louis, Missouri, USA; section Appendix Section 5.1).

Plate 7.1 Filtration of the soil and extraction solution through Whatman 1 filter paper.

The solution was then analysed for Al using ICP-OES analysis (Varian 720-ES ICP-OES; Varian Pty Ltd, Melbourne, Australia). For each run (treatment combination), two replicates of an internal QC check soil were analysed and one blank (solution through filter paper) to monitor the continued accuracy of the methodology. The Al_{CaCl2} concentration (mg kg^{-1}) was calculated using the weight of the soil and the Al concentration from the ICP-OES as per Equation 7.1 (Analytical Research Laboratory, 2014).

Equation 7.1

$$Al_{CaCl2} = \frac{[Al] \times V}{m}$$

Where:

Al_{CaCl2}= extractable Al (mg kg^{-1})

[Al]= Al in the extract, determined by the ICP-OES (mg L^{-1})

V= volume of extractant (mL)

m= mass of soil (g).

7.2.3.2 KCl Extraction

The KCl method was conducted using four different concentrations of potassium chloride (KCl). The concentrations of the KCl salt used were 0.2 M, 0.5 M, 1 M and 2 M. These concentrations were selected to encompass the standard concentration for the KCl test of 1 M (Blakemore *et al.*, 1987; Rayment & Lyons, 2011a; Sims, 1996; Van Lierop, 1990), and higher and lower concentrations as comparisons. The 1:10 ratio for the KCl was implemented in this experiment, as this is the standard test ratio in the literature and used in the past by commercial laboratories in New Zealand (Rayment & Lyons, 2011a; Sims, 1996; Van Lierop, 1990).

2.5 g (±0.005 g) of soil was weighed into a 50 mL falcon tube and the exact weight of soil used was recorded as accurate to three decimal places. 25 mL of KCl extractant was added to the soil through a bottle top dispenser. The samples were placed in sample racks and shaken on an end- over- end shaker for 5 minutes, 30 minutes or 60 minutes. The standard extraction time for this soil test is 30 minutes (Van Lierop, 1990). After shaking, samples were filtered into 30 mL vials which were placed in a stand with funnels in the top (Plate 7.1) holding Whatman 1 filter paper. For the KCl Al extraction, Blakemore *et al.* (1987) reported using Whatman 42 filter paper, however, the other methods of KCl extraction did not specify the filter paper used. The Whatman 42 filter paper has an Al content of 2 mg L^{-1}, which is too high for running Al tests through this filter paper. Whatman 1 was selected, as it has a lower Al content (<0.5 µg g^{-1} of paper) and although a medium to fast filter, it produced a clear solution to be run on the ICP-OES (Whatman, 2007). Using the Whatman 1 paper maintained consistency between the two extraction methods ($CaCl_2$ and KCl), which both used Whatman 1, and reduced the potential contamination of samples from the filter paper. The solution was then analysed on the ICP-OES.

For each run (treatment combination), there were two replicates of an internal QC control soil were analysed and one blank (solution through filter paper) to ensure the continued accuracy of the

methodology. In addition, a primary reference soil from the Australasian Soil and Plant Analysis Council (ASPAC) was used. This soil has been measured for KCl Al by the traditional method of 1 M KCl, 1:10 and a 30 minute extraction time used by multiple laboratories around the world. This soil was measured for the 1 M KCl treatments at all three extraction times in this experiment. The Al_{KCl} concentrations are presented in $cmol_c/kg$ and were calculated using a modified equation from Blakemore *et al.* (1987) due to the different soil: solution ratio.

Equation 7.2

$$Al_{KCl} = \frac{[Al] \times z \times V}{(MwAl \times m \times 10)}$$

Where:

Al_{KCl}= extractable Al concentration ($cmol_c/kg$).

[Al]= Al in the extract, determined by the ICP-OES ($mg\ L^{-1}$).

z= charge on the Al^{3+} ion

V= volume of extractant (mL).

MwAl= Al molecular weight

10= conversion from 1000 g to 100 g.

m= mass of soil (g).

7.2.4 Statistical Analysis

The $CaCl_2$ ($mg\ kg^{-1}$) and KCl ($cmol_c/kg$) datasets were $\log_{10}$ transformed in order to achieve a normal distribution to satisfy the assumptions for analysis of variance (ANOVA). The statistical analysis of the $CaCl_2$ and the KCl datasets were conducted separately, as they are different tests with different ratios of soil to extractant. A general ANOVA was conducted in 'Genstat 16.0' (VSN International) for $CaCl_2$ and KCl separately, to determine differences in the soil Al concentration between the soils for molarities and extraction times for each extractant. Results indicated that soil was a significant factor, both as a main effect and in interactions, affecting Al concentrations for both $CaCl_2$ and KCl extractions. In order to investigate the effects of molarity and extraction time on individual soils, the five soils were analysed individually using a two-way ANOVA for the $CaCl_2$ and KCl datasets. The log soil Al concentration was the response variable and molarity and extraction time were the predictor variables. The interaction of extraction time and molarity was also included. The values presented in Table 7.2-Table 7.5 are the back-transformed mean Al concentrations. The significance level (*P* values) represent the results from the ANOVA on the log transformed data. The standard error of the mean

(SEM), upper and lower, were calculated using the general mean and standard error from the ANOVA outputs on the log transformed data. The SEM were also back transformed which is why the error bars are asymmetric, both in the tables and the graphs. The standard deviation (SD) upper and lower for each mean Al concentration were back-transformed and presented in Appendix Tables 5.1 and 5.2. For the significant results, a Fisher's protected LSD 5% pairwise comparison was conducted between the means to identify differences (α =0.05). A *P* value of >0.05 was regarded as statistically non-significant in this experiment. The amount of Al extracted (mg kg^{-1}) was compared between the two extractants, $CaCl_2$ and KCl, for the standard tests using a one-way ANOVA. The extractable Al was log transformed for analysis, as per the previous analyses, to achieve normal distribution.

7.3 Results

7.3.1 $CaCl_2$ soil Al extraction

7.3.1.1 Overall treatment effects

The Al_{CaCl2} concentration differed (*P*<0.001) among soils and molarities (*P*<0.001) in this experiment. However, there was no difference (*P*>0.05) in the Al_{CaCl2} concentrations measured across the three extraction times (Table 7.2).

7.3.1.1.1 Soils

The Al_{CaCl2} concentrations ranged from 4.3 mg kg^{-1} from the WT soil to 14.7 mg kg^{-1} for the MO soil. The MO soil measured higher (*P*<0.05) Al_{CaCl2} concentrations than all other soils. There was no difference (*P*>0.05) in the mean Al_{CaCl2} concentration between the AR and OM soils, according to Fisher's protected LSD (Table 7.2). The mean Al_{CaCl2} concentration of the MO soil was more than triple that of the WT soil. The Al_{CaCl2} concentration was affected (*P*<0.01) by the interaction of soil and molarity and affected (*P*<0.05) by the interaction of soil and extraction time. The Al_{CaCl2} concentration was affected (*P*<0.01) by the three way interaction of soil, molarity and extraction time (Table 7.2). This analysis confirmed that the Al_{CaCl2} concentrations differed among soils. These treatment effects are examined in more detail for individual soils in section 7.3.1.2.

7.3.1.1.2 Molarity

The Al_{CaCl2} concentration increased (*P*<0.001) with an increase in the molarity of $CaCl_2$. The mean Al_{CaCl2} extracted ranged from 3.4 mg kg^{-1} to 15.2 mg kg^{-1} for the 0.01 M and 0.05 M $CaCl_2$ respectively. However, the Al_{CaCl2} concentration was not affected (*P*>0.05) by the interaction of molarity and extraction time (Table 7.2).

Table 7.2 Back-transformed mean Al_{CaCl2} concentrations (mg kg^{-1}) extracted by $CaCl_2$ for individual soils, WT, AR, MO, OM and GF with increasing molarity (3 levels of molarity; ranging from 0.01-0.05 M) and increasing extraction time (3 levels; ranging from 20-180 minutes). Soil acronyms are described in Table 5.1.

		Mean Al_{CaCl2} (mg kg^{-1})
soils		
	WT	4.3 $_{d}$
	AR	6.5 $_{c}$
	MO	14.7 $_{a}$
	OM	5.8 $_{c}$
	GF	10.5 $_{b}$
	SEM (upper/lower)	(8.43, 6.86)
molarity (M)		
	0.01	3.4 $_{c}$
	0.02	8.5 $_{b}$
	0.05	15.2 $_{a}$
	SEM (upper/lower)	(8.23, 7.02)
	Grand mean	7.6
***P* values**		
	soil	***
	molarity	***
	extraction time	ns
	soil x molarity	**
	soil x extraction time	*
	molarity x extraction time	ns
	soil x molarity x extraction time	**

*** Significant at $P<0.001$ level, ** significant at $P<0.01$ level, * significant at $P< 0.05$ level, ns- no significant difference. Numbers with letter subscripts in common are not different ($\alpha= 0.05$) based on the Fisher's protected LSD.

7.3.1.2 Treatment effects within Soils

7.3.1.2.1 Molarity

The amount of Al extracted from the WT, AR, OM and GF soils was influenced ($P<0.001$) by the molarity of $CaCl_2$ (Table 7.3). However, there was no difference ($P>0.05$) in the amount of Al extracted from the MO soil with changes in the molarity of $CaCl_2$.

The Al_{CaCl2} concentrations increased with an increase in the molarity of $CaCl_2$. Aluminium concentrations ranged from 1.7 mg kg^{-1} at 0.01 M $CaCl_2$ for the WT soil to a peak Al_{CaCl2} concentration of 22.5 mg kg^{-1} extracted by 0.05 M $CaCl_2$ for the GF soil. The largest difference between the soil Al

extracted by 0.01 M and 0.05 M $CaCl_2$ was 17.1 mg kg^{-1} on the GF soil, while the smallest difference was 8.7 mg kg^{-1} on the AR soil. The soil Al concentration extracted by the 0.05 M $CaCl_2$ was more than four and three times higher than the extractable Al concentration extracted by the 0.01 M $CaCl_2$ on these soils respectively. For the WT, AR and GF soils, the soil Al concentration extracted by the standard test molarity (0.02 M) was different (P<0.05) to Al extracted by the lower (0.01 M) and higher (0.05 M) molarity $CaCl_2$. The OM soil differed, as although the Al_{CaCl2} concentration increased with an increase in molarity, there was no difference (P>0.05) between the 0.01 M and 0.02 M $CaCl_2$.

Table 7.3 Back-transformed mean Al_{CaCl2} concentrations (mg kg^{-1}) extracted by $CaCl_2$ for individual soils, WT, AR, MO, OM and GF with increasing molarity (3 levels of molarity; ranging from 0.01-0.05 M) and increasing extraction time (3 levels; ranging from 20-180 minutes). Soil acronyms are described in Table 5.1.

		Mean Al_{CaCl2} (mg kg^{-1})				
	Soil Type	WT	AR	MO	OM	GF
	Grand mean	4.3	6.5	14.7	5.8	10.5
molarity (M)	0.01	1.7 $_c$	3.6 $_c$	8.8	1.8 $_b$	4.8 $_c$
(mean of the three extraction times, *n* = 6)	0.02	4.0 $_b$	6.4 $_b$	21.0	7.6 $_b$	10.7 $_b$
	0.05	11.8 $_a$	12.3 $_a$	17.1	14.7 $_a$	22.5 $_a$
	SEM (upper/lower)	(4.45,4.23)	(7.00,6.15)	(20.24,10.71)	(7.12,4.73)	(11.64,9.46)
	P value molarity	***	***	ns	***	***
extraction time	20	5.2 $_a$	6.6	8.1	5.0	11.6
(min)	60	4.4 $_b$	6.6	21.0	7.0	9.3
(mean of the three molarities, *n* = 6)	180	3.5 $_c$	6.4	18.7	5.6	10.7
	SEM[@] (upper/lower)	(4.45,4.23)	(7.00,6.15)	(20.24,10.71)	(7.12,4.73)	(11.64,9.46)
	P value extraction time	***	ns	ns	ns	ns
	P value molarity x extraction time	*	ns	*	ns	ns

Note: *** Significant at *P*< 0.001 level, ** significant at *P*<0.01 level, * significant at *P*< 0.05 level, ns- no significant difference. Numbers with letter subscripts in common are not different (α= 0.05) based on the Fisher's protected LSD. The upper and lower SEM are reported for each soil in relation to the grand mean and back-transformed. [@] The SEM (upper and lower) are the same for molarity and extraction time, this is a genuine effect of this dataset. The standard deviation (upper and lower) of the means (replicates) are presented in Appendix Table 5.1. The mean Al concentration for 0.02 M and the 60 minute extraction were the same, this was a genuine result.

7.3.1.2.2 Extraction time

The amount of Al extracted was not affected by extraction time for the AR, MO, OM and GF soils for the $CaCl_2$ extraction (P>0.05). However, the WT soil was different. The Al_{CaCl2} concentration decreased (P<0.001) with an increase in extraction time from 5.2 mg kg^{-1} at the 20 minute extraction time to 3.5 mg kg^{-1} at 180 minutes extraction time (Table 7.3).

7.3.1.2.3 Molarity x Extraction time Interactions

There was no interaction effect of molarity and extraction time on the Al_{CaCl2} concentration for the AR, OM and GF soils (P>0.05). However, the WT and MO soils differed, as there was an interaction effect (P<0.05) of molarity and extraction time on the Al_{CaCl2} concentration (Table 7.3)

The WT soil Al_{CaCl2} concentrations ranged from 1.3 mg kg^{-1} to 12.7 mg kg^{-1} across all molarity and extraction time combinations (Figure 7.2a). Peak Al_{CaCl2} concentrations were measured with 0.05 M $CaCl_2$ across the three extraction times (20, 60 and 180 minutes) at 12.7, 11.8 and 10.9 mg kg^{-1} respectively. The Al_{CaCl2} concentrations were higher (P<0.05) than the extractable Al measured by all other treatment combinations. The Al extracted by 0.02 M and 0.01 M $CaCl_2$ decreased with an increase in extraction time on the WT soil, and were lowest at the treatment combination of 0.01 M and 180 minute extraction time at 1.3 mg kg^{-1}. The standard Al test (0.02 M and a 60 minute extraction time) extracted 4.1 mg kg^{-1} Al, which was higher (P<0.05) than the Al concentrations extracted by 0.02 M $CaCl_2$ at the 180 minute extraction time and 0.01 M $CaCl_2$ across all extraction times.

The MO soil Al_{CaCl2} concentrations ranged from 2.2 mg kg^{-1} (0.05 M and 20 minute extraction) to 50.4 mg kg^{-1} (0.05 M 60 minute extraction) across the treatment combinations (Figure 7.2c). The standard test (0.02 M and 60 minute extraction) measured a mean Al_{CaCl2} concentration of 20.0 mg kg^{-1}, which was no different (P>0.05) to all other treatment combinations except the 0.05 M and 20 minute extraction time, which was lower (P<0.05).

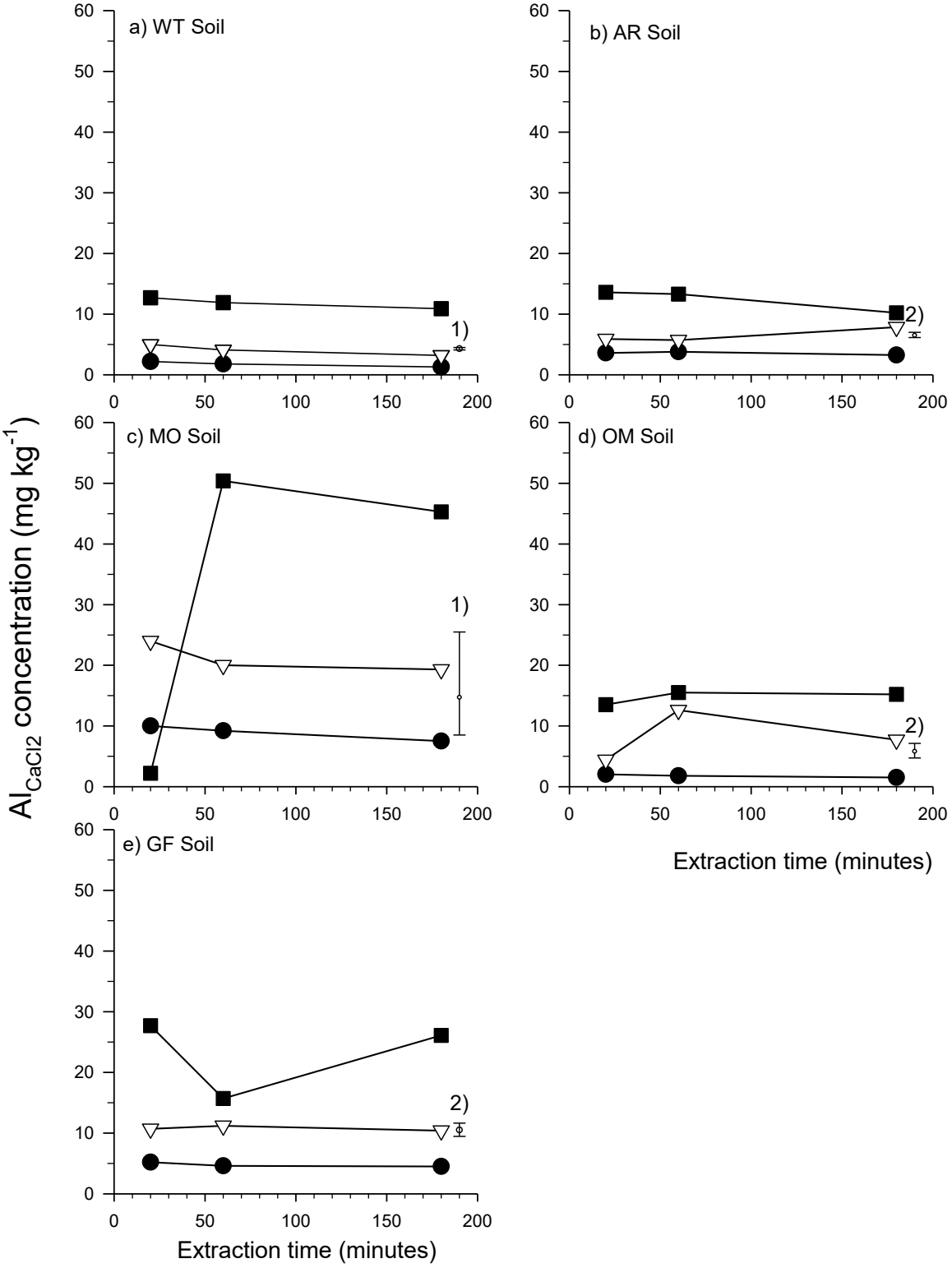

Figure 7.2 Back-transformed mean Al_{CaCl2} concentrations (mg kg^{-1}) across molarities (M) of 0.01 (●), 0.02 (▽) and 0.05 (■) and three extraction times of 20, 60 or 180 minutes on the (a) WT soil, (b) AR soil, (c) MO (d) OM and (e) GF soil. Values are means ± SEM (*upper and lower bounds),* calculated from grand mean are for 1) the interaction of molarity and extraction time and 2) the main effect of molarity, where the interaction was non-significant. At no time was there a main effect of extraction time. Soil acronyms are described in Table 5.1.

7.3.2 KCl soil Al extraction

7.3.2.1 Overall treatment effects

The KCl extractable Al concentration differed (P<0.001) among soils and molarities (P<0.001) in this experiment. There was no difference (P>0.05) in Al_{KCl} measured across the three extraction times (Table 7.4).

7.3.2.1.1 Soils

The KCl extractable concentration differed (P<0.001) among soils (Table 7.4). The mean Al_{KCl} concentration of the MO soil was more than four times higher than the AR soil. The Al_{KCl} concentration was affected (P<0.001) by the interaction of soil and molarity and affected (P<0.05) by the interaction of soil and extraction time. The Al_{KCl} concentration was affected (P<0.01) by the three way interaction of soil, molarity and extraction time (Table 7.4). This analysis demonstrated that the Al_{KCl} concentrations differed among soils. These treatment effects are examined in more detail for individual soils in 7.3.2.2.

7.3.2.1.2 Molarity

The Al_{KCl} concentration increased (P<0.001) with an increase in the molarity of KCl. The mean Al_{KCl} extracted ranged from 0.6 $cmol_c$/kg to 1.5 $cmol_c$/kg for the 0.2 M and 2 M KCl respectively. However, there was no difference (P>0.05) in the mean Al_{CaCl2} concentration between the 1 M and 2 M KCl, according to Fisher's protected LSD (Table 7.4). The soil Al_{Kcl} concentration was affected (P<0.001) by the interaction of molarity and extraction time.

Table 7.4 Back-transformed mean Al_{KCl} concentrations ($cmol_c/kg$) extracted by KCl for individual soils, WT, AR, MO, OM and GF with increasing molarity (4 levels of molarity; ranging from 0.2-2 M) and increasing extraction time (3 levels; ranging from 5-60 minutes). Soil acronyms are described in Table 5.1.

		Mean Al_{KCl} ($cmol_c/kg$)
soils		
	WT	1.0_{b}
	AR	0.4_{d}
	MO	2.2_{a}
	OM	0.7_{c}
	GF	1.9_{a}
	SEM (upper/lower)	(1.07, 0.99)
molarity (M)		
	0.2	0.6_{c}
	0.5	0.9_{b}
	1.0	1.4_{a}
	2.0	1.5_{a}
	SEM (upper/lower)	(1.07, 0.99)
	Grand mean	1.0
***P* values**		
	soil	***
	molarity	***
	extraction time	ns
	soil x molarity	***
	soil x extraction time	*
	molarity x extraction time	***
	soil x molarity x extraction time	**

*** Significant at $P< 0.001$ level, ** significant at $P<0.01$ level, * significant at $P< 0.05$ level, ns- no significant difference. Numbers with letter subscripts in common are not different ($\alpha= 0.05$) based on the Fisher's protected LSD.

7.3.2.2 Treatment effects within Soils

7.3.2.2.1 Molarity

The amount of Al extracted from the WT, AR, MO, OM and GF soils was influenced ($P<0.001$) by the molarity of KCl (Table 7.5). The Al_{KCl} concentrations increased with an increase in the molarity of KCl. Aluminium concentrations ranged from 0.2 $cmol_c/kg$ at 0.2 M KCl for the AR soil to a peak Al_{KCl} concentration of 3.0 $cmol_c/kg$ extracted by 2 M KCl for the MO soil. The largest difference between the Al extracted by 0.2 M and 2 M KCl was 1.5 $cmol_c/kg$ on the MO soil, while the smallest difference was 0.4 $cmol_c/kg$ [1] on the AR soil. The Al_{KCl} concentrations from the WT, AR and OM soils peaked at a molarity of 1 M KCl. A further increase in the molarity of the extractant resulted in no difference ($P>0.05$) in the Al_{KCl}. The Al_{KCl} concentration for the MO soil peaked at 2 M KCl, which was no different

(*P*>0.05) to the standard (1 M KCl) test, and there was no difference (*P*>0.05) between the Al extracted by the standard test or the 0.5 M KCl (Table 7.5). However, there was a difference (*P*<0.05) between the Al extracted by the 0.5 M and 2 M KCl. The GF soil Al concentration extracted by the 2 M KCl was higher (*P*<0.05) than the Al extracted at 1 M KCl (standard test), while there was no difference (*P*>0.05) between the concentration extracted by 0.5 M and 1 M KCl.

Table 7.5 Back-transformed mean Al_{KCl} concentrations ($cmol_c/kg$) extracted by KCl for individual soils, WT, AR, MO, OM and GF with increasing molarity (4 levels of molarity; ranging from 0.2-2 M) and increasing extraction time (3 levels; ranging from 5-60 minutes). Soil acronyms are described in Table 5.1.

		Mean Al_{KCl} ($cmol_c/kg$)				
	Soil Type	WT	AR	MO	OM	GF
	Grand mean	1.0	0.4	2.2	0.7	1.9
molarity (M) (mean of the three extraction times, *n* = 6)	0.2	0.5 $_c$	0.2 $_c$	1.5 $_c$	0.4 $_c$	1.5 $_c$
	0.5	0.9 $_b$	0.3 $_b$	2.0 $_b$	0.6 $_b$	1.8 $_b$
	1.0	1.5 $_a$	0.7 $_a$	2.5 $_{ab}$	1.1 $_a$	2.0 $_b$
	2.0	1.6 $_a$	0.6 $_a$	3.0 $_a$	1.0 $_a$	2.5 $_a$
	SEM (upper/lower)	(1.05,0.96)	(0.40,0.37)	(2.37,1.97)	(0.80,0.63)	(2.00,1.88)
	P value molarity	***	***	***	***	***
extraction time (min) (mean of the four molarities, *n* = 8)	5	1.1 $_a$	0.4	2.3	0.7	1.6 $_b$
	30	1.0 $_{ab}$	0.4	2.0	0.8	2.0 $_a$
	60	0.9 $_b$	0.4	2.2	0.7	2.2 $_a$
	SEM[@] (upper/lower)	(1.05,0.96)	(0.40,0.37)	(2.34,1.99)	(0.80,0.64)	(1.99,1.89)
	P value extraction time	*	ns	ns	ns	***
	P value molarity x extraction time	***	***	*	ns	***

Note: *** Significant at $P<0.001$ level, ** significant at $P<0.01$ level, * significant at $P<0.05$ level, ns- no significant difference. Numbers with letter subscripts in common are not different ($\alpha= 0.05$) based on the Fisher's protected LSD. The upper and lower SEM are reported for each soil in relation to the grand mean and back-transformed. [@] The SEM (upper and lower) are similar for molarity and extraction time, this is a genuine effect of this dataset. The standard deviation of the means (replicates) are presented in Appendix Table 5.2.

7.3.2.2.2 Extraction time

The amount of Al extracted was not affected by extraction time for the AR, MO and OM soils for the KCl extraction (P>0.05). However, the WT and GF soils were different. The WT soil Al_{KCl} concentration decreased (P<0.05) with an increase in extraction time from 1.1 $cmol_c$/kg at the 5 minute extraction time to 0.9 $cmol_c$/kg at 60 minute extraction time (Table 7.5). There was a difference (P<0.05) between the shortest and longest extraction times, however, there was no difference (P>0.05) between the soil Al extracted by the 30 minute (standard) extraction time and the 5 minute extraction, and there was no difference (P>0.05) between the 30 minute extraction and the 60 minute extraction time. The GF soil Al_{KCl} concentration increased (P<0.001) with an increase in extraction time from 1.6 $cmol_c$/kg at a 5 minute extraction to 2.0 $cmol_c$/kg at a 30 minute (standard) extraction time. Further shaking led to no difference (P>0.05) in the soil Al concentration extracted (Table 7.5).

7.3.2.2.3 Molarity x Extraction time Interactions

There was no interaction effect (P>0.05) of molarity and extraction time on the soil Al_{KCl} concentration for the OM soil (Table 7.5, Figure 7.3d). However, for the WT, AR, MO and GF soils, there was a molarity by extraction time interaction. The significance level of the interaction differed among soils, from P<0.05 for the MO soil to P<0.001 for the WT, AR and GF soils (Table 7.5).

The WT soil Al concentration ranged from 0.4 $cmol_c$/kg to 1.8 $cmol_c$/kg (Table 7.5, Figure 7.3a). The standard test, 1 M KCl and a 30 minute extraction time, measured an Al_{KCl} concentration of 1.6 $cmol_c$/kg, which was no different (P>0.05) to the Al extracted by 2 M KCl (all extraction times) and 1M (5 and 60 minute extraction times). The lowest Al_{KCl} concentrations were measured by 0.2 M at all extraction times and 0.5 M at the 60 minute extraction time (ranging from 0.4-0.6 $cmol_c$/kg) on the WT soil.

The AR soil Al_{KCl} concentration ranged from 0.1 $cmol_c$/kg (0.2 M, 5 minute extraction) to 0.8 $cmol_c$/kg (1 M, 5 minute extraction) (Figure 7.3b). The standard 1 M KCl test measured a mean Al concentration of 0.7 $cmol_c$/kg, which was no different (P>0.05) from the Al_{KCl} concentrations measured by 1 M KCl (5 minute extraction) and 2 M KCl (30 and 60 minute extraction times). The lowest concentrations of Al_{KCl} were measured at the 0.2 M (all extraction times) and 0.5 M 60 minute extraction time.

Extractable Al_{KCl} concentrations for the MO soil ranged from 1.2 $cmol_c$/kg to 3.5 $cmol_c$/kg (Figure 7.3c). The highest Al_{KCl} concentrations were measured at 2 M KCl and an extraction time of 60 minutes at

3.5 $cmol_c$/kg. There was no difference (P>0.05) between the Al measured by the standard test (1 M KCl and 30 minute extraction), at 2.0 $cmol_c$/kg and all other treatment combinations except for the 2 M and 60 minute extraction, which was higher (P<0.05).

The GF soil Al_{KCl} concentration ranged from 0.9 $cmol_c$/kg (0.2 M at a 5 minute extraction) to 3.1 $cmol_c$/kg (2 M KCl at a 60 minute extraction) (Figure 7.3e). The mean Al_{KCl} concentration extracted by the standard 1 M KCl test (30 minute extraction) was 2.3 $cmol_c$/kg, which was lower (P<0.05) than the peak Al at 2 M and 60 minute extraction. The standard test extracted higher (P<0.05) soil Al_{KCl} concentrations than the 0.5 M (5 and 30 minute extraction times), 1 M (60 minute extraction) and 0.2 M (5 minute extraction) on the GF soil.

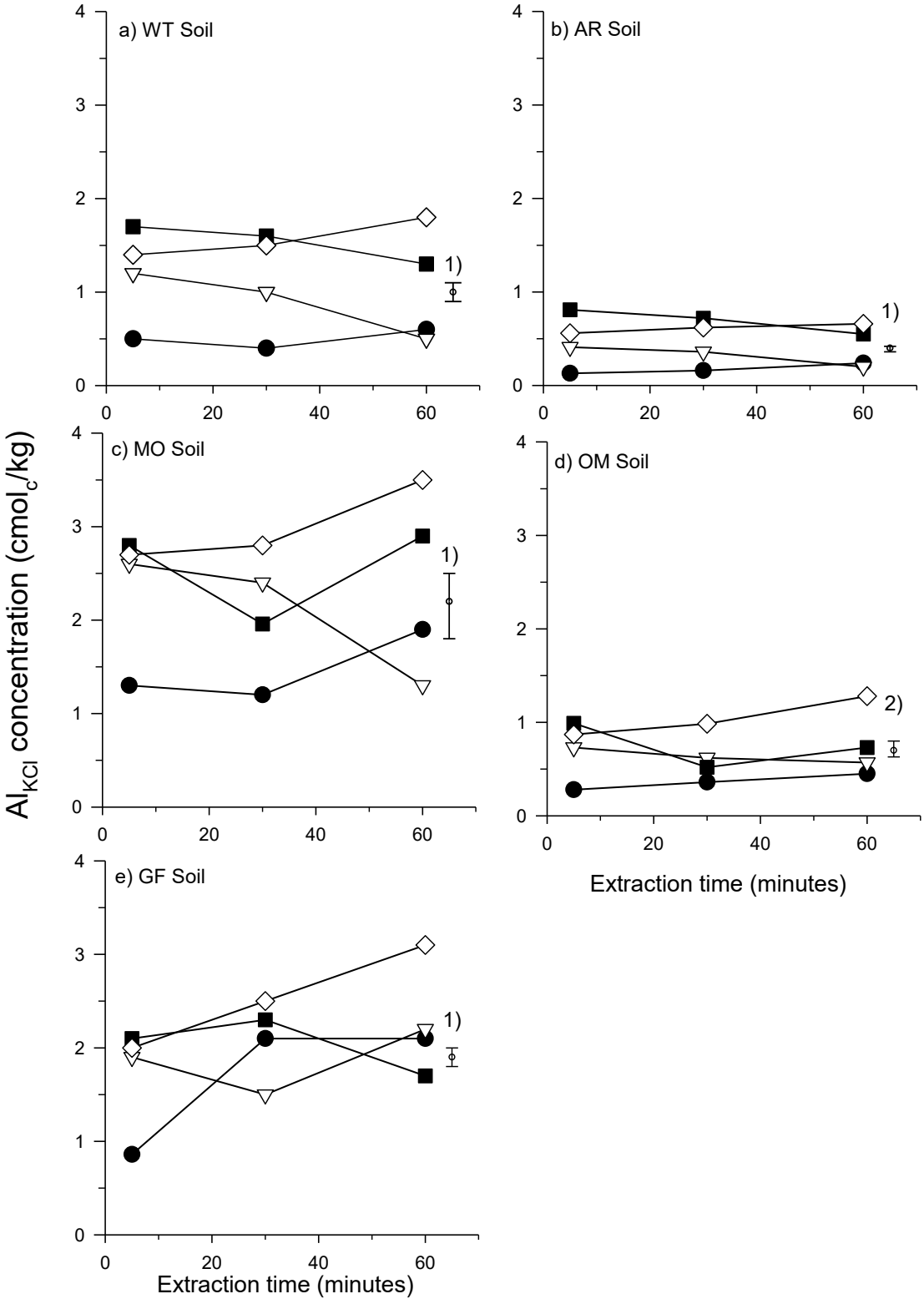

Figure 7.3 Back-transformed mean Al_{KCl} concentrations ($cmol_c/kg$) across four molarities (M) of 0.2 (●), 0.5 (▽), 1 (■) or 2 M (◇) and three extraction times of 5, 30 or 60 minutes on the a) WT soil, (b) AR soil, (c) MO soil, (d) OM soil and (e) GF soil. Values are means ± 1 SEM (*upper and lower bounds),* calculated from grand mean are for 1) the interaction of molarity and extraction time and 2) the main effect of molarity, where the interaction was non-significant. At no time was there a main effect of extraction time. Soil acronyms are described in Table 5.1.

7.3.3 A comparison of the quantity of Al extracted by the standard $CaCl_2$ and KCl tests

The mean Al concentration extracted by the 1 M KCl was 16 times higher (P<0.001) than the Al extracted by the 0.02 M $CaCl_2$ across the five soils (Table 7.6).

Table 7.6 Back-transformed mean Al concentrations (mg kg^{-1}) extracted by 0.02 M $CaCl_2$ and 1 M KCl across all soils.

		Mean extractable Al (mg kg^{-1})
extractants		
	$CaCl_2$	9.2 $_b$
	KCl	142.9 $_a$
	SEM (upper/lower)	(24.47, 5.46)
	Grand mean	36.3
***P* value**	extractant	***

*** Significant at P<0.001 level, ** significant at P<0.01 level, * significant at P< 0.05 level, ns- no significant difference.

7.4 Discussion

7.4.1 $CaCl_2$ extractions

7.4.1.1 Molarity

The molarity of $CaCl_2$ plays an important role in determining the Al_{CaCl2} concentrations on the WT, AR and GF soils. An increase in the Al_{CaCl2} extracted with increasing molarity indicates that an increase in the concentration of $CaCl_2$ salt solutions result in more Ca sorbed and more Al forced into soil solution. In contrast, on the OM soil, there was no statistical difference in the Al extracted by the 0.01 M and 0.02 M $CaCl_2$, however, the extractable Al increased with a further increase in the $CaCl_2$ molarity (Table 7.3). This was likely to be the result of the variability in the sample replicates for the 0.02 M extraction. Hoyt and Webber (1974) found that for 33 Canadian soils, the amount of Al extracted by 0.01 M $CaCl_2$ was half that of the 0.02 M $CaCl_2$, and that the extractable Al concentration at each molarity was similar regardless of extraction time (5 minutes and 60 minutes). However, the authors made no suggestions as to the cause for these findings. This laboratory study found similar results for four New Zealand soils, with an increase of approximately double (determined by soil type) the Al concentration extracted from each soil between 0.01 M and 0.02 M $CaCl_2$. The Al concentration extracted seems to be directly proportional to the molarity of the $CaCl_2$ used in the extraction.

In contrast, the Al_{CaCl2} concentration extracted from the MO soil with an increase in molarity of $CaCl_2$ was not statistically significant. This is an Allophanic Brown Soil which had the highest Al_{CaCl2} concentration and a large amount of exchangeable Al, indicated by the low base saturation (Table 5.2). It may be that because the Al_{CaCl2} is so high, that regardless of the molarity of the extractant, the extractable Al concentrations obtained are within a similar range and were not considered significantly different. Results for this soil were variable and further work is required to explain and confirm these findings.

7.4.1.2 Extraction time

The WT soil is an Allophanic Soil of volcanic origin and has properties that could have contributed to a decrease in the extractable Al measured in solution with an increase in extraction time, through re-adsorption to binding sites. These soil properties include texture (28% clay), mineralogy (allophane) and organic matter content (22%) (carbon), which affect the CEC (Table 5.2 and Appendix Table 3.2). Specific matrix effects, including re-adsorption by cation exchange sites if there are sites present that adsorb the metal more strongly (e.g. organic matter and hydrous oxides), can cause soluble and exchangeable forms of metals in the soil to decrease over time (increased extraction time) (Hlavay & Polyák, 2004). The implication of our finding is that the $CaCl_2$ test, with a 60 minute extraction time, may not give an accurate measure of the extractable Al concentration on a soil with such characteristics as the WT soil. An equilibrium may not have been reached due to the absorptive characteristics of this soil and the buffering capacity of the soil to added salts. However, no other have studies reported a time at which equilibrium was reached for a corresponding soil. Therefore, this needs to be investigated further with a larger number of soil samples included in the study.

Hoyt and Nyborg (1972) found that when adjusting the extraction time between 0 and 128 hours for the 0.01 M $CaCl_2$ extraction, the first few hours had the greatest effect on the Al concentration extracted. Amounts of Al extracted were not increased at extraction times greater than 16 hours. When 1 hour and the longer 16 hour extraction, which was previously recommended (Hoyt & Nyborg, 1971), were compared for the 0.01 M, 0.02 M and 0.04 M $CaCl_2$, the 1 hour extraction gave a better correlation with plant data compared to the 16 hr extraction and is more economical in a laboratory analysis. The differences in extraction time were smaller in our study but each increment of extraction time from 20 minutes to 60 minutes and to 180 minutes was sufficient to decrease the Al_{CaCl2} concentration on the WT soil. This is a relatively small difference (1.7 mg kg^{-1}) in the Al_{CaCl2} concentration across the extraction time sequence, however, the change in Al concentration could have very different effects on the plants growing in these soils. This is determined by the threshold for

Al toxicity and is species specific (Edmeades *et al.*, 1983; Moir *et al.*, 2016). However, differences in the Al concentration measured by extraction time were not compared to plant growth in this experiment.

7.4.1.3 Molarity X extraction time

The molarity of the $CaCl_2$ and the length of extraction time were important determinants of the Al_{CaCl2} concentrations extracted on the WT and MO soils but not the AR, OM and GF soils (Table 7.3). A decrease in Al_{CaCl2} extracted with an increase in extraction time at each of the molarities indicates that there has been some re-adsorption of Al to the WT soil. The absorption properties are likely related to the high organic matter content and the clay minerals present (Blakemore *et al.*, 1987), as mentioned previously in Section 7.4.1.2. The high clay and organic matter content provide binding sites for cation exchange and can increase the reserve acidity (Al^{3+} and H^{+}) in the soil (Soon *et al.*, 2008). The concentration of Al measured is controlled by the sorption onto and desorption from mineral phases. The concentration of the extractant stays almost constant and the metal concentration is affected by the soil type, in particular the binding strength and solubility of Al in different soils (Houba *et al.*, 2000; van Veen & Lottermoser, 2017). With an increase in extraction time, the WT soil appears to remove Al from solution, which could be explained by binding to the exchange sites. Soil organic matter strongly influences the solubility of Al by forming complexes with Al and provides a natural buffer to acidity and Al toxicity (Alleoni *et al.*, 2010; Brown *et al.*, 2008). Soils with a higher OM content are less likely to be toxic in Al, as the OM binds Al and makes it unavailable to plants. Zołotajkin *et al.* (2011) found that for two forest soils at the same pH_{H2O}, the site with the higher OM and Ca^{2+} content had reduced exchangeable Al concentrations, 800 mg kg^{-1} compared to 1101 mg kg^{-1} (0.01 M $BaCl_2$). However, these soils are from a forest environment and had much higher OM present (36 and 44%) than the soil in this study. Also, soils were more acidic (pH_{H2O} 3.6 and 3.7) and much higher Al concentrations were measured than those in our study. Zołotajkin *et al.* (2011) concluded that the reduction in Al seemed to be related to the sorption characteristics of the organic matter or the formation of Al-OM complexes. This reduces the effect of acidity and contributes to a soil buffering effect. Many other studies have reported the effects of organic matter and organic acids on exchangeable Al concentrations in soils (Bloom *et al.*, 1979; Hue *et al.*, 1986; Lundström, 1993; Powell & Hawke, 1995). The organic matter content could be a reason for the lower Al_{CaCl2} concentrations in the WT soil compared to the other soils. Even so, the standard test method extracted 4.1 mg kg^{-1} Al, above the toxicity threshold for many legumes (Moir *et al.*, 2016).

The Al_{Cacl2} concentration extracted by the standard test method was 20.0 mg kg^{-1} on the MO soil (Figure 7.2c), which is well above the toxicity threshold for many legumes (Moir *et al.*, 2016). This

concentration was not statistically different to all other treatments, except for the 0.05 M $CaCl_2$ with a 20 minute extraction time, at 2.2 mg kg^{-1}. It appears that the highest molarity had insufficient time to equilibrate with the soil and less Al had moved into solution. The concentration measured was not statistically different to that extracted by 0.01 M $CaCl_2$ at all extraction times. The reason for this result remains unclear. On three of the five soils, the different combinations of molarity and extraction time for the $CaCl_2$ extraction did not measure a statistically significant difference in the Al_{CaCl2} concentration. Studies relating the different extraction methodologies of the current $CaCl_2$ Al test are scarce for New Zealand soils. This is important new information and requires further work to confirm key findings.

7.4.2 KCl extractions

7.4.2.1 Molarity

The molarity of KCl was an important determinant of the Al_{KCl} concentrations extracted from all soils (Table 7.5). The Al_{KCl} concentration generally peaked at the standard test molarity (1 M KCl) and a further increase in molarity resulted in no increase in the Al_{KCl} extracted. On the WT, AR, OM and MO soils, this trend was observed, which indicates that at 1 M KCl (standard test), has removed all exchangeable Al from cation exchange sites. In contrast, on the GF soil, the molarity of KCl at which maximum Al_{KCl} concentrations were extracted was at 2 M KCl, which was 0.5 $cmol_c$/kg higher than that extracted by the 1 M KCl. This difference in the amount of Al extracted across the four molarities among soils, suggested that differences in the soil properties could be affecting the Al measured. As a result, the molarity of KCl at which maximum Al concentration was extracted, differed among the soils. On the GF soil, it appears that the stronger salt solution displaced more Al from exchange sites, which were otherwise held, when the lower concentration of extractant were used. This could be possible, as the GF soil is from Central Otago and derived from schist parent rock (an interlayer mineral). The 2 M KCl is a strong salt solution and outside the range used in most studies. It could be that the 2 M KCl is measuring Al from interlayer minerals. This has been suggested for 1 M KCl by several studies in New Zealand and soils in Brazil (Lee 1988; Marques *et al.*, 2002; Percival *et al.*, 1996), however, there is no literature on 2 M KCl. A higher salt concentration would be more likely to extract more Al from the soil. However, whether this process is occurring in the GF soil cannot be confirmed by this dataset.

An increase in the concentration of KCl had a significant effect on the amount of Al extracted from two Oklahoma acidic soils, pH_{H2O} 4.5 and 4.8 (McElreath *et al.*, 1992). On each soil, there was an increase in the Al extracted with an increase in the concentration of KCl from 0.125 M- 1 M, with the highest concentrations extracted at 1 M KCl. There was no suggestion of any reasons for this trend. Kachurina *et al.* (2000) compared the Al extracted by 1 M and 2 M KCl on 35 soils from Oklahoma varying in

location and extractable Al concentration. They found that the Al extracted by 2 M KCl was only half that of the 1 M KCl, showing a decrease in the Al extracted with an increase in molarity. The authors were unable to explain this unexpected trend. This is a contrasting result to those obtained in this laboratory study, in which for four out of the five soils there was no statistically significant difference in the Al_{KCl} concentration between the 1 M and 2 M KCl extractions. The GF soil measured an increase (P<0.05) in the Al_{KCl} concentration between the two molarities. The different trend observed between our study and Kachurina *et al.* (2000) is likely attributed to differences between the American and New Zealand soils tested. However, there was no information provided about the chemistry or physical properties of the soils sampled. Samples were from different locations which implies different soil types and land- use. However, due to the lack of information, the reason for the different trends between the studies could not be elucidated. The 1 M KCl is the most common test, as Al_{KCl} concentrations measured have been related to plant growth. Whether the Al extracted by the 2 M KCl is readily exchangeable Al, and relates to plant growth, is unknown. Further study of a wider range of New Zealand soils is required to confirm these findings.

7.4.2.2 Extraction time

The extraction time was an important determinant of the extractable Al_{KCl} concentrations on the WT and GF soils. However, the general trends between the two soils are different. On the WT soil the decrease in Al_{KCl} concentration with an increase in extraction time, which was thought to be a result of the characteristics associated with this volcanic soil readsorbing Al. This has been discussed previously in sections 7.4.1.2 and 7.4.1.3. In contrast, the Al_{KCl} concentration extracted from the GF soil increased with an increase in extraction time (Table 7.5).

Close and Powell (1989a) suggested a rapid method for estimating weathering processes in soils with 1 M KCl and a 5 minute extraction and using the metallochromic reagent CAS to detect a colour change and 30 minute CAS, compared to 16 hour concentrations measured using AAS. Their study was conducted on 17 New Zealand soils of different depths in the profile and the authors found, that the Al extracted in 5 minutes is correlated to the 16 hr extraction time (r^2=0.96) and the 30 minute extraction was also strongly correlated with the 16 hour extraction (r^2=0.97), with a few exceptions. Overall there was a good agreement between the field test (5 minute and 30 minute) and those determined by the 16 hour extraction time, the original extraction time for the KCl test (Blakemore *et al.*, 1987). There was a strong positive linear relationship between the two measures, however, whether the Al concentration was higher for the 16 hour extraction compared to the 5 minute extraction differed among the soils tested. In our study the Al_{KCl} on the GF soil increased with an

increase in extraction time, but only between the 5 minute and 30 minute extraction times. Their study compared the Al concentration extracted by the 5 minute and the 16 hour (AAS) extraction times and the 30 minute to the 16 hour (AAS), but did not compare the 5 minute to the 30 minute. There was no literature comparing the soil Al extracted by 5 minute and 30 minute extraction times for the standard KCl test.

The increase in exposure time of soil to extractant resulted in no statistical difference (Table 7.5) in the Al concentration extracted by KCl on the AR, MO and OM soil. This was similar to findings by McElreath *et al.* (1992) who found that altering the extraction time (5, 10, 20 and 40 minutes) for the KCl extraction did not affect the concentration of the Al extracted on two Oklahoma acidic soils. The differences in Al extracted were not significant, however, on one of the soils they measured the lowest concentrations of Al at 40 minutes extraction time, which they attributed to a possible secondary reaction with clay minerals (McElreath *et al.*, 1992). In contrast, Naidu (1985) compared the effect of extraction time on the Al_{KCl} concentration measured for six acidic soils from Fiji. The soils ranged in Al concentration (1 M KCl) from 0.3-35.6 mmol kg^{-1}. Five methods of shaking, leaching and standing were compared in this study. There was a significant difference in the Al extracted by the different extraction time methods, which followed an increase in the order of short term shake (5 seconds)<long term shake (16 hr)<long standing (5 second shake, 16 hour stand)<2 x 1 hour shake<leaching for 2 hours. For the lowest Al soil (0.3 mmol kg^{-1}), they found that there was no statistical difference in the Al_{KCl} concentration extracted between the shortest and longest shake times. These results suggest that the amount of Al which is extracted among different extraction times is affected by both the soil type and the range of extraction times included in the study.

Statistical differences in the Al_{KCl} concentration across the extraction times were observed for the WT and GF soils, however, the differences in the concentrations were small. Within this range of extraction times the differences in Al concentration were 0.2 $cmol_c/kg$ and 0.4 $cmol_c/kg$ for the WT and GF soil respectively. These differences are unlikely to be a meaningful biologically for plants growing in this soil, due to the error associated with the test and the range at which Al is toxic.

7.4.2.3 Molarity X extraction time

The molarity of the KCl and the length of extraction time are important determinants of the Al_{KCl} concentrations extracted for four of the five soils (Figure 7.3a, b, c and e). On the WT, AR, MO and GF soils there was an increase in the Al_{KCl} concentration extracted with an increase in molarity, with the

highest concentrations of Al extracted with 2 M KCl. This was likely due to the highest molarity KCl extracting more Al from the exchange sites because of the higher concentrations of the competing K^+ ion. Therefore, more of the sorbed Al is released into solution to allow for an equilibrium to exist. However, on these soils the Al_{KCl} concentration extracted by the 2 M KCl at different extraction times were not different to many of the combinations with other molarities, mainly the 1 M KCl at various extraction times.

There was an increase in the Al_{KCl} concentration extracted with increased extraction time for the highest molarity on the GF soil. It appears that this trend has also occurred on the WT and MO soils, however, there was not a significant difference between the Al_{KCl} concentrations across the different extraction times. The reason for the higher Al_{KCl} concentration extracted with a longer extraction time on the GF soil, may be related to the schist parent rock. As suggested in section 7.4.2.1, the 2 M KCl may be extracting Al from the interlayers of the schist and the longer extraction time may allow more of the Al to move into solution.

There were several soils on which the Al_{KCl} concentration decreased at the longest extraction time at various molarities, including the WT, MO and GF soils. On the WT soil, for the molarity of the standard 1 M test, the Al_{KCl} concentration was lower at the longest extraction time compared to the shortest extraction time (Figure 7.3a). As discussed in sections 7.4.1.2 and 7.4.1.3, specific soil properties which provide a large surface area for cation exchange are likely attributing to the difference in Al across extraction times at certain molarities due to re-adsorption onto exchange sites. However, only the 1 M KCl and 0.5 M KCl showed a decrease in Al concentration with an increase in extraction time. For the 2 M KCl there was no statistical difference across extraction times and it appears that this strong extractant extracted all exchangeable Al from this soil, regardless of the extraction time. Many studies have questioned the reliability of the 1 M KCl extraction in soils with high organic matter or variable charge, as the organic matter and Al complexes are not readily exchangeable with neutral salts such as KCl (Bloom *et al.*, 1979; Hargrove & Thomas, 1984; Takahashi & Dahlgren, 1998). This extraction has therefore been reported to underestimate the Al concentration on Allophanic and variable charge soils (Dahlgren & Walker, 1993, 1994). The Al extracted by KCl would be expected to increase with an increase in the molarity of KCl and the extraction time. However, this did not occur on the MO soil, which decreased at the highest extraction time for the 0.5 M KCl extraction and the GF soil Al_{KCl} concentration decreased at the highest extraction time for the 1 M KCl. These results were inconsistent with other soils and combinations for these soils. This may be related to re-adsorption of the Al to the

soil, however, this is uncertain and it is more likely that this result is an artefact of this experiment and requires further testing.

The lowest Al_{KCl} concentrations were extracted by the combination of the lowest molarity and shortest extraction time on the GF soil. This was expected, as the lower molarity KCl has lower concentrations of K^+ ions to compete with the Al, therefore less Al from exchange sites move into solution and the shortest extraction time means less time for the soil and extractant to equilibrate. On all other soils the lowest Al_{KCl} concentration was extracted with 0.2 M KCl, however, the extraction time and whether the Al_{KCl} concentration extracted was different to that extracted by other molarity and extraction time combinations was soil dependent.

The AR soil had the lowest overall concentrations of Al_{KCl} extracted (Figure 7.3b) and showed the least variation in Al_{KCl} extracted across all molarity and extraction time combinations. This is a Pumice Soil, and had the highest base saturation compared to the other three soils (Table 5.2). All soils had a similar pH_{H2O}, therefore the reduced Al concentrations on this soil were likely attributed to the higher base saturation and to a lesser extent the lower CEC.

The Al concentration extracted by the standard test (1 M KCl and 30 minute extraction) was measured at concentrations that could be toxic to sensitive legume species for the WT, MO and GF soils (Moir *et al.*, 2016). However, the Al_{KCl} concentration for the AR soil was below this threshold.

The Al_{KCl} concentration extracted from the OM soil was not statistically different across all molarity and extraction time combinations for the KCl extraction. The OM soil is a Recent Soil, which are characterised as young and weakly developed soils (Hewitt, 2013). This soil had the lowest organic matter content of all the soils (Table 5.2), coupled with a low clay content (Appendix Table 3.2). This may have contributed to there being no statistical difference (Table 7.5) in the exchangeable Al extracted across the treatment combinations, as a result of low presence of Al on the exchange sites. However, this needs to be investigated further. This was similar to findings by McElreath *et al.* (1992) on the two acidic Oklahoma soils, where there was no concentration and extraction time interaction observed for extractable Al on either of the soils tested. Their study was conducted for a range of 0.125-1 M KCl and extraction times of 5, 10, 20 and 40 minutes, molarities and extraction times were lower than those used in this experiment.

An important finding of this study was that unlike the $CaCl_2$ test, the Al_{KCl} concentration on most soils was affected by the interaction of molarity and extraction time. It could be an effect of the variability of the test on the soils or a result of including an additional molarity treatment to the KCl extraction. The next step in this field of research should be to carry out these extractions on a larger number of soils and to link the concentrations extracted to plant growth data.

7.4.3 A comparison of the quantity of Al extracted by the standard $CaCl_2$ and KCl tests

The extractable Al concentration for this suite of soils, was 16 times higher with the KCl standard test compared to the $CaCl_2$ (Table 7.6). This was slightly lower than other studies conducted comparing the tests, however, the result was still in a similar range to other studies. Venter (2017a) found that for a total of 200 soil samples, the 1 M KCl extracted an average of 20 times more Al from the soil compared to the 0.02 M $CaCl_2$ test. Venter (2017b) compared the Al concentration extracted by the standard 0.02 M $CaCl_2$ test and the 1 M KCl test for an acidic Brown Soil in the Kakanui ranges at varying depths in the soil profile. The extractable Al concentration in the top 15 cm (30 samples) of an unlimed soil was 25 times higher when extracted with 1 M KCl. At depths between 15 cm and 60 cm in the profile (15 cm increments), the difference ranged from 16 to 21 times, with the higher concentrations of Al continually extracted by the 1 M KCl. In another study, Manoharan *et al.* (1995) measured 12 times more Al with the 1 M KCl from a 0-7.5 cm control treatment of a Pallic Soil compared to the 0.02 M $CaCl_2$. It appears that regardless of the soil type and depth of sampling, differences between the 0.02 M and 1 M KCl are consistently within a similar range. However, not many comparative studies of the two tests have been conducted.

Higher Al concentrations extracted by the KCl compared to the $CaCl_2$ have been observed in other studies (Bertsch & Bloom, 1996; Close & Powell, 1989a; Manoharan, 1997; Venter, 2017b). The exchanging cations for the two extractions are different, K^+ is monovalent while Ca^{2+} is divalent. The equilibrium between cations on the exchange surface and those in solution, depends on the concentration of the cations and their relative affinity for adsorption surfaces in the soil (McLaren & Cameron, 1996a). Cations have different energy for adsorption, and as such, certain cations are more strongly attracted to exchange surfaces, which is partly related to the charge of the hydrated ion. This is influenced by both the valence (charge) of the ion and the mass of the ion. A trivalent cation such as Al^{3+} and divalent cations such as Ca^{2+} have a greater energy for adsorption than monovalent cations (McLaren & Cameron, 1996a). Al^{3+} is more strongly sorbed than Ca^{2+} or Na^+ due to its 3+ charge and is a strong competitor for cation adsorption on clays (Thomas & Hargrove, 1984). The charge density of Al^{3+} is important, as it has a similar high charge density to H^+ and therefore a small radius for the

hydrated ion. In order to bind more strongly to the soil, the cation requires a high mass: charge ratio, a smaller mass and a higher charge. This means that Al^{3+} binds strongly to exchange sites in soils and requires a stronger salt solution to remove the Al^{3+} into solution. The Ca^{2+} when added to the soil will have a stronger energy for adsorption than the K^{+}, however, the concentration of Ca^{2+} in the extract (0.02 M) was chosen to be so low as to cause minimal disturbance to the ionic equilibrium (Soon *et al.*, 2008). Even though the K^{+} has a lower affinity for exchange sites, the high concentration (1 M) of the extractant, removes more Al^{3+} from soil exchange sites and therefore measures higher concentrations of Al compared to the lower ionic strength test (Close & Powell, 1989a; Venter, 2016).

7.5 Conclusions

- Literature which examines differences in the Al extracted by the two extractants by altering the methodology is scarce for New Zealand soils.
- This laboratory investigation identified that changes in the molarity and extraction time of the $CaCl_2$ and KCl extraction methods affected the soil Al concentrations in extracts. Differences in soil response (Al concentration measured), were found even with small changes in the molarity and extraction time for the two extraction methods.
- The effect of molarity and extraction time on the Al concentrations extracted, differed among the five soils tested in this experiment. This was an important finding, as this experiment used contrasting New Zealand soil orders, with similar pH_{H2O} and differing Al concentrations, to see if the soils reacted in the same way. This finding suggests that the Al concentrations measured by the two test methods are affected in the topsoil by properties related to soil order.
- Additional studies are required on New Zealand soils with a larger number of soils and wider variety of soil types to determine whether trends observed in this study of five soils remain the same for a wider range of acidic NZ soils.
- A future research step would be to assess the relationship between different test combinations of $CaCl_2$ and KCl in a range of New Zealand soils to determine which of these soil tests best relates to Al toxicity, using plant growth as a bio-indicator. Species of interest could include legumes such as lucerne and lupin, which vary in their sensitivity to Al.

8 General discussion

The overarching aim of this research was to improve the understanding of soil extractable Al and to investigate and determine the key factors that drive the variation in soil extractable Al in New Zealand high and hill country soils. Soil extractable Al was studied in a number of experiments at different scales (national, catchment, glasshouse, laboratory and rhizosphere) to achieve the research aim. Soil acidification and associated Al toxicity have been identified as critical issues which are widespread in New Zealand (Chapter 2). In high and hill country areas, this is of particular concern, as soils continue to weather over time and the application of lime is often uneconomic. In particular, it was important to focus on different New Zealand soil orders, to determine which soils and areas may be more susceptible to high concentrations of extractable Al that could be toxic to plants. Legumes were then grown as bioindicators in a suite of New Zealand soils to determine plant responses to extractable Al concentrations. The findings of this research programme provide new information surrounding soil extractable Al concentrations and potential for toxicity in New Zealand soils, which will be valuble to both scientists and farmers.

8.1 Chapter 3: National Soils Database Analysis

Research question 1) "What are the key covariates of soil Al_{KCl} in New Zealand soils that enable exchangeable Al to be predicted?"

In Chapter 2, a review of the literature identified possible variables that could influence soil extractable Al concentrations in New Zealand soils. Data from a large database of New Zealand soils were analysed in order to determine, of the pre-selected environmental, soil chemical and physical variables, which variables were important for influencing the soil extractable Al_{KCl} concentration. In addition, to determine if soil order was effective in partitioning soils based on their soil Al_{KCl} variability. Base saturation, soil pH_{H2O}, cation exchange capacity, total N, total C and soil order were strongly associated with soil Al_{KCl} concentrations in three horizon depth zones in New Zealand soils. There were important differences in the relationship between extractable Al_{KCl} concentrations and different soil variables, particularly between the top 20 cm depth zone and the two deeper depth zones (20-50 cm and 50-120 cm). The 20 cm depth zone was more sensitive to BS and total N and less sensitive to CEC and pH_{H2O}, while non-sensitive to carbon. Acidity and high CEC contributed to high extractable Al concentrations, while high BS and total C had a negative effect. The major difference in carbon points towards carbon being a modulator of soil Al concentrations.

Carbon had a dual effect on Al_{KCl} concentrations, where in deeper horizons there was less C and increased Al at any given pH_{H2O} (Figure 3.5a and b), however, in the top 20 cm there was higher carbon

but a lack of relationship with C. The relationship between total C and Al_{KCl} suggests that complexation of Al to OM may have occurred, which makes Al non-extractable by KCl (non-exchangeable) (Bhumbla & Mclean, 1965; Schnitzer & Gupta, 1965). Organic matter and several low molecular weight organic acids in soil solution can form strong complexes with soluble Al and reduce bioavailability (Hue *et al.*, 1986; Ismail *et al.*, 1994; McColl & Pohlman, 1986; van Hees *et al.*, 2000). However, whether there are high Al_{KCl} concentrations depends on the pH of the soil, and the presence of organic matter strongly alters the relationship between pH and Al (van Hees *et al.*, 2000).

There was a strong relationship between total N in the 20 cm depth zone, which decreased with increasing Al_{KCl} (Figure 3.2a), likely a reduction in legume abundance and N fixation as a result of Al toxicity. Legume shoot growth, nodulation and N fixation are restricted and less effective on acidic and high Al soils (Berenji *et al.*, 2017; Helyar & Anderson, 1971; Moir & Moot, 2010; Scott *et al.*, 2008; Wigley, 2017), leading to less N in the soil. It is also possible that the strong relationship between total N and Al_{KCl} is a function of long term vegetation growth and acidification (both current and historic). However, there was no information in the database that could confirm the vegetation growth or type (e.g. legumes) or N fixation at these sites and literature relating N content of the soil to exchangeable Al is scarce. Higher Al_{KCl} concentrations in the two deeper depth zones highlight the issues of subsoil acidity and Al toxicity in New Zealand soils, which is more difficult to mitigate.

Rainfall did not appear as a significant variable in the multiple regression model, in contrast to other studies (Harrison *et al.*, 1990; Webb *et al.*, 1986). This was likely because other variables including pH_{H2O}, CEC and BS more effectively represented the effects that rainfall is often a proxy for, which is weathering/acidification and leaching intensity. The effects of rainfall on extractable Al concentrations was investigated further in a finer (catchment-scale) study in the subsequent chapter, to provide a comparison to this analysis at a national scale.

Soil order was identified as a significant variable that influenced the extractable Al_{KCl}, which achieved the second objective of this chapter (Table 3.3). The results confirmed that Brown Soils and Podzols had the highest mean Al_{KCl} concentration across all depths, compared to Pallic Soils and Recent soils, which were lowest. Brown Soils and Podzols had a mean Al_{KCl} concentration that could be toxic to many legumes (>1.0 $cmol_c$/kg)(Edmeades *et al.*, 1983; Moir *et al.*, 2016). The results were not unexpected, as they follow a general development sequence in soil formation. This is an important result as Brown Soils are the most widespread soil order in New Zealand (43%, Hewitt, 2013). This strongly highlights the extent of this issue. This is an important piece of information as farmers with Brown Soils or Podzols

on their farms are likely to have higher extractable Al_{KCl} concentrations compared to other soil orders. A disadvantage of using the database was that some soils were poorly represented, including Organic Soils, Pumice Soils and Allophanic Soils, and as a result, extractable Al trends could not be elucidated on these soils.

8.2 Chapter 4: A field survey of soil pH and extractable Al in the Ashburton Lakes catchment, Canterbury, New Zealand.

Hypothesis 1) Extractable Al_{CaCl2} increases with increasing soil and landform age.

Hypothesis 2) Extractable Al_{CaCl2} increases with increasing mean annual rainfall.

Chapter 4 involved a catchment field study, which represents a high and hill country environment with commonly farmed landforms. Studies have been conducted investigating soil chemistry among different landforms and aged surfaces along rainfall or age gradients in New Zealand (Dixon *et al.*, 2016; Harrison *et al.*, 1990; Webb *et al.*, 1986), and indicated potential variability in exchangeable Al concentrations related to landscape scale effects. However, there has been no systematic study of exchangeable Al variability at this scale. The scope of this study was soil Al_{CaCl2} variability within a single catchment and was focussed on determining which key factors drive pH_{H2O} and Al_{CaCl2} at a landscape scale. There was a distinct rainfall gradient in this catchment and the age of land surfaces have been well defined in previous studies (Barrell *et al.*, 2011). The study involved soil sampling at 21 sites across the catchment.

The main finding was that depth was the strongest explanatory variable for soil pH_{H2O} and extractable Al_{CaCl2} in this landscape. Soil pH_{H2O} increased with profile depth and extractable Al declined (Figure 4.6 and Figure 4.7). The pH_{H2O} trend was consistent with the trend observed in the NSD analysis, however, the Al_{CaCl2} trend was the opposite to that of Al_{KCl} revealed in the NSD analysis. This may be related to the NSD analysis encompassing many different soil orders and sites with different combinations of soil forming factors contributing to the chemistry and formation of the soil. These factors will differ from those specific factors which influence the formation and the chemistry of the Brown Soils (Acidic Orthic and Typic Orthic Brown Soils) in the Ashburton Lakes catchment. Rainfall was a significant factor for Al_{CaCl2} in this catchment, however, there was not an overall trend of an increase in Al_{CaCl2} concentration with an increase in rainfall (Figure 4.5). This contrasts with the hypothesis and results that have been reported in the literature (Dixon *et al.*, 2016; Harrison *et al.*, 1990; Webb *et al.*, 1986). Rainfall at the sites sampled ranged from 1128 mm to 1558 mm yr^{-1}, which is above the pedogenic threshold established in the study by Dixon *et al.* (2016) of 800 mm yr^{-1} which was associated with cation weathering, acidification, release and rapid mobilisation of trivalent metallic ions. The trend for rainfall was unexpected, however, it was significant, which was different to the NSD study. It was hypothesised

that extractable Al_{CaCl2} increases with increasing soil and landform age. However, there were no systematic patterns of Al_{CaCl2} and pH_{H2O} on different age surfaces. This contrasts with chronosequence studies which show increased Al_{CaCl2}, and decreased pH_{H2O} with increasing soil age (Harrison *et al.*, 1990).

Of the soil chemical variables, total N was the best explanatory variable of soil pH_{H2O} and negatively correlated, whereas the opposite trend was revealed in the large scale NSD analysis. The reason for this remains unexplained, but may involve the interaction of other correlated factors. It should be noted that there was a much smaller range of total N in the Brown Soils in the Ashburton Lakes catchment compared to the NSD analysis. However, the main trend in raw data from the NSD mirrors these findings, showing a distinct decrease in pH_{H2O} with an increase in total N. This may be related to long term vegetation growth and acidification (both current and historic), in which total N is correlated to pH_{H2O}, however, the relationship between total N and soil pH_{H2O} was weak in the Ashburton Lakes study (Figure 4.9). Moreover, the link between total N and vegetation growth and acid generation (current and historic) was unable to be confirmed in either study. The pH_{CaCl2} was the best predictor of Al_{CaCl2}. This was an expected outcome and was also found to be an important variable for Al_{KCl} in the NSD analysis, however, the measure was pH_{H2O}. Soil pH affects the solubility of Al and therefore is a primary control in soils. Unlike the NSD analysis, total C was not a significant variable for the Al_{CaCl2} in this study. This could be an aspect worth exploring further for soils in this catchment, especially given the curve in the data of soil extractable Al concentrations (Figure 4.7), which indicates that there may be some complexation of Al in the topsoil at some sites.

Decision trees were used as a tool to split the data for soil pH_{H2O} and extractable Al in each depth zone and the rules associated with the top 20 cm of the profile were used to create maps of the catchment. The maps showed that soil pH_{H2O} and extractable Al are strongly related and sites with higher Al_{CaCl2} concentrations were in areas with higher rainfall, low elevation and south facing sites (Figure 4.18). The rainfall trend contrasted the trend in the linear model. Al_{CaCl2} was measured at concentrations that could be toxic to legumes in this catchment, which ranged from 1.2 mg kg^{-1} to 39.1 mg kg^{-1}. The fact that rainfall, aspect and elevation were shown to be the most important factors in the decision tree indicates that pH_{H2O} and extractable Al are more sensitive to factors affecting leaching rate rather than duration (i.e. landform age). These findings provide information for farmers and fertiliser companies as to areas in the landscape that are likely to have higher extractable Al concentrations and may be prone to toxicity.

8.3 Chapter 5: A glasshouse experiment growing legumes as bioindicators of Al toxicity in a suite of acidic New Zealand soils.

Hypothesis 1) Legume yield is strongly associated with soil extractable Al_{CaCl2} concentrations.

Hypothesis 2) The relationship between soil Al_{CaCl2} and plant growth differs among soils at the same pH.

Lucerne shoot yields were strongly associated with soil extractable Al_{CaCl2} concentrations on a suite of acidic high and hill country soils. A strong relationship between Al and yield was found for lucerne but not for Caucasian clover. Shoot yield increased with lime application, particularly between the L0P0 and 2 t lime ha^{-1} treatment, which reduced the extractable Al concentration on all soils to below toxic concentrations (≤2.5 mg kg^{-1}) (Figure 5.4b, d and f and Figure 5.3). However, the relationship between soil Al and plant growth differed among the 10 soils, which included Pumice, Allophanic, Brown, Recent and Pallic Soils. Shoot K% and B concentrations were also significant covariates associated with lucerne shoot yield in the low lime treatments. However, at the lowest (potentially deficient) concentrations in the herbage, the lucerne shoot yields were highest which indicates that these were unlikely to be limiting in the low lime treatments in this experiment.

The shoot N% in the herbage increased with lime applied, particularly between the L0P0 and 2 t lime ha^{-1} treatment, which coincided to not only an increase in pH_{H2O} but a substantial reduction in soil extractable Al concentration. This trend was the same as the relationship between soil total N and extractable Al in the NSD analysis, but differed to the relationship reported between soil pH_{H2O} and total N in the Ashburton lakes catchment study, where the pH_{H2O} increased and the soil total N decreased. While one is a soil measurement and the other a plant N measurement, both imply the link and importance of pH and extractable Al to N fixation and the growth of legumes. This has been reported in several other studies (Berenji *et al.*, 2017; Helyar & Anderson, 1971; Moir & Moot, 2010; Scott *et al.*, 2008; Wigley, 2017).

On most soils, the yield response by lucerne was higher with lime applied compared to P, indicating the effects of extractable Al in the L0P0 soils. However, in this study, an important finding was that the relationship between lucerne shoot yield and soil extractable Al for the low lime and P datasets differed. It was suggested that this may be related to the test possibly measuring Al-phosphates that are in a form not detrimental to the plant, but result in a higher concentration of Al measured by the test. This may explain the higher yields in the higher Al treatments on some soils in the P dataset (which was unexpected). However, future research is required to address this finding, as this was beyond the scope of this experiment.

The Caucasian clover yields were consistent and generally less affected by lime applications than lucerne, and showed a minimal response to P applied, which supports other studies that report Caucasian clover as a promising species for high country of New Zealand (Figure 5.4a, c and e and Figure 5.8a, c and e).

For plants growing for 10 months, yields were reasonable on most soils and treatment combinations equivalent to 840-18092 kg DM ha^{-1} for lucerne and 1527-12824 kg DM ha^{-1} for CC, however, yields were consistently low across all lime and P treatments on two of the volcanic soils (WT and PK). The reasons for this could not be elucidated in this experiment and this could be an area of future research. It is important to recognise the limitations of measuring pasture yields for plants grown in pots in the glasshouse. Further work is required to quantify the effects of extractable Al on pasture growth in high and hill country soils in in-situ field experiments.

8.4 Chapter 6: A rhizobox experiment investigating the effects of legumes and pH on Al at the root-soil interface.

Hypothesis 1) Plants influence the soil extractable Al and pH_{H2O} at the root-soil interface and that the degree of influence differs between Russell lupin and lucerne.

This work is the first study that has shown, in high resolution, distinct patterns of soil Al mobilisation, induced by the roots of important pasture species in New Zealand. In this study a rhizobox experiment was used to investigate the influence of plants on soil extractable Al and soil pH_{H2O} at the root-soil interface in an acidic high country soil. Focusing on both lucerne and Russell lupin enabled the effects to be compared. The passive sampling technique, diffusive gradient in thin-films (DGT) was used, coupled with soil measurements of the bulk and rhizosphere soil, to determine the diffusive flux of Al from the soil environment to the adjacent resin gel. The size of the flux can be related to the concentration at the interface between the soil and resin gel. While DGT has been used to measure Al in aqueous systems (Panther *et al.*, 2012), it has not been measured in soils. This is the first time that DGT has been used to measure the mobilisation of Al in soil at high resolution around the roots of pasture species across a liming sequence.

The pH was more acidic in the rhizosphere and Al_{CaCl2} concentrations were higher in the rhizosphere compared to the bulk soil, particularly in lupin plants. Other studies have measured differences in soil pH and Al concentrations between the bulk soil and the rhizosphere of many different plant species (Blossfeld *et al.*, 2010; Collignon *et al.*, 2012; George *et al.*, 2002; Göttlein *et al.*, 1999; Li *et al.*, 1997; Van Breemen *et al.*, 1983; Youssef & Chino, 1989), however, this is study differs in that DGT and LA-ICP-MS measurements were also taken. The Al flux was effectively measured in proximity to plant

roots using DGT and LA-ICP-MS, which was used to produce an image of the spatial variation of Al across the DGT resin. DGT results showed depletion of Al along segments of the plant root, indicating previous removal by the plant from the soil (Figure 6.5a). Distinct zones of metal depletion around the plant roots and an increase with distance away from the root has also been reported for Mn and P (Hoefer *et al.*, 2015; Santner *et al.*, 2012). There were hotspots of mobilisation at the root tip, where most of the metal uptake is carried out by plants. Mobilisation of Al expectedly declined with increased lime applied, which was evident in the DGT results. However, even with an acidic rhizosphere and high Al concentrations, the lupin yields appeared to be unaffected by Al_{CaCl2}. This could be explained by the release of organic acids under high Al conditions, which bind Al, making it unavailable to plants. The hot water extractable carbon was consistently higher in the rhizosphere of both species. It seemed to increase with lime application, likely released due to a decline in nutrient availability with an increase in soil pH_{H2O}. However, a limitation of this study was that the specific organic acids released by the roots were unable to be identified and would have been a useful measure to relate to extractable Al_{CaCl2} concentrations and the potential remediation of toxicity. The differences in Al concentrations between the lupin rhizosphere and bulk soil were greater than the lucerne. This could be linked partially to the higher biomass production of lupin. The high lupin yields, particularly in the lowest lime treatment, show its ability to grow in acidic and high Al soils, in contrast to sensitive lucerne, which had very low yields. Measurements were taken along a liming sequence, however, this is one study and lacks replication, and results need to be confirmed in further replicated experiments.

8.5 Chapter 7: A laboratory investigation of the soil aluminium test on contrasting New Zealand soils

Hypothesis 1) The concentration of extractable Al from the soil varies with the type of extractant, molarity and extraction time.

In Chapter 7, a detailed laboratory investigation was conducted on five of the glasshouse soils from Chapter 5 to assess the effect of the methodology (molarity and extraction time) of the standard soil $CaCl_2$ and KCl soil Al tests and whether this altered the Al concentration extracted. All soils had a similar pH_{H2O} (5.2 and 5.3) but ranged in soil Al_{CaCl2} concentrations (3.8-13.3 mg kg^{-1}). Different soil orders were represented including two Brown Soils, a Recent Soil, a Pumice Soil and an Allophanic Soil, all from high and hill country areas. This laboratory investigation has identified that the effect of molarity and extraction time on the Al concentrations extracted, differed among the five soils tested. This was an important finding, as this experiment used contrasting New Zealand soil orders, with similar pH_{H2O} and differing Al concentrations, to see if the soil reacted in the same way. This finding suggests that the Al concentrations measured by the two test methods are affected in the topsoil by properties related to

soil order. Differences in soil response, Al concentration measured, were found even with small changes in the molarity and extraction time for the two extraction methods.

On most soils an increase in molarity of the extractant ($CaCl_2$ and KCl), the Al concentration extracted increased due to the higher concentrations of competing cations (Ca^{2+} and K^+). More Al^{3+} from exchange sites is released into solution to allow for equilibrium. However, the concentration extracted from the MO soil was not statistically different across the molarities of $CaCl_2$. For KCl most soils increased to a peak Al concentration at 1 M KCl and no more Al was removed with a further increase in the extractant molarity (Table 7.5). The exception to this was the GF soil, which may have been a result of the schist parent rock and the possible removal of Al from the interlayer minerals by 2 M KCl. This was also thought to be the reason behind the increase in Al extracted with an increase in extraction time on this soil for the KCl test. In contrast, for both extractions, the Al extracted decreased with an increase in extraction time for the WT soil (Table 7.3 and Table 7.5), which was attributed to the specific matrix effects including readsorption of Al onto exchange sites (clay and OM) during the extraction. There were differences in the Al concentration extracted across the different molarity and extraction time combinations for different soils. Unlike the $CaCl_2$ test, most soils were affected by the interaction of molarity and extraction in terms of the Al concentration extracted.

Much higher Al concentrations were extracted by KCl than $CaCl_2$, 16 times higher overall (Table 7.6), and was consistent with the few studies in which both measurements were taken (Manoharan *et al.*, 1995; Venter, 2017b). This is not unexpected as although Ca^{2+} has a higher energy for adsorption than K^+ (due to the charge on the ion), the higher concentration of KCl has more K^+ ions in solution and therefore measures higher concentrations than the lower ionic strength test. Differences in properties of the soils and the extraction of Al therefore influences the effectiveness of chemical measurement. This is an important finding, as this soil test is the current measure that farmers have available to inform them of potential soil Al toxicity on their farms and to assist in land-use decisions. Additional studies are required on New Zealand soils with a larger number of soils and wider range of soil types to determine whether trends observed in this study are similar for other acidic New Zealand soils. The next step would be to assess the relationship between the Al concentration extracted by the different test combinations for $CaCl_2$ and KCl and plant growth, to determine which of these tests best relates to Al toxicity for a range of New Zealand soils.

9 Conclusions and Future research

- Lucerne shoot yields were found to be strongly associated with soil extractable Al_{CaCl2} on a suite of New Zealand soils. In contrast, this same relationship was not found for Caucasian clover. This is a critical result which demonstrates that soil extractable Al may be the key factor driving legume yield on acid New Zealand soils. Further work is required to quantify the effects of extractable Al on legume growth in high and hill country soils in an in-situ field experiment.

- The relationship between soil pH_{H2O} and Al was quantified on 10 soils (main New Zealand soil orders) with lime applied in the glasshouse experiment. However, between the 0 and 2 t lime ha^{-1} treatments, the Al_{CaCl2} concentration of all soils dropped to <3 mg kg^{-1}, which was unexpected. This was coupled with a substantial increase in lucerne yield on most soils. Future research should investigate the application of lower lime rates (between the 0 and 2 t lime ha^{-1} treatments) to determine where the critical change point is for the Al_{CaCl2} concentration for this suite of New Zealand soils, and how this impacts on the legume shoot yield.

- Soil order has predictive ability that allows a first order identification of areas prone to toxic concentrations of Al. On acidic and high Al volcanic soils, particularly those low in Olsen P, even when Al toxicity was overcome by lime additions and P was applied, legumes yields were low. These soils were also not well represented in the NSD and behaved differently to the other soils in the laboratory study, particularly the Allophanic soil. The relationship between extractable Al concentration and plant yield in New Zealand volcanic soils needs to be investigated further, to determine what is limiting legume yields.

- Further exploration, through denser and more rigorous sampling, is required in the Ashburton Lakes catchment of these spatial patterns of soil properties which affect acidification and soil extractable Al. It is important to note that the results from this field study cannot be extrapolated to other catchments in New Zealand, as the combination of soil forming factors such as climate, relief, parent material differ from those in the Ashburton Lakes catchment.

- In the Ashburton Lakes catchment study, the effects of rainfall on extractable Al showed the opposite trend to what was hypothesised. This could have been due to the rainfall range being above the pedogenic threshold identified by Dixon *et al.* (2016). A future area of research could

be using extractable Al as a possible indicator of thesholds being crossed. From climosequence and chronosequence studies extractable Al varies systematically with rainfall and soil age, but it seems that there have to be other drivers responsible for short range variability.

- Based on findings from the NSD analysis, catchment study and laboratory experiment, the relationship between soil carbon and extractable Al is worth exploring further in New Zealand soils.

- Variables, including environmental, soil chemical and physical were determined, both in the NSD analysis and the Ashburton Lakes catchment study, which helped to identify under which set of conditions extractable Al concentrations are highest in New Zealand soils and in a specific catchment area. This is important, as by identifying areas that have high extractable Al concentrations, land previously unable to grow lucerne because of Al may be remediated if Al is isolated.

- Both the NSD and catchment studies used depth as a variable and determined the depth in the profile that extractable Al concentrations were highest. In the NSD study of many soil orders, the higher Al_{KCl} concentrations in the two deeper depth zones (50 cm and 120 cm) highlighted issues of subsoil acidity and Al toxicity. In the Ashburton Lakes catchment, the Al_{CaCl2} was highest in the top soil and reduced with increased depth in the Brown Soils sampled. However, the analyses highlighted that the depth at which extractable Al concentrations are high varies among sites and soil types, and local assessments of profile variability of Al are necessary. Identifying the location in the profile that high extractable Al occurs could enable solutions to be developed across large areas.

- For the rhizobox study, although measurements were taken along a liming sequence, this is one study and lacks replication, and results need to be confirmed in further replicated experiments. In these experiments the measurement of specific organic acids would be advised in order to determine if detoxification of Al has occurred through chelation.

- The rhizobox experiment was unable to assess the effects of moisture in addition to pH_{H2O}, on the rhizosphere and soil Al, therefore this is an aspect that could be investigated in future studies.

- The next step after the laboratory investigation would be to assess the relationship between the Al concentration extracted by the different test combinations for $CaCl_2$ and KCl and plant growth, to determine which of these tests best relates to Al toxicity for a range of New Zealand soils. Species of interest could include legumes such as lucerne and lupin, which vary in their sensitivity to extractable Al. While many initial studies overseas linked plant growth to the extractable Al concentration measured by the test extracted, there needs to be a comparison between these methods on New Zealand soils.

- This research has resulted in determining the key drivers of soil extractable Al concentrations on a national scale, in a single catchment; has assessed the effects of extractable Al on legume growth in the glasshouse; plant effects on soil chemistry at a rhizosphere scale in the rhizobox experiment and examined differences in the Al extracted by the $CaCl_2$ and KCl tests, which differed among the New Zealand soils tested, and combinations of molarity and extraction time. The findings from this thesis will be valuble to both scientists and farmers.

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1 Appendix A National Soils Database

1.1 Final multiple linear regression Genstat outputs for the 20 cm, 50 cm and 120 cm depth zones.

1.1.1 20 cm depth zone

Regression analysis

Response variate: $logAl_{KCl}$
Fitted terms: Base_saturation_% + CEC_me% + Nitrogen_% + PH_H2O + NZ_Revised_order

Summary of analysis

Source	d.f.	s.s.	m.s.	v.r.	F pr.
Regression	15	43.93	2.92863	39.90	<.001
Residual	148	10.86	0.07340		
Total	163	54.79	0.33615		
Change	-11	-2.14	0.19498	2.66	0.004

Percentage variance accounted for 78.2
Standard error of observations is estimated to be 0.271.

Estimates of parameters

Parameter	estimate	s.e.	t(148)	t pr.
Base_saturation_%	-0.01728	0.00144	-11.99	<.001
CEC_me%	0.02087	0.00388	5.37	<.001
Nitrogen_%	-0.935	0.168	-5.56	<.001
PH_H2O	-0.2680	0.0598	-4.48	<.001
NZ_Revised_order B	2.009	0.283	7.09	<.001
NZ_Revised_order E	1.691	0.319	5.31	<.001
NZ_Revised_order G	1.895	0.298	6.37	<.001
NZ_Revised_order L	1.773	0.312	5.68	<.001
NZ_Revised_order M	1.698	0.409	4.15	<.001
NZ_Revised_order N	1.715	0.398	4.31	<.001
NZ_Revised_order O	1.728	0.332	5.20	<.001
NZ_Revised_order P	1.936	0.278	6.97	<.001
NZ_Revised_order R	1.943	0.280	6.94	<.001
NZ_Revised_order U	1.905	0.296	6.44	<.001
NZ_Revised_order X	1.607	0.324	4.97	<.001
NZ_Revised_order Z	1.672	0.262	6.38	<.001

Accumulated analysis of variance

Change	d.f.	s.s.	m.s.	v.r.	F pr.
+ Base_saturation_%	1	35.68931	35.68931	486.24	<.001
+ CEC_me%	1	0.22411	0.22411	3.05	0.083
+ Nitrogen_%	1	4.45804	4.45804	60.74	<.001
+ PH_H2O	1	1.41323	1.41323	19.25	<.001
+ NZ_Revised_order	11	2.14475	0.19498	2.66	0.004
Residual	148	10.86306	0.07340		
Total	163	54.79250	0.33615		

1.1.2 50 cm depth zone

Regression analysis

Response variate: $logAl_{KCl}$
Fitted terms: BS_% + CEC_me% + Carbon_% + PH_H2O + NZ_Revised_order

Summary of analysis

Source	d.f.	s.s.	m.s.	v.r.	F pr.
Regression	15	53.448	3.56322	61.93	<.001
Residual	160	9.206	0.05754		
Total	175	62.655	0.35803		
Change	-11	-6.037	0.54882	9.54	<.001

Percentage variance accounted for 83.9
Standard error of observations is estimated to be 0.240.

Estimates of parameters

Parameter	estimate	s.e.	t(160)	t pr.
BS_%	-0.01238	0.00142	-8.72	<.001
CEC_me%	0.04079	0.00370	11.02	<.001
Carbon_%	-0.1179	0.0134	-8.77	<.001
PH_H2O	-0.5107	0.0649	-7.87	<.001
NZ_Revised_order B	2.975	0.326	9.14	<.001
NZ_Revised_order E	2.793	0.337	8.28	<.001
NZ_Revised_order G	2.856	0.328	8.71	<.001
NZ_Revised_order L	2.252	0.368	6.13	<.001
NZ_Revised_order M	2.618	0.422	6.20	<.001
NZ_Revised_order N	2.818	0.372	7.57	<.001
NZ_Revised_order O	3.214	0.389	8.26	<.001
NZ_Revised_order P	3.061	0.324	9.44	<.001
NZ_Revised_order R	2.928	0.325	9.01	<.001
NZ_Revised_order U	3.115	0.322	9.68	<.001
NZ_Revised_order X	2.711	0.397	6.83	<.001
NZ_Revised_order Z	2.890	0.311	9.29	<.001

Accumulated analysis of variance

Change	d.f.	s.s.	m.s.	v.r.	F pr.
+ BS_%	1	27.28446	27.28446	474.19	<.001
+ CEC_me%	1	2.05577	2.05577	35.73	<.001
+ Carbon_%	1	4.96584	4.96584	86.30	<.001
+ PH_H2O	1	13.10518	13.10518	227.76	<.001
+ NZ_Revised_order	11	6.03707	0.54882	9.54	<.001
Residual	160	9.20618	0.05754		
Total	175	62.65451	0.35803		

1.1.3 120 cm depth zone

Regression analysis

Response variate: $logAl_{KCl}$
Fitted terms: BS_% + CEC_% + Carbon_% + Nitrogen_% + PH_H2O + NZ_Revised_order

Summary of analysis

Source	d.f.	s.s.	m.s.	v.r.	F pr.
Regression	16	59.48	3.71776	59.54	<.001
Residual	162	10.12	0.06244		
Total	178	69.60	0.39101		
Change	-11	-5.21	0.47373	7.59	<.001

Percentage variance accounted for 84.0
Standard error of observations is estimated to be 0.250.

Estimates of parameters

Parameter	estimate	s.e.	t(162)	t pr.
BS_%	-0.00709	0.00134	-5.30	<.001
CEC_%	0.03849	0.00366	10.52	<.001
Carbon_%	-0.1283	0.0156	-8.21	<.001
Nitrogen_%	1.105	0.417	2.65	0.009
PH_H2O	-0.5103	0.0583	-8.75	<.001
NZ_Revised_order B	2.713	0.300	9.03	<.001
NZ_Revised_order E	2.655	0.326	8.15	<.001
NZ_Revised_order G	2.719	0.300	9.05	<.001
NZ_Revised_order L	2.121	0.345	6.15	<.001
NZ_Revised_order M	2.405	0.409	5.87	<.001
NZ_Revised_order N	2.376	0.381	6.23	<.001
NZ_Revised_order O	2.869	0.330	8.69	<.001
NZ_Revised_order P	2.932	0.303	9.67	<.001
NZ_Revised_order R	2.667	0.305	8.75	<.001
NZ_Revised_order U	2.901	0.294	9.87	<.001
NZ_Revised_order X	2.793	0.318	8.80	<.001
NZ_Revised_order Z	2.628	0.295	8.92	<.001

Accumulated analysis of variance

Change	d.f.	s.s.	m.s.	v.r.	F pr.
+ BS_%	1	26.64343	26.64343	426.71	<.001
+ CEC_%	1	6.17857	6.17857	98.95	<.001
+ Carbon_%	1	6.44041	6.44041	103.15	<.001
+ Nitrogen_%	1	0.89614	0.89614	14.35	<.001
+ PH_H2O	1	14.11465	14.11465	226.05	<.001
+ NZ_Revised_order	11	5.21103	0.47373	7.59	<.001
Residual	162	10.11513	0.06244		
Total	178	69.59936	0.39101		

1.2 Correlation of soil variables

Table 1.1 A correlation matrix for the variables in the 20 cm depth zone (two-sided, Pearson).

	1	2	3	4	5	6	7	8	9	10	11	12
1. Al %	-											
2. BS %	0.169*	-										
3. CEC	0.293**	-0.931^{ns}	-									
4. C %	-0.011^{ns}	-0.195*	0.905***	-								
5. Rainfall	-0.430***	-0.471***	0.001^{ns}	0.211**	-							
6. Temperature	-0.075^{ns}	0.248**	0.159*	0.244**	0.187*	-						
7. N %	0.206**	-0.077^{ns}	0.862***	0.901***	0.083^{ns}	0.352***	-					
8. pH	0.421***	0.633***	0.101^{ns}	-0.052^{ns}	-0.474***	0.302***	0.131^{ns}	-				
9. P retention	0.501***	-0.347***	0.643***	0.594***	0.035^{ns}	0.150^{ns}	0.715***	0.184*	-			
10. clay %	0.388***	0.053^{ns}	0.289***	-0.272^{ns}	-0.272***	-0.074^{ns}	0.108^{ns}	0.191*	0.287***	-		
11. log Al_{KCl}	-0.148^{ns}	-0.807***	0.012^{ns}	0.289^{ns}	0.289***	-0.434***	-0.14^{ns}	-0.666***	0.099^{ns}	0.055^{ns}	-	
12. precipitation variability	-0.397***	-0.358***	-0.040^{ns}	0.827^{ns}	0.827***	0.255***	0.056^{ns}	-0.354***	0.004^{ns}	-0.261***	0.176*	-
Mean	6.7	50.8	21.2	6.2	1419	11.8	0.4	5.3	39.9	24	-0.4	337
Range	0.33-15.7	6.0-100	4.0-88.6	0.4-31.3	555-4043	5.5-15.5	0.03-1.77	4.0-6.7	0-97.5	5-70	-1-1.06	130-781

Note: ***P<0.001, ** P<0.01, *P<0.05, ns=non-significant P>0.05.

Table 1.2 A correlation matrix for the variables in the 50 cm depth zone (two-sided, Pearson).

	1	2	3	4	5	6	7	8	9	10	11	12
1. Al %	-											
2. BS %	0.156*	-										
3. CEC	0.159*	-0.115^{ns}	-									
4. C %	-0.076^{ns}	-0.280***	0.877***	-								
5. Rainfall	-0.404***	-0.509***	-0.121^{ns}	0.069^{ns}	-							
6. Temperature	-0.154*	0.148^{ns}	-0.006^{ns}	0.096^{ns}	0.191*	-						
7. N %	0.246**	-0.213**	0.688***	0.769***	-0.019^{ns}	0.089^{ns}	-					
8. pH	0.428***	0.709***	-0.148*	-0.307***	-0.481***	0.103^{ns}	-0.080^{ns}	-				
9. P retention	0.549***	-0.467***	0.482***	0.432***	0.027^{ns}	0.003^{ns}	0.686***	-0.027^{ns}	-			
10. clay %	0.303***	0.171*	0.264***	-0.021^{ns}	-0.262***	-0.026^{ns}	0.039^{ns}	0.053^{ns}	0.145^{ns}	-		
11. log Al_{KCl}	-0.222**	-0.660***	0.256***	0.213**	0.228**	-0.290***	-0.002^{ns}	-0.764***	0.123^{ns}	0.174*	-	
12. precipitation variability	-0.444***	-0.470***	-0.155*	-0.009^{ns}	-0.009***	0.298***	-0.069^{ns}	-0.420***	0.014^{ns}	-0.182*	0.250***	-
Mean	7.4	37.2	13.9	2.5	1362	11.7	0.2	5.4	41	24.6	-0.04	328
Range	0.6-12.3	1.0-100	0.9-132.0	0.2-43	555-4043	5.5-15.4	0.0-0.8	4.0-6.8	1-99	5-70	-1.0-1.2	131-781

Note: ***P<0.001, ** P<0.01, *P<0.05, ns=non-significant P>0.05.

Table 1.3 A correlation matrix for the variables in the 120 cm depth zone (two-sided, Pearson).

	1	2	3	4	5	6	7	8	9	10	11	12
1. Al %	-											
2. BS %	0.010^{ns}	-										
3. CEC	0.015^{ns}	-0.110^{ns}	-									
4. C %	-0.151^{*}	-0.208^{**}	0.881^{***}	-								
5. Rainfall	-0.22^{**}	-0.507^{***}	-0.037^{ns}	0.102^{ns}	-							
6. Temperature	0.087^{ns}	0.129^{ns}	0.142^{ns}	0.156^{*}	0.186^{*}	-						
7. N %	0.009^{ns}	-0.207^{**}	0.784^{***}	0.872^{***}	0.043^{ns}	0.103^{ns}	-					
8. pH	0.110^{ns}	0.742^{***}	-0.281^{***}	-0.345^{***}	-0.400^{***}	-0.043^{ns}	-0.213^{**}	-				
9. P retention	0.475^{***}	-0.517^{***}	0.372^{***}	0.288^{***}	0.118^{ns}	0.126^{ns}	0.469^{***}	-0.238^{**}	-			
10. clay %	0.3820^{***}	-0.078^{ns}	0.474^{***}	0.206^{**}	-0.140^{ns}	0.185^{*}	0.188^{*}	-0.213^{**}	0.355^{***}	-		
11. log Al_{KCl}	-0.010^{ns}	-0.619^{***}	0.364^{***}	0.246^{***}	0.174^{*}	-0.122^{ns}	0.190^{*}	-0.802^{***}	0.268^{***}	0.430^{***}	-	
12. precipitation variability	-0.161^{*}	-0.484^{***}	-0.003^{ns}	0.055^{ns}	0.804^{***}	0.286^{***}	0.037^{ns}	-0.400^{***}	0.153^{*}	0.034^{ns}	0.260^{***}	-
Mean	8.2	37.8	12.4	1.5	1364	11.8	0.1	5.6	39.9	25	-0.1	330
Range	3-18	0-100	1-137	0.1-42	555-4043	5.5-15.5	0.0-0.9	3.8-7.7	1-99	5-85	-1-1	130-781

Note: ***P<0.001, ** P<0.01, *P<0.05, ns=non-significant P>0.05.

2 Appendix B Ashburton Lakes Catchment study

Table 2.1 Abbreviations of the different soil characteristics presented in the soil description tables.

Horizon Boundary		Texture	
distinct	d	silt loam	zl
gradual	g	loamy silt	lz
wavy	w	silty clay loam	zcl
diffuse	dif	gravel	g
irregular	ir	sandy loam	sl
smooth	s	loamy sand	lsa
Roots		Structure	
many	many	strong	4
common	many	moderate	2
few	few	weak	1
very fine	VF	well developed	3
fine	few	fine	fi
medium	many	medium	med
medium and fine	M-F	coarse	co
very fine to medium	VF-M	blocky	blk
coarse	C	nutty	nty
Strength		granular	gr
loose	loose		
weak	weak		
slightly firm	sl firm		
moderately firm	mode firm		
very firm	v firm		
very weak	vweak		

Table 2.2 Soil descriptions of the alluvial fan sites in the Ashburton Lakes catchment.

Horizon	Depth	Boundary	Colour (moist)	Field Texture	Structure	Soil Strength	Roots	Site Notes
AF1								
Ah	0 - 16	d,s	10YR 3/2	zl	2,gr	vweak	many, VF-M	
BW1	16 - 34	d,w	10YR 3/4	zl	2,gr	weak	many, VF-M	Worm mixing in the BW.
BW2	34 - 63		10YR 3/4	sl	1,sg		many, VF-F	Subrounded alluvial gravels. Some coarse gravels (~60%). Weak, loose structure. Held together by gravels.
MO8								
Ah	0 - 20	d,s	10YR 3/3	zl	2,med & co,blk	sl firm	many, VF-M	Some fines in the structure.
Bw	20 - 84		2.5Y 5/4	zl	2,med & co,blk	sl firm	common, VF-F	Silt loam with estimated 15% clay. One subrounded cobble in this horizon. Some fines in the structure
Gravels								A gravel layer.
AF3								
Ah	0 - 11	d,s	7.5YR 3/2	lz	2, vfi & fi,blk	weak	many, VF-F	
BW	11 cm - 22	d,s	10YR 3/4	sl	2,fi,blk	weak	many, VF-F	Worm mixing.
B/C1	22 - 34	d,s	10YR 3/4	sl	1	loose	many, VF-F	Structureless (unconsolidated). Medium angular gravels 50%.
B/C2	34 - 56		10YR 3/4	g	1	loose	common, VF-F	Structureless (unconsolidated). Fine gravels 50%.

AF4								
Ah	0 - 17	d,s	10YR 3/2	lz	2, fi, gr	weak	many, M-F	
Bw1	17 - 35	d,s	10YR 5/4	sl	2, med, blk	weak	common, F	Angular coarse gravels 20%.
Bw2	35 - 55		10YR 5/6	sl	2,fi,gr	vweak	common, F	Angular 10-100 mm. 30% medium gravels to cobbles.
AF5								
Ah	0 - 12	d,s	10YR 3/2	sl	2,fi,gr	vweak	many, VF	Roots - 60-70% top 10 cm. 30% 10-35 cm and less below.
AB	12- 1 8	d,s	7.5YR 3/2	lz	2,fi&med,gr	vweak	common, VF	
BW1	18 - 45	d,w	10YR 4/4	sl	2,fi,blk	weak	common, VF	
BW2	45 - 60		2.5Y 5/6	zl	2,fi,blk	weak	few, VF	Angular coarse gravel to boulders, (70-80%), 5-10 mm angular clasts. 80-90% small loose aggregates

Table 2.3 Soil descriptions of the outwash surface sites in the Ashburton Lakes catchment.

Horizon	Depth	Boundary	Colour (moist)	Texture	Structure	Strength	Roots	Site Notes
OF2								
Ah	0 - 12	d,s	10YR 3/2	sl	2,co,gr	weak	many, VF-F	Fluffy soils.
BW1	12- 3 0	d,w	10YR 3/4	sl	2,co,gr	vweak	common, VF-F	
BW2	30 - 60		10YR 3/4	sl	1,sg	loose	many, VF-F	Structureless, held between rocks. Subrounded 50% course gravels, med gravels, cobbles and stones. Weathered.
MO1								
Ah	0 - 10	d,s	10YR 3/2	zl	2,med,gr	sl firm	many, VF-F	
BW1	10- 5 2	d,w	10YR 4/4	zl	2,med,gr	weak	few, VF-F	Worm mixing in the top of this horizon.
BW2	52 - 69		10YR 4/4	lsa	1,fi&med,gr	loose	many, VF	Subrounded cobbles 25% stones, coarse gravels.
MO7								
Ah	0 - 19	d,w	10YR 3/2	zl	4,fi&med,blk	sl firm	many, VF-M	
BW1	19 - 77	d,w	2.5YR 5/4	zl	2,fi&med,blk	weak	common, VF-F	Piece of charcoal at 36cm. Worm holes present in the BW horizon and mixing in the top of the BW. Silt loam - 15% clay.
BW2	77 - 105		2.5YR 5/4	zl	2,co,blk	weak	common, VF-F	Silt loam - 15% clay Subrounded cobbles and coarse gravels.

Gravels								
OF3								
Ah	0 - 17	g,w	10YR 4/3	zl	1,Vfi&fi,blk	vweak	many,VF-F	Coarse gravel to boulders 10-25%, subrounded.
Bw1	17 - 37	d,s	2.5Y 6/4	zl	1,Vfi&fi,blk	weak	many,VF-F	Coarse gravel to boulders 10-25%, subrounded.
Bw2	37 - 40		2.5Y 6/4	zl	1,sg	vweak	many,VF-F	Fine gravels to medium boulders. Loose fine angular gravel 75% (structureless).
OF4								
Ah	0 - 13	g,w	10YR 4/3	zl	2,Vfi&fi,blk	loose	many,VF-M	Base of the Ah horizon, evidence of worm mixing.
Bw1	13 - 30	d,w	2.5Y 6/4	zl	2,Vfi&med,blk	vweak	many,VF-F	Secondary colour is worm mixing in this horizon. This horizon was texturally slightly more sticky than the Ah horizon.
Bw2 gravels	30 - 50		2.5Y 6/4	zl	2,fi & med,blk	vweak	common,VF-F	Boulders, spherical, medium gravels to boulders 75%.
OF5								
Ah	0 - 12	g,s	10YR4/3	zl	2,fi & med,blk	vweak	many,VF-M	Worm mixing of Ah into BW
BW	12- 6 0	d,s	2.5Y 5/4	zl	1,fi & med,blk	sl firm	common,VF-F	
Gravels	60 +							Fine subangular gravels

Table 2.4 Soil descriptions of the moraine sites in the Ashburton Lakes catchment

Horizon	Depth	Boundary	Colour (moist)	Texture	Structure	Strength	Roots	Site Notes
OF1								
Ah	0 - 11	d,s	10YR 3/2	zl	2,med&co,gr	sl firm	many, VF-F	There were some larger, subangular peds.
BW1	11- 3 5	d,w	10YR 4/3	zl	1,vf&fi,gr	weak	common, VF-F	
BW2	35 - 55		10YR 4/4	lsa	1,vf&fi,gr	loose	many, VF-F	Subrounded 70-75% coarse gravels. 2% cobbles and medium gravels too.
MO2								
Ah	0 - 18	g,w	10YR 4/3	zl	2, fi& med,gr	weak	many, VF-M	
BW1	18 - 37	d,s	2.5Y 6/4	zl	2,vf&fi,blk	weak	common, VF-M	Worm mixing between the Ah and B horizons. Subrounded boulder. Med-coarse gravels 1-2%.
BW2	37 - 65		2.5Y 6/4	lz	1,sg	loose	common, VF-M	Few boulders. Gravels 50%, VF. Structureless, loose.
MO3								
Ah	0 - 26	g,w	10YR 4/3	zl	4,fi,fi	sl firm	many, VF-M	Top 3 cm colluvial gravels.
BW	26 - 65		2.5Y 6/6	zcl	2,fi,gr	vweak	common, VF-F	10% medium gravels to boulders.
MO5								
Ah	0 - 24	d,w	10YR 4/3	zl	2, fi & med, blk	sl firm	many, M-F	Soil held together well by plant roots.
BW1	24 - 55	g,w	10YR 4/4	zl	2, fi & med, blk	sl firm	many, VF-F	Worm mixing.
BW2	55 - 65		2.5Y 5/4	zl	2, fi & med, blk	sl firm	common, VF-F	Boulders.

MO4								
Ah	0 - 22	d,s	10YR 4/3	zl	4,med ,blk	weak	many, VF-M	Strong structure-held together by roots.
BW1	22 - 79	g,s	2.5 Y 6/6	zl	2,med&co,blk	sl firm	common, VF-F	
BW2	79 - 106		2.5Y 5/4	zl	2,co ,blk	weak	few, VF-F	
Gravels								Subangular and subrounded gravels at 100 cm (coarse).
MO6								
Ah	0 - 17	d,s	10YR 5/3	zl	4,fi &med, gr	vweak	many, VF-M	Semi-deformable but only because of roots holding.
BW	17 - 66		10YR 6/6	zl	2,med&co,blk	vweak	common, VF-F	Silt loam -15-20% clay. Worm mixing in the top of the BW horizon.
Gravels								
MO9								
Ah	0 - 13	d,w	10YR 4/4	zl	4,Fi &med, blk	sl firm	many, VF-F	Charcoal was found at 5 cm depth.
AB	13 - 24	d,w	2.5Y 5/6	zcl	2,med&co, blk	sl firm	few, VF-F	Worm mixing. Large native earthworms at this site.
BW	24 - 80		2.5Y 5/6	zcl	2,med&co, blk	sl firm	common, VF-F	
MO10								
Ah	0 - 22	g,s	10YR3/2	zl	4,fi&med,blk	weak	many, VF-C	Surface-Angular medium stones. 50%. Worm mixing between horizons. Higher OM in Ah.
BW	22 - 50		10YR 5/4	zl	2,blk	sl firm	few, VF-F	Subsurface rounded stones to boulders. 50%

MO11								
Ah	0 - 25	g,s	10YR 4/3	zl	2,fi&med,blk	vweak	many, F-C	
BW	25 - 62		2.5Y 5/4	zl	2,vfi&med,blk	weak	common, VF-F	There was a firm layer in the B horizon, like in the MO12.
gravels								>62 cm gravels. Rounded boulders 15-25%, rounded coarse cobbles ~10 cm
MO12								
Ah	0 - 15	g,s	10YR 3/3	zl	2,fi, blk	vweak	many, VF-M	
Bw1	15 - 35	d,s	2.5Y 5/4	zl	2,fi&med,blk	weak	common, VF-F	
Bw2	35 - 54		2.5Y 6/6	zl	1,blk	sl firm	few, VF	At 35 cm a hard pan. Angular medium gravels to stones, boulders? But didn't dig out.

Table 2.5 The Akaike information criterion (AIC) and *P* value for each factor model for pH_{H2O} and Al_{CaCl2} in the Ashburton Lakes Catchment.

Terms	AIC pH_{H2O}	P value (pH)	AIC $LogAl_{CaCl2}$	P value ($LogAl_{CaCl2}$)
rainfall	-409	0.029	-303	0.003
landform	-436	<0.001	-305	0.017
age	-483	<0.001	-335	<0.001
depth	-658	<0.001	-481	<0.001

Note: In the depth model site code was used as a random variable.

Table 2.6 The Akaike information criterion (AIC) for combined factor models for pH_{H2O} and Al_{CaCl2} in the Ashburton Lakes Catchment.

Terms	AIC pH_{H2O}	AIC $LogAl_{CaCl2}$
depth	-658	-481
depth + age	-654	-467
depth + landform	-650	-471
depth + rainfall	-642	-465
depth + age + landform + rainfall	-632	-452

Note: Values in the table are from a general linear model (site as a random variable).

Table 2.7 The Akaike information criterion (AIC) and *P* value for linear models involving individual factors applied to the testing subset for pH_{H2O} and Al_{CaCl2}.

Terms	AIC pH_{H2O}	P value (pH)	AIC $LogAl_{CaCl2}$	P value ($LogAl_{CaCl2}$)
rainfall	-114	0.479	-99	0.451
landform	-122	0.062	-105	0.240
age	-133	<0.001	-107	0.400
depth	-165	0.007	-111	0.131

Note: In the depth model site code was used as a random variable.

Table 2.8 The Akaike information criterion (AIC) and *P* value for linear models involving individual factors applied to the testing subset for pH_{H2O} and Al_{CaCl2}.

Terms	AIC pH_{H2O}	*P* value (pH)	AIC $LogAl_{CaCl2}$	P value ($LogAl_{CaCl2}$)
pH_{CaCl2}	-	-	-215	<0.001
carbon	-167	0.021	-115	0.169
nitrogen	-173	0.013	-120	0.165
CEC	-164	0.051	-112	0.625
BS	-166	0.388	-119	0.139

Note: Values in the table are from a general linear model (site code was used as a random variable).

3 Appendix C Glasshouse Experiment

3.1 Soil total metal and texture analyses

Total metal analysis for each soil (Table 3.1) was determined by microwave digestion of the soil (CEM MARS Xpress, CEM Corporation, USA) and measured on the ICP-OES (Varian 720 ICP-OES, Varian Australia Pty Ltd, Melbourne).

The proportion of sand, silt and clay were determined using Laser diffraction particle size analysis on a Malvern Mastersizer 2000 at the University of Waikato (Earth Science Department, School of Science, Faculty of Science and Engineering). Soil texture was determined using the New Zealand Soil Desciption Handbook (Milne *et al.*, 1995) and is presented in Table 3.2.

Table 3.1 The concentration of total elements (mg kg^{-1}) for the 10 native New Zealand soils used in this experiment**.**

Element concentration (mg kg^{-1})	WT	AR	PK	MO	GM	BD	OM	LP	GF	MG
Al	43047	7742	8038	33081	27373	35410	25000	26619	24367	26137
Ca	1779	1939	1499	1896	9504	7069	8752	10024	7098	7271
Fe	25524	7263	4722	22933	23462	26509	22878	24347	23791	27136
K	692	484	542	4889	3505	4258	3320	3913	3755	4203
Mg	1209	371	236	4173	5083	5173	4533	4055	3067	4178
Mn	289	260	729	304	347	429	382	568	364	681
Na	321	919	1581	134	148	130	106	148	99	136
S	854	407	554	302	584	202	149	274	221	283
Zn	40	28	28	55	64	69	67	56	48	76

Soil codes are listed by their geographic location from North to South.

Table 3.2 The particle size distribution analysis (soil texture) for the 10 glasshouse soils, giving the exact percentage of clay, silt and sand.

Soil	Clay % (0.05- 2 µm)	Silt % (2-20 µm)	Sand % (20-200 µm)	Texture (NZSC)
WT	27.8	38.3	33.5	Clay loam
AR	0.6	13.7	85.7	Sand
PK	0.7	14.1	85.2	Sand
MO	17.0	31.8	50.9	Sandy loam
GM	12.9	39.3	47.6	Sandy loam
BD	13.8	36.6	49.4	Sandy loam
OM	9.3	35.9	54.5	Sandy loam
LP	5.8	32.3	62.0	Loamy sand
GF	4.1	43.7	52.2	Loamy silt
MG	3.3	46.8	49.9	Loamy silt

Soil codes are listed by their geographic location from North to South.

3.2 Soil moisture

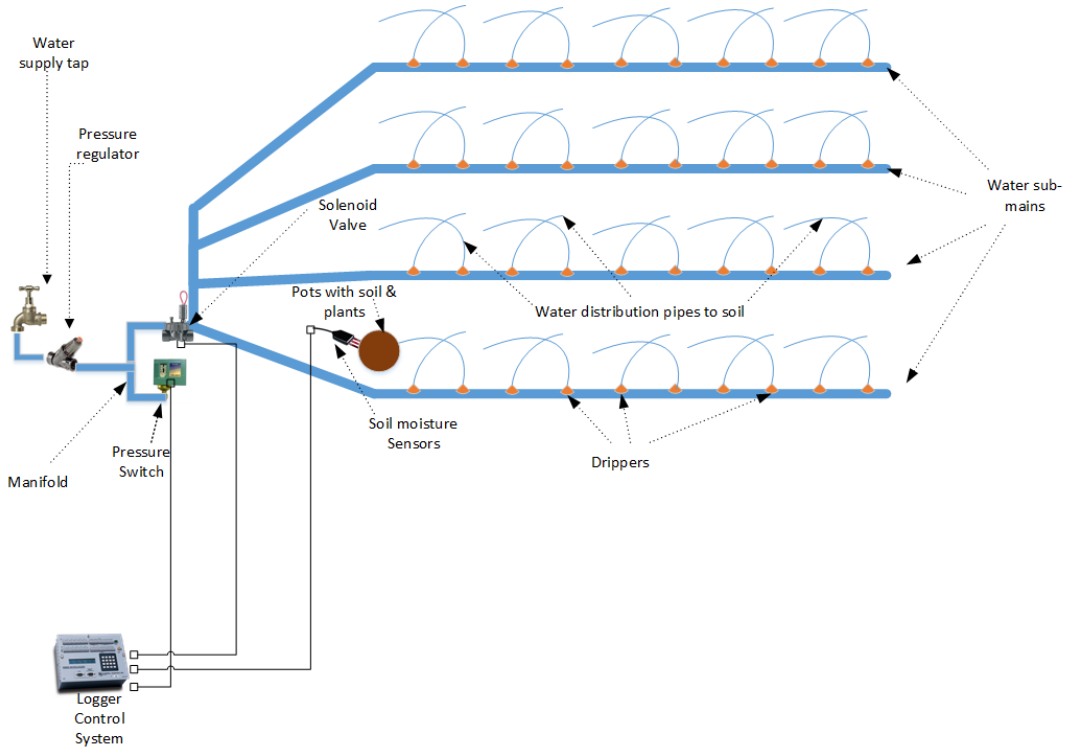


Figure 3.1 A diagram showing the automatic dripper irrigation system set up in the glasshouse.

Table 3.3 Average daily Volumetric Water Content (%) for WT, AR, PK, MO, OM, LP and MG soils over the experimental period, as recorded by the data logger and the moisture/temperature sensors. The maximum VWC % was set at 50%, as this value is above soil field capacity and near soil saturation.

Soil	Mean daily Volumetric Water Content (%)
WT	36.2
AR	29.2
PK	24.9
MO	25.9
OM	22.0
LP	18.8
MG	37.0
Grand mean	27.7

The irrigation regime was designed to provide water so that there was no significant limitation to plant growth due to water stress throughout the experiment. The values reflect that there was a constant supply of water to the plants for all soils that was maintained in this experiment.

Table 3.4 Mean soil pH_{H2O} (n=8) at the lime rates of 0, 2, 4, 8 or 12 t lime ha^{-1} and across all soils.

Soil	Lime rate applied (t lime ha^{-1})				
	0	2	4	8	12
WT	5.2_{wx}	5.5_{s}	5.9_{n}	6.4_{jk}	6.7_{h}
AR	5.4_{tuv}	5.9_{no}	6.6_{i}	7.3_{bc}	7.5_{a}
PK	5.1_{y}	5.8_{pq}	6.1_{m}	6.9_{g}	7.4_{b}
MO	$5.0y_{z}$	5.5_{st}	5.9_{no}	6.7_{h}	7.2_{cd}
GM	5.0_{z}	5.3_{vw}	5.8_{pqr}	6.5_{ij}	7.0_{f}
BD	5.4_{tu}	5.8_{op}	6.2_{lm}	6.8_{g}	7.1_{ef}
OM	5.3_{uvw}	5.9_{no}	6.5_{j}	7.2_{cd}	7.3_{bc}
LP	5.7_{qr}	6.2_{lm}	6.6_{i}	7.2_{de}	7.2_{de}
GF	$5.0y_{z}$	5.7_{r}	6.1_{m}	6.9_{g}	7.2_{de}
MG	5.1_{xy}	5.8_{pq}	6.3_{kl}	7.1_{ef}	7.2_{cd}

Numbers with letter subscripts in common are not different (α=0.05) based on Fisher's protected LSD. Statistically significant differences for soil pH are not always visible due to rounding.

Table 3.5 Back-transformed mean Olsen P at the lime rates of 0, 2, 4, 8 or 12 t lime ha^{-1} a and across all soils.

Soil	Lime rate applied (t lime ha^{-1})				
	0	2	4	8	12
WT	8_{wx}	7_{A}	7_{B}	6_{CD}	6_{CD}
AR	31_{a}	22_{b}	16_{fgh}	17_{de}	20_{c}
PK	17_{d}	14_{jk}	11_{op}	10_{rs}	13_{l}
MO	18_{d}	12_{no}	9_{uv}	11_{pq}	17_{de}
GM	21_{bc}	16_{fg}	14_{ijk}	14_{k}	16_{ef}
BD	9_{uv}	8_{zA}	7_{B}	8_{xyz}	9_{v}
OM	9_{v}	5_{E}	5_{F}	6_{DE}	6_{C}
LP	9_{v}	8_{yzA}	8_{w}	10_{st}	11_{qr}
GF	13_{lm}	11_{pq}	8_{wxy}	12_{mn}	15_{hi}
MG	11_{pqr}	10_{tu}	10_{tu}	15_{hij}	15_{gh}

Mean Olsen P have been back-transformed from the log form which was analysed. Numbers with letter subscripts in common are not different (α=0.05) based on Fisher's protected LSD. Statistically significant differences for Olsen P are not always visible due to rounding.

Table 3.6 Equations for fitted lines for each soil in Figure 5.3 in the thesis.

Soil	Equation type	Equation	R^2	P value
WT	Exponential Decay, Single, 2 Parameter	$y = 3.074*10^8*\exp(-3.501*x)$	0.96	0.0031
AR	Exponential Decay, Single, 2 Parameter	$y = 1.906*10^8*\exp(-3.318*x)$	0.99	0.0007
PK	Exponential Decay, Single, 2 Parameter	$y = 1.906*10^6*\exp(-2.474*x)$	1.00	<0.0001
MO	Exponential Decay, Single, 2 Parameter	$y = 3.481*10^8*\exp(-3.511*x)$	0.97	0.0027
GM	Exponential Decay, Single, 2 Parameter	$y = 2.579*10^8*\exp(-3.457*x)$	0.94	0.0064
BD	Exponential Decay, Single, 2 Parameter	$y = 1.364*10^6*\exp(-2.485*x)$	0.87	0.0218
OM	Exponential Decay, Single, 2 Parameter	$y = 3.023*10^7*\exp(-3.051*x)$	0.94	0.0063
LP	Exponential Decay, Single, 2 Parameter	$y = 5.127*10^2*\exp(-1.195*x)$	0.87	0.202
GF	Exponential Decay, Single, 2 Parameter	$y = 2.158*10^7*\exp(-3.014*x)$	1.00	<0.0001
MG	Exponential Decay, Single, 2 Parameter	$y = 8.020*10^6*\exp(-2.887*x)$	0.99	0.0003

Table 3.7 Equations for fitted lines for Caucasian clover yields and soil pH each soil in Figure 5.4 a, c and e) in the thesis.

Soil	Equation type	Equation	R^2	P value
WT	Polynomial, Quadratic	y = -14.846+5.282*x-0;.366*x^2	0.64	0.3567
AR	Polynomial, Quadratic	y = 23.931+-4.092*x+0.223*x^2	0.93	0.0684
PK	Polynomial, Quadratic	y =-20.920+9.688*x-0.897*x^2	0.95	0.0478
MO	Polynomial, Quadratic	y = -226.753+80.994*x-6.785*x^2	0.99	0.0069
GM	Polynomial, Quadratic	y = -3.123+5.744*x-0.537*x^2	0.52	0.4836
BD	Polynomial, Quadratic	y = -38.576+13.398*x-0.790*x^2	1.00	0.0004
OM	Polynomial, Quadratic	y = -19.492+8.865*x-0.616*x^2	0.84	0.1577
LP	Polynomial, Quadratic	y = -15.149+10.937*x-0.995*x^2	0.82	0.1795
GF	Polynomial, Quadratic	y = -155.355+57.390*x-4.922*x^2	1.00	0.0029
MG	Polynomial, Quadratic	y = -86.939+35.618*x-3.201*x^2	0.99	0.0093

Table 3.8 Equations for fitted lines for lucerne yields and soil pH each soil in Figure 5.4b,d and f in the thesis.

Soil	Equation type	Equation	R^2	P value
WT	Exponential rise to maximum, single, 3 parameter	$y=-2.119*10^{1}+2.528*10^{4}*(1-\exp(-1.685*10^{-4}*x))$	0.99	0.0075
AR	Exponential rise to maximum, single, 3 parameter	$y=-6.508*10^{7}+6.508*10^{7}*(1-\exp(-2.927*x))$	0.97	0.0292
PK	Polynomial, Quadratic.	y=-66.328+24.179*x-1.996 *x^2	0.83	0.1741
MO				
GM	Exponential rise to maximum, single, 3 parameter	$y=-7.658*10^{7}+7.658*10^{7}*(1-\exp(-3.207*x))$	0.93	0.0725
BD	Polynomial, Quadratic.	y= -262.188+82.805*x-6.120 *x^2	0.94	0.0647
OM	Polynomial, Quadratic.	y=-107.162+34.228*x-2.404 *x^2	0.96	0.0439
LP	Polynomial, Quadratic.	$y=-1.3*10^{2}+4.539*10^{1}*x-3.318 *x^2$	0.97	0.0311
GF	Peak, Pseudo-Voigt, 4 parameter	y = 12.821*(0.074*(1/(1+((x-6.195)/0.849)^2))+(1-0.074)*exp(-0.5*((x-6.195)/0.849)^2))	0.97	0.2357
MG	Polynomial, Quadratic.	y=--113.604+45.987*x-4.120 *x^2	0.93	0.0661

Table 3.9 Equations for fitted lines for Caucasian clover root yields for each soil in Figure 5.5a, c and e in the thesis.

Soil	Equation type	Equation	R^2	P value
WT	Polynomial, Quadratic.	y = -17.898+6.068*x-0.451*x^2	0.91	0.0951
AR	Polynomial, Quadratic.	y = 23.636-6.082*x+0.467*x^2	0.101	0.8990
PK	Polynomial, Quadratic.	y = -15.375+6.527*x-0.582*x^2	0.89	0.1087
MO	Polynomial, Quadratic.	y = -57.394+21.763*x-1.858*x^2	0.82	0.1810
GM	Polynomial, Quadratic.	y = 15.988-2.405*x+0.131*x^2	0.71	0.2947
BD	Polynomial, Quadratic.	y = -15.132+6.145*x-0.409 *x^2	0.69	0.3081
OM	Polynomial, Quadratic.	y = 28.158-6.715 *x+0.492*x^2	0.41	0.5897
LP	Polynomial, Quadratic.	y = -74.546+25.943*x-0.244*x^2	0.93	0.0703
GF	Polynomial, Quadratic.	y = -64.569+24.886*x-2.194*x^2	0.99	0.0151
MG	Polynomial, Quadratic.	y = 18.654+-1.178*x-0.160*x^2	0.98	0.0178

Table 3.10 Equations for fitted lines for each soil in Figure 5.5b, d and f in the thesis.

Soil	Equation type	Equation	R^2	P value
WT	Polynomial, Quadratic.	y = 15.498-6.262 *x+0.679*x^2	0.92	0.0786
AR	Polynomial, Quadratic.	y= -48.163+16.654*x-1.299 *x^2	0.67	0.3317
PK	Polynomial, Quadratic.	y = -26.820+9.655*x-0.781 *x^2	0.64	0.3628
MO	Peak, Log Normal, 3 Parameter	y = if(x<=0, 0,68.075*exp(-0.5*(ln(x/6.359)/0.109)^2)/x)	0.98	0.0214
GM	Polynomial, Quadratic.	y = -110.002+38.507*x-3.118 *x^2	0.85	0.1509
BD	Polynomial, Quadratic.	y= -199.495+63.121*x-4.726 *x^2	0.98	0.0196
OM	Polynomial, Quadratic.	y= -37.225+11.878*x-0.790 *x^2	1.00	0.0050
LP	Peak, Weibull, 4 parameter.	y = if(x<=6.651-4.051e+4*((3.3193+4-1)/3.3193+4)^(1/3.3193+4), 0, 12.713*((3.3193+4-1)/3.3193+4)^((1-3.3193+4)/3.3193+4) * (abs((x-6.651)/4.051e+4((3.3193+4-1)/3.3193+4)^(1/3.3193+4))^(3.3193+4-1)) * exp(-abs((x-6.651)/ 4.051e+4((3.3193+4-1)/3.3193+4)^(1/3.3193+4))^3.3193+4+(3.3193+4-1)/3.3193+4))	0.46	0.8408
GF	Peak, Gaussian, 4 Parameter.	y= -1.460+9.331*exp(-.5*((x-6.107)/0.829)^2)	0.99	0.1190
MG	Polynomial, Linear.	y= 34.227-4.529 *x	0.99	0.0004

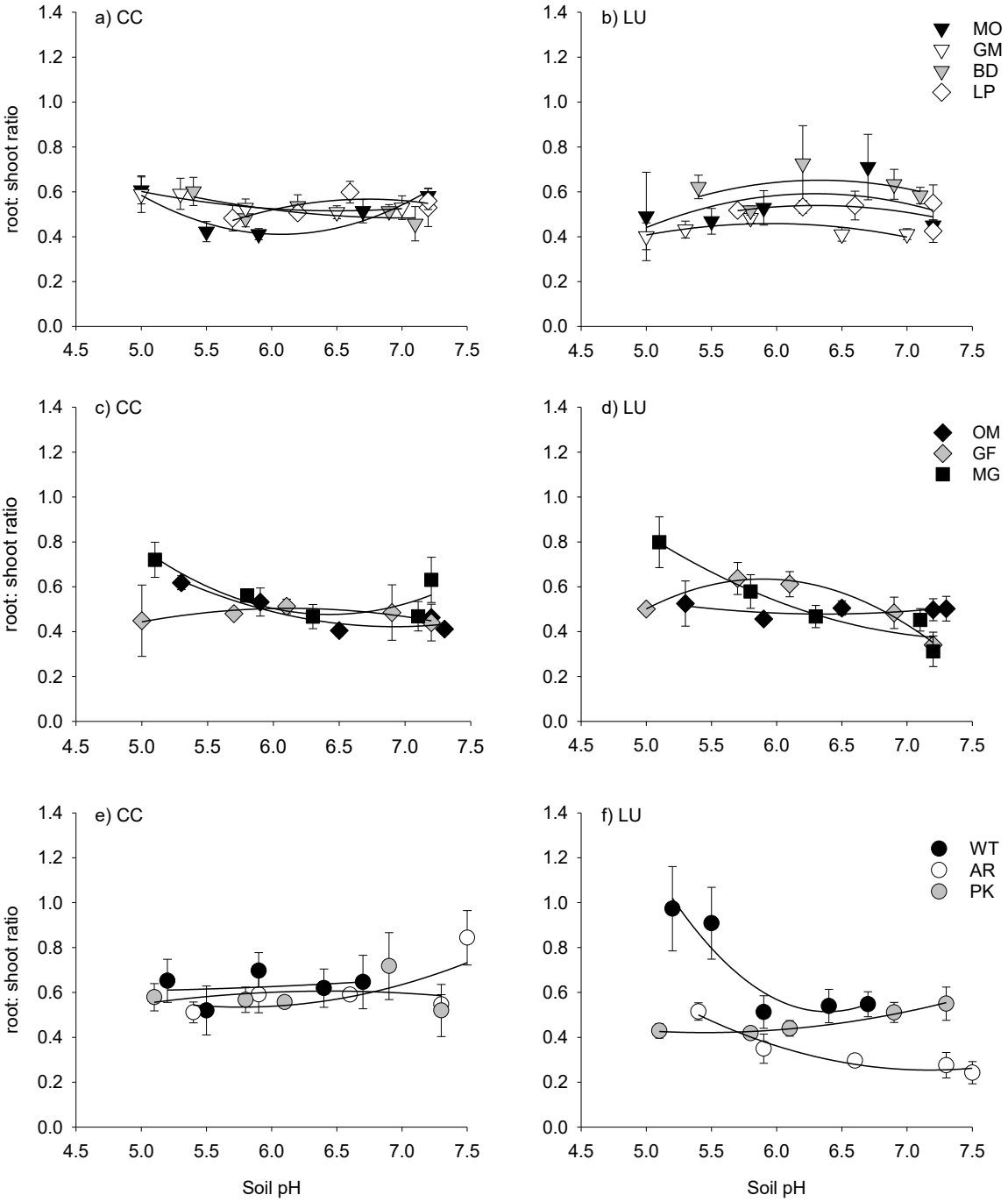


Figure 3.2 Mean total root: shoot ratio of Caucasian clover (e) and lucerne (f) and soil pH_{H2O} obtained with the application of 0, 2, 4, 8 and 12 t lime ha^{-1} on all soils. Soil acronyms are described in Table 5.2. The standard error bars indicate the standard error of the mean ± one SEM (n=4). The equations for fitted lines, R^2 and P value are presented in Table 3.11 and Table 3.12 in the appendix.

Table 3.11 Equations for fitted lines for Caucasian clover root: shoot ratio and pH for each soil in Figure 3.2a, c and e in the appendix.

Soil	Equation type	Equation	R^2	P value
WT	Polynomial, Quadratic.	y = 0.727-0.059*x+0.007*x^2	0.05	0.9469
AR	Polynomial, Quadratic.	y = 2.718-0.758*x+0.066*x^2	0.47	0.5351
PK	Polynomial, Quadratic.	y= -0.550+0.359*x-0.028*x^2	0.06	0.9370
MO	Polynomial, Quadratic.	y= 5.999-1.841*x+0.152*x^2	0.87	0.1346
GM	Polynomial, Quadratic.	y= 2.148-0.503*x+0.039*x^2	0.89	0.1130
BD	Polynomial, Quadratic.	y = 2.216-0.492*x+0.035*x^2	0.52	0.4815
OM	Polynomial, Quadratic.	y= 4.154-1.078 *x+0.078*x^2	0.90	0.1111
LP	Polynomial, Quadratic.	y = -3.439+1.189*x-0.088 *x^2	0.65	0.3551
GF	Polynomial, Quadratic.	y=-1.292+0.587*x-0.048 *x^2	0.85	0.1458
MG	Polynomial, Quadratic.	y= 6.429-1.852 *x+0.144*x^2	0.77	0.2345

Table 3.12 Equations for fitted lines for lucerne root:shoot ratio and pH for each soil in Figure 3.2b, d and f in the appendix.

Soil	Equation type	Equation	R^2	P value
WT	Polynomial, Quadratic.	y= 15.090-4.562 *x+0.357*x^2	0.89	0.1128
AR	Polynomial, Quadratic.	y= 4.276-1.121 *x+0.078*x^2	0.95	0.0493
PK	Polynomial, Quadratic.	y= 1.578-0.425 *x+0.039*x^2	0.99	0.0113
MO	Polynomial, Quadratic.	y= -2.976+1.134*x-0.090 *x^2	0.29	0.7151
GM	Polynomial, Quadratic.	y= -1.522+0.665*x-0.056 *x^2	0.56	0.4437
BD	Polynomial, Quadratic.	y= -2.753+1.076*x-0.085*x^2	0.13	0.8708
OM	Polynomial, Quadratic.	y = 1.776-0.407 *x+0.032*x^2	0.34	0.6560
LP	Polynomial, Quadratic.	y= -1.997+0.805*x-0.064 *x^2	0.23	0.7713
GF	Polynomial, Quadratic.	y= -5.159+1.966*x-0.167*x^2	0.99	0.0135
MG	Polynomial, Quadratic.	y = 4.465-1.087 *x+0.072*x^2	0.93	0.0702

3.2.1 Nodule scores (lime and low lime datasets)

3.2.1.1 Main effects of lime, soil and species

Table 3.13 Mean nodule score for legumes grown on 10 New Zealand high and hill country soils supplied with 0, 2, 4, 8 or 12 t lime ha^{-1}.

		Lime dataset *nodule score
Lime (t lime ha^{-1})	*P* value	ns
Soil type	*P* value	***
Plant species	*P* value	***
Interactions	Soil x species	$**_i$
	Soil x lime/P	*
	Species x lime/P	ns
	Soil x lime/P x species	ns

Note: *** Significant at *P*<0.001 level, ** significant at *P*<0.01 level, * significant at *P*<0.05 level, ns- no significant difference. * Nodule score refers to specific numbers and attributes described in Table 5.7 of the thesis. The i symbol refers to important effects (high F value) and the d refers to discounted effects.

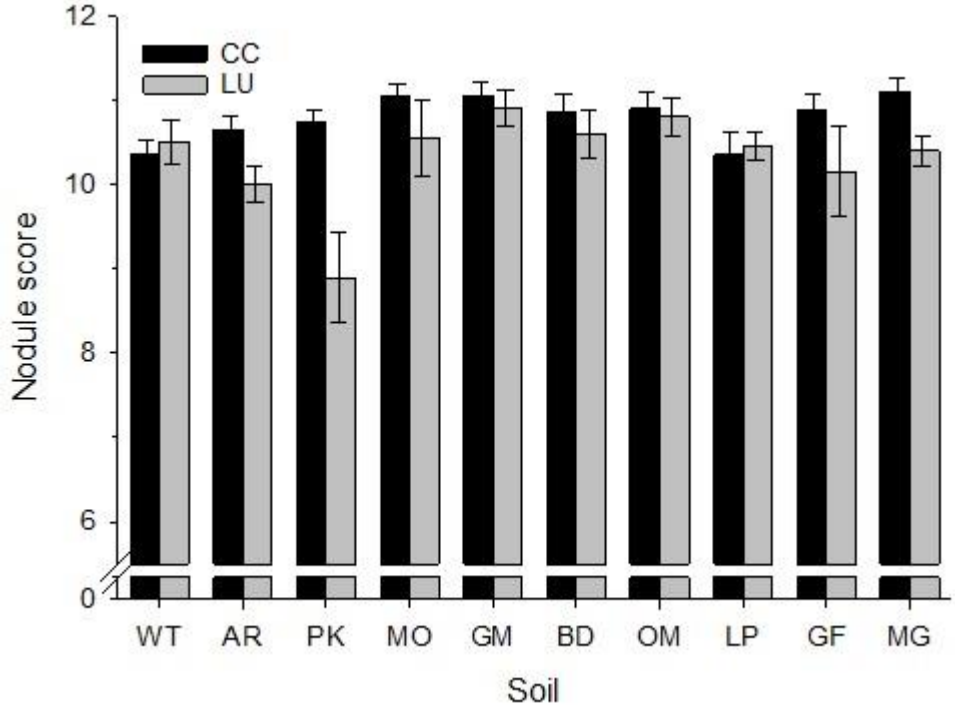


Figure 3.3 Mean nodule score for Caucasian clover (CC) and lucerne plants (LU) growing in all 10 soils for the lime dataset. The standard error bars indicate ± one SEM (n=20).

3.2.1.2 Other variables that influence nodule score (low lime dataset)

Table 3.14 The effect of soil type and lime applied (0, 2, 4, 8 or 12 t lime ha^{-1}) on the lucerne nodule score and the influence of covariates including soil measurements (log Al and log Olsen P) and shoot nutrient concentrations (K, log S, B, log Mn, log Zn, P, Mo, N and log Al).

			Low lime (0, 2, 4 t lime ha^{-1})
Main treatment effects			
Lime (t lime ha^{-1})/ P (mg P L^{-1} soil)		*P* value	ns
Soil type		*P* value	ns
Interaction		Soil x lime/P	ns
covariates	Log Soil Al		ns
	Shoot K%		ns
	Log shoot S (mg kg^{-1})		*
	Shoot B (mg kg^{-1})		ns
	Log shoot Mn (mg kg^{-1})		ns
	Log shoot Zn (mg kg^{-1})		ns
	Shoot P%		ns
	Shoot Mo (mg kg^{-1})		ns
	Log soil Olsen P		ns
	Shoot N%		ns
	Log shoot Al (mg kg^{-1})		*

Note: *** Significant at *P*<0.001 level, ** significant at *P*<0.01 level, * significant at *P*<0.05 level, ns- no significant difference. Soil pH was not included as a covariate for lime datasets due to the strong correlation between the treatment factor and the covariate.

3.3 Equations for the lines fitted for covariate graphs for the low lime dataset

Table 3.15 Linear trend lines and R^2 values for the relationship between lucerne shoot yield and log soil Al presented in Figure 5.6a in the thesis.

Soil	Equation for fitted linear trendline	R^2
WT	y = -2.1374x + 2.3116	1.00
AR	y = -8.2866x + 13.162	0.96
PK	y = -0.9583x + 6.245	0.55
MO	y = -13.527x + 14.783	0.91
GM	y = -9.9892x + 19.348	0.91
BD	y = -12.942x + 10.01	1.00
OM	y = -6.5041x + 9.3551	1.00
LP	y = -7.3834x + 17.717	0.99
GF	y = -6.576x + 9.6464	0.99
MG	y = 0.2187x + 13.816	0.90

Table 3.16 Linear trend lines and R^2 values for the relationship between lucerne shoot yield and shoot K% presented in Figure 5.6b in the thesis.

Soil	Equation for fitted linear trendline	R^2
WT	y = -3.68x + 9.4405	0.73
AR	y = -13.03x + 23.875	0.99
PK	y = -6.8313x + 13.398	0.93
MO	y = -22.386x + 33.451	0.99
GM	y = -21.283x + 35.393	0.98
BD	y = -27.817x + 58.722	1.00
OM	y = -20.333x + 36.873	0.99
LP	y = -11.879x + 38.384	0.94
GF	y = -27.817x + 58.722	1.00
MG	y = 0.8292x + 12.188	0.84

Table 3.17 Linear trend lines and R^2 values for the relationship between lucerne shoot yield and shoot B (mg kg^{-1}) presented in Figure 5.6c in the thesis.

Soil	Equation for fitted linear trendline	R^2
WT	y = -0.1497x + 6.5175	0.93
AR	y = -0.5747x + 24.592	0.98
PK	y = -0.0746x + 7.7987	0.48
MO	y = -0.9346x + 37.361	0.94
GM	y = -0.6876x + 27.633	0.93
BD	y = -0.488x + 28.222	0.84
OM	y = -0.3675x + 22.42	0.95
LP	y = -0.2166x + 30.345	0.97
GF	y = -0.5312x + 20.185	0.98
MG	y = 0.016x + 13.422	0.98

Table 3.18 Linear trend lines and R^2 values for the relationship between lucerne root yield and log soil Al presented in Figure 5.7a in the thesis.

Soil	Equation for fitted linear trendline	R^2
WT	y = -0.7698x + 1.6705	0.63
AR	y = -1.2406x + 4.6768	0.54
PK	y = -0.4546x + 2.669	0.67
MO	y = -7.1341x + 7.3959	0.85
GM	y = -5.5945x + 9.1795	0.99
BD	y = -7.8353x + 6.1945	0.81
OM	y = -2.6861x + 4.7047	0.92
LP	y = -4.7956x + 8.9451	0.98
GF	y = -4.4398x + 5.8399	0.97
MG	y = 3.3826x + 9.2018	1.00

Table 3.19 Linear trend lines and R^2 values for the relationship between lucerne root yield and shoot N% presented in Figure 5.7b in the thesis.

Soil	Equation for fitted linear trendline	R^2
WT	y = 1.4063x - 3.0119	R^2 = 0.8849
AR	y = 2.4464x - 5.0325	R^2 = 0.8882
PK	y = 1.6425x - 3.4719	R^2 = 0.8684
MO	y = 7.1028x - 19.818	R^2 = 0.8675
GM	y = 16.575x - 57.999	R^2 = 0.4726
BD	y = 6.1239x - 14.99	R^2 = 0.6803
OM	y = 7.9036x - 25.408	R^2 = 0.6322
LP	y = 9.65x - 26.068	R^2 = 0.4871
GF	y = 6.5144x - 18.007	R^2 = 0.9893
MG	y = -7.56x + 35.858	R^2 = 0.8969

Table 3.20 Linear trend lines and R^2 values for the relationship between lucerne root yield and shoot P% presented in Figure 5.7c in the thesis.

Soil	Equation for fitted linear trendline	R^2
WT	y = 2.446-3.644*x	0.01
AR	y = 24.048-57.626*x	0.45
PK	y = 17.538-55.375*x	0.13
MO	y = 103.499-439.539*x	0.94
GM	y = 21.795-67.944*x	0.99
BD	y = 27.565-91.829*x	0.77
OM	y = 1.110+19.433*x	0.01
LP	y = -5.226+73.762*x	0.92
GF	y = 20.297-62.011*x	0.99
MG	y = -7.776+74.495*x	0.34

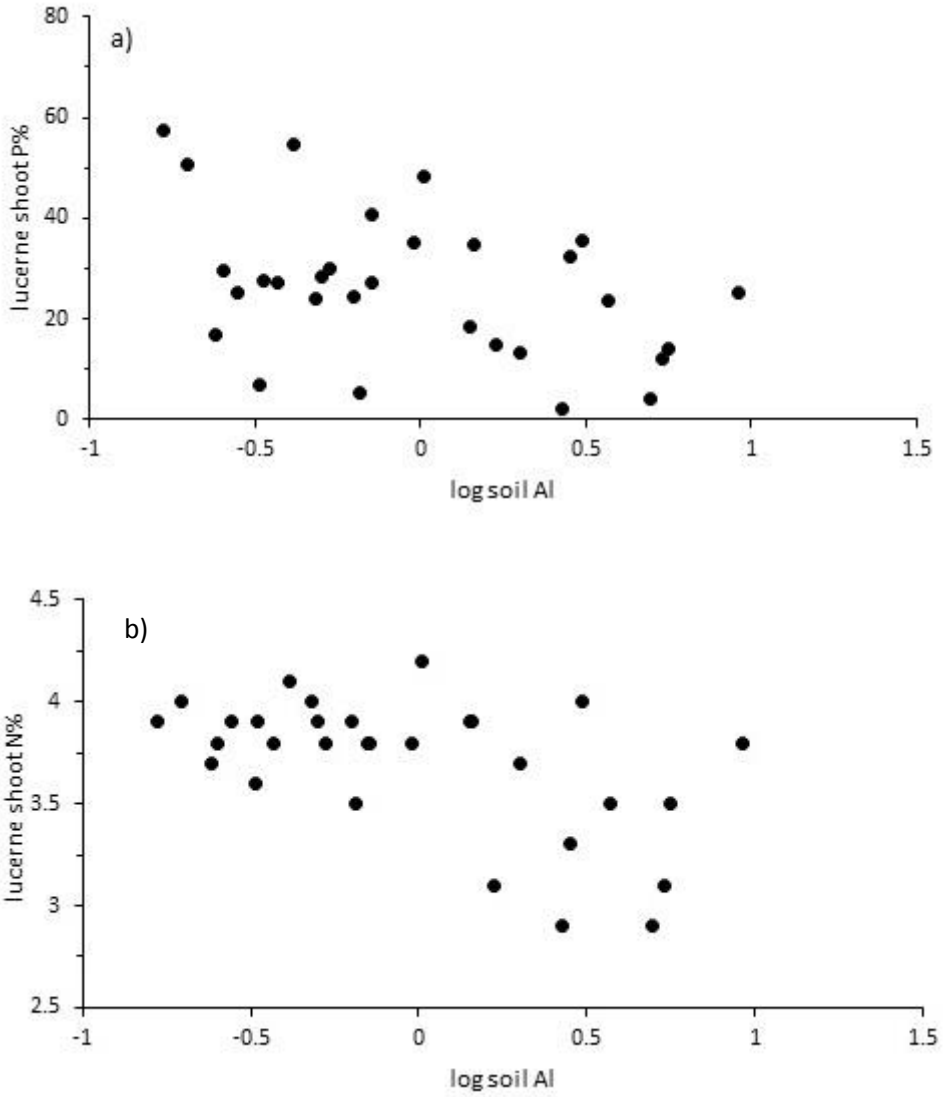


Figure 3.4 Mean log soil Al and shoot P% (a) and shoot N% (b) for lucerne plants growing in the low lime treatments (0, 2 and 4 t lime ha^{-1}) across all soils.

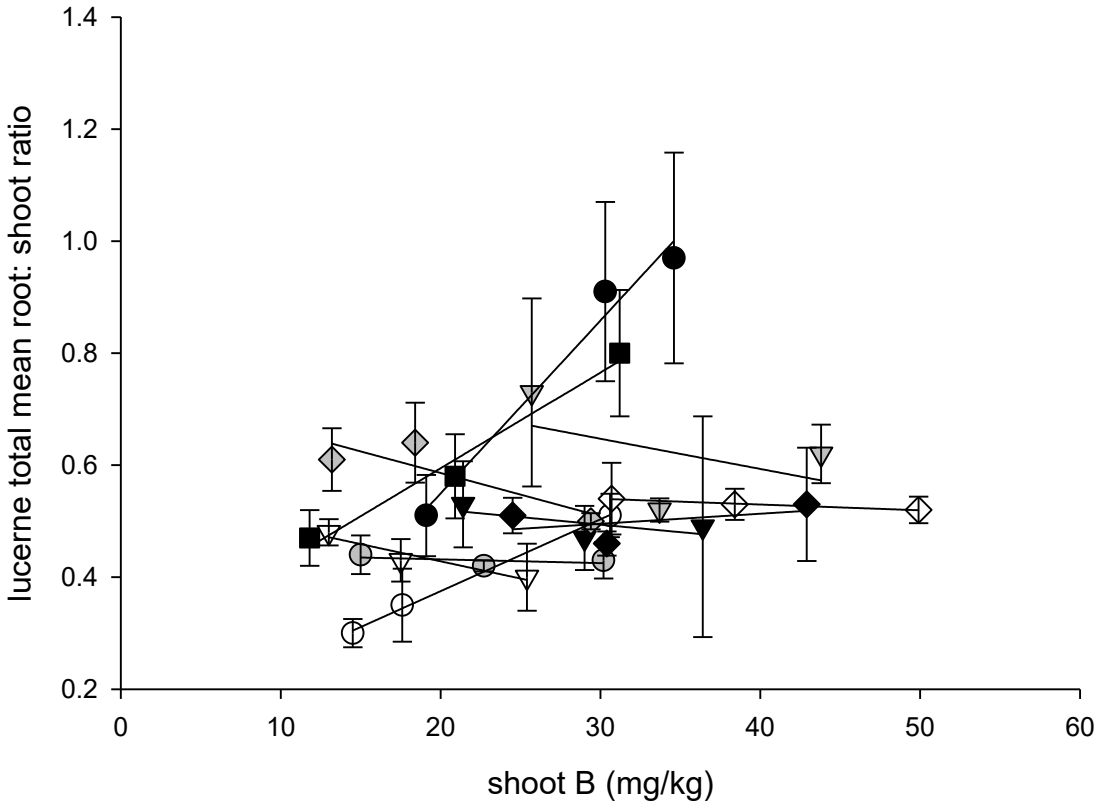


Figure 3.5 Mean total lucerne root: shoot ratio and shoot B concentration (mg kg^{-1}) with 0, 2 and 4 t lime ha^{-1} applied across all soils. Soil acronyms are described in Table 5.2 Linear trendlines have been fitted and equations are presented in Table 3.21 in the Appendix.

Table 3.21 Linear trend lines and R^2 values for the relationship between lucerne root: shoot ratio and B concentration (mg kg^{-1}) presented in Figure 3.5 in the appendix.

Soil	Equation for fitted linear trendline	R^2
WT	y = 0.0309x - 0.0683	0.98
AR	y = 0.0128x + 0.1186	1.00
PK	y = -0.0007x + 0.4451	0.26
MO	y = -0.0027x + 0.5745	0.44
GM	y = -0.0062x + 0.5513	0.91
BD	y = -0.0054x + 0.8088	0.22
OM	y = 0.0018x + 0.4416	0.22
LP	y = -0.001x + 0.5708	0.99
GF	y = -0.0077x + 0.7402	0.75
MG	y = 0.0171x + 0.2523	0.98

3.4 Shoot macronutrients concentrations and uptake (lime dataset)

Table 3.22 Mean total shoot N %, total shoot P (%), total shoot K (%) and total log S (mg kg^{-1}) concentration for legumes grown on 10 New Zealand high and hill country soils supplied with 0, 2, 4, 8 or 12 t lime ha^{-1}.

		Total shoot N (%)	Total shoot P (%)	Total shoot K (%)	Total log S
Lime (t lime ha^{-1})	P value	***	***	***	***
Soil type	P value	***	***	***	***
Plant species	P value	***	***	***	$***_i$
Interactions	Soil x species	***	***	***	**
	Soil x lime	***	***	***	***
	Species x lime	$***_i$	ns	$***_i$	***
	Soil x lime x species	$*_i$	$**_i$	*	ns

Note: *** Significant at *P*<0.001 level, ** significant at *P*<0.01 level, * significant at *P*<0.05 level, ns- no significant difference. The i symbol refers to important effects (high F value) and the d refers to discounted effects.

Table 3.23 Mean total shoot N uptake (+fixation) (mg pot^{-1}), total shoot P uptake (mg pot^{-1}), total shoot K uptake (mg pot^{-1}) and total shoot S uptake (mg pot^{-1}) for legumes grown on 10 New Zealand high and hill country soils supplied with 0, 2, 4, 8 or 12 t lime ha^{-1}.

		Total shoot N uptake (+fixation) (mg pot^{-1})	Total shoot P uptake (mg pot^{-1})	Total shoot K uptake (mg pot^{-1})	Total shoot S uptake (mg pot^{-1})
Lime (t lime ha^{-1})	P value	***	***	***	***
Soil type	P value	***	***	***	***
Plant species	P value	***	***	***	$***_i$
Interactions	Soil x species	$***_i$	***	***	***
	Soil x lime	***	***	***	***
	Species x lime	$***_i$	***	***	***
	Soil x lime x species	$***_d$	$***_i$	$***_i$	***

Note: *** Significant at *P*<0.001 level, ** significant at *P*<0.01 level, * significant at *P*<0.05 level, ns- no significant difference. Numbers with letter subscripts in common are not different (α=0.05) based on Fisher's protected LSD. The i symbol refers to important effects (high F value) and the d refers to discounted effects.

3.5 Shoot micronutrients concentrations and uptake (lime dataset)

Table 3.24 Mean shoot concentrations of Al, Mo, B, Mn and Zn (mg kg^{-1}) for legumes grown on 10 New Zealand high and hill country soils supplied with 0, 2, 4, 8 or 12 t lime ha^{-1}.

		mg kg^{-1}				
		Log Al	Mo	B	Log Mn	Log Zn
Lime (t lime ha^{-1})	*P* value	ns	***	$***_i$	$***_i$	$***_i$
Soil type	*P* value	***	***	***	***	***
Plant species	*P* value	ns	***	***	ns	***
Interactions	Soil x species	ns	***	ns	***	***
	Soil x lime	$*_i$	$***_i$	***	***	***
	Species x lime	ns	***	ns	***	***
	Soil x lime x species	ns	$***_d$	**	ns	ns

Note: *** Significant at *P*<0.001 level, ** significant at *P*<0.01 level, * significant at *P*<0.05 level, ns- no significant difference. The i symbol refers to important effects (high F value) and the d refers to discounted effects.

Table 3.25 Mean total shoot uptake concentrations of Mo, B and Zn (mg pot^{-1}) for legumes grown on 10 New Zealand high and hill country soils supplied with 0, 2, 4, 8 or 12 t lime ha^{-1}.

		Uptake mg pot^{-1}		
		Mo	B	Log Zn
Lime (t lime ha^{-1})	*P* value	***$_i$	***$_i$	***
Soil type	*P* value	***$_i$	***$_i$	***
Plant species	*P* value	***	***	***
Interactions	Soil x species	***	***	***
	Soil x lime	***	***	***
	Species x lime	***	***	***
	Soil x lime x species	ns	***$_d$	*$_i$

Note: *** Significant at *P*<0.001 level, ** significant at *P*<0.01 level, * significant at *P*<0.05 level, ns- no significant difference. The i symbol refers to important effects (high F value) and the d refers to discounted effects.

Table 3.26 Mean shoot N% concentration for lucerne growing in 0, 2 and 4 t lime ha^{-1} treatments on 10 New Zealand high and hill country soils.

	Lime rate (t lime ha^{-1})		
Soil	0	2	4
WT	2.9	3.5	3.6
AR	3.5	4.2	4.1
PK	3.5	3.9	3.7
MO	2.9	3.8	3.8
GM	3.8	4.0	3.9
BD	3.1	3.9	3.8
OM	3.7	4.0	3.9
LP	3.8	4.0	3.9
GF	3.1	3.9	3.9
MG	3.3	3.8	3.8

Table 3.27 Mean shoot P% concentration for lucerne growing in 0, 2 and 4 t lime ha^{-1} treatments on 10 New Zealand high and hill country soils.

	Lime rate (t lime ha^{-1})		
Soil	0	2	4
WT	0.22	0.22	0.19
AR	0.34	0.33	0.35
PK	0.27	0.27	0.27
MO	0.23	0.22	0.22
GM	0.26	0.21	0.20
BD	0.26	0.21	0.21
OM	0.21	0.20	0.22
LP	0.21	0.23	0.25
GF	0.29	0.22	0.20
MG	0.23	0.20	0.22

Table 3.28 Mean shoot S (mg kg^{-1}) concentration for lucerne growing in 0, 2 and 4 t lime ha^{-1} treatments on 10 New Zealand high and hill country soils.

	Lime rate (t lime ha^{-1})		
Soil	0	2	4
WT	5012	3981	3981
AR	3981	3981	3981
PK	3981	5012	3981
MO	3981	3162	3981
GM	5012	3981	3981
BD	3981	3981	3981
OM	3981	3981	3981
LP	3981	3981	3981
GF	5012	5012	3981
MG	3981	3981	3981

Mean S concentrations have been back-transformed from the log form which was analysed.

Table 3.29 Mean shoot Zn (mg kg^{-1}) concentration for lucerne growing in 0, 2 and 4 t lime ha^{-1} treatments on 10 New Zealand high and hill country soils.

	Lime rate (t lime ha^{-1})		
Soil	0	2	4
WT	100.0	63.1	39.8
AR	100.0	50.1	39.8
PK	125.9	50.1	39.8
MO	199.5	50.1	31.6
GM	100.0	50.1	39.8
BD	63.1	39.8	31.6
OM	63.1	31.6	31.6
LP	31.6	25.1	20.0
GF	125.9	39.8	25.1
MG	63.1	31.6	31.6

Mean Zn concentrations have been back-transformed from the log form which was analysed.

Table 3.30 Mean shoot Mo (mg kg^{-1}) concentration for lucerne growing in 0, 2 and 4 t lime ha^{-1} treatments on 10 New Zealand high and hill country soils.

	Lime rate (t lime ha^{-1})		
Soil	0	2	4
WT	0.062	0.001	0.096
AR	0.085	0.357	1.196
PK	0.051	0.236	0.902
MO	0.001	0.001	0.481
GM	0.087	0.256	0.432
BD	0.001	0.053	0.109
OM	0.025	0.341	0.860
LP	0.001	0.028	0.255
GF	0.001	0.001	0.235
MG	0.007	0.001	0.014

Table 3.31 Mean shoot Mn (mg kg^{-1}) concentration for lucerne growing in 0, 2 and 4 t lime ha^{-1} treatments on 10 New Zealand high and hill country soils.

	Lime rate (t lime ha^{-1})		
Soil	0	2	4
WT	158.5	79.4	50.1
AR	199.5	79.4	50.1
PK	631.0	251.2	199.5
MO	158.5	125.9	79.4
GM	251.2	79.4	63.1
BD	158.5	63.1	39.8
OM	158.5	79.4	63.1
LP	79.4	50.1	50.1
GF	199.5	100.0	63.1
MG	125.9	63.1	50.1

Mean Mn concentrations have been back-transformed from the log form which was analysed.

3.6 Analyses and equations for the lines fitted to the shoot yield graphs for the P dataset

Table 3.32 Mean soil pH_{H2O} for each species and across all soils for the P dataset (0, 30 and 150 mg P L^{-1} soil).

	Plant species	
Soil	CC	LU
WT	5.2_{cd}	5.4_{bc}
AR	5.4_{b}	5.2_{de}
PK	5.3_{cd}	5.2_{de}
MO	4.9_{hi}	5.3_{cd}
GM	4.8_{i}	5.0_{gh}
BD	5.3_{cd}	5.3_{bc}
OM	5.3_{bc}	5.3_{cd}
LP	5.6_{a}	5.6_{a}
GF	5.0_{ghi}	5.0_{fgh}
MG	5.1_{efg}	5.1_{ef}

Numbers with letter subscripts in common are not different (α=0.05) based on Fisher's protected LSD. Statistically significant differences for soil pH and Olsen P aren't always visible due to rounding.

Table 3.33 Mean soil pH_{H2O} for each P rate applied (0, 30 and 150 mg P L^{-1} soil) and across all soils.

Soil	P rate applied (mg P L^{-1} soil)		
	0	30	150
WT	5.2_{defghi}	5.2_{defgh}	5.4_{cd}
AR	5.4_{c}	5.2_{efghi}	5.3_{cdefg}
PK	5.1_{hijk}	5.2_{efghi}	5.3_{cdefg}
MO	5.0_{jklm}	5.1_{hijkl}	5.1_{ijkl}
GM	5.0_{klm}	4.8_{o}	4.8_{no}
BD	5.4_{bc}	5.3_{cdefg}	5.2_{defghi}
OM	5.3_{cdef}	5.4_{cde}	5.2_{efghi}
LP	5.7_{a}	5.6_{ab}	5.6_{ab}
GF	5.0_{jklm}	4.9_{mno}	4.9_{lmno}
MG	5.1_{ghijk}	5.1_{hijkl}	5.0_{klmn}

Numbers with letter subscripts in common are not different (α=0.05) based on Fisher's protected LSD. Statistically significant differences for soil pH and Olsen P aren't always visible due to rounding.

Table 3.34 Back-transformed mean soil extractable Al_{CaCl2} for each P rate applied (0, 30 and 150 mg P L^{-1} soil) and across all soils growing Caucasian clover plants (CC) and lucerne (LU).

Soil	P rate applied (mg P L^{-1} soil)					
	CC			LU		
	0	30	150	0	30	150
WT	$5.4_{ijklmnop}$	$5.2_{ijklmnop}$	$4.2_{lmnopqrst}$	3.0_{tuvw}	2.9_{uvw}	2.8_{uvw}
AR	6.9_{fghij}	6.0_{ghijkl}	3.3_{stuv}	$4.1_{mnopqrstu}$	6.9_{fghij}	6.7_{fghijk}
PK	6.5_{ghijk}	$4.9_{jklmnopqr}$	$5.6_{hijklmno}$	6.2_{ghijk}	9.6_{bcdef}	6.8_{fghij}
MO	12.8_{ab}	13.8_{ab}	11.7_{abc}	$5.5_{hijklmnop}$	$4.9_{ijklmnopqr}$	$5.0_{ijklmnopq}$
GM	6.5_{ghijk}	7.9_{defgh}	$5.8_{ghijklmn}$	10.2_{abcde}	13.1_{ab}	14.6_{a}
BD	2.4_{vwx}	3.5_{qrstu}	3.1_{tuvw}	1.8_{x}	$4.7_{klmnopqrs}$	$3.9_{opqrstu}$
OM	3.8_{pqrstu}	$5.8_{ghijklmn}$	$5.0_{ijklmnopqr}$	2.2_{wx}	$4.0_{opqrstu}$	6.9_{fghij}
LP	0.5_{B}	0.5_{AB}	0.7_{zAB}	0.7_{yzA}	1.0_{y}	1.0_{yz}
GF	6.4_{ghijk}	10.7_{abcd}	11.9_{abc}	$6.0_{ghijklm}$	8.2_{cdefg}	7.1_{efghi}
MG	3.4_{rstuv}	$4.1_{nopqrstu}$	$4.3_{lmnopqrst}$	3.1_{tuvw}	$4.0_{opqrstu}$	$3.9_{opqrstu}$

Mean extractable soil Al have been back-transformed from the log form which was analysed. Numbers with letter subscripts in common are not different (α=0.05) based on Fisher's protected LSD.

Table 3.35 Back-transformed Olsen P for each P rate applied (0, 30 and 150 mg P L^{-1} soil) across all soils.

Soil	P rate applied (mg P L^{-1} soil)		
	0	30	150
Olsen P	13_{c}	17_{b}	42_{a}

Mean Olsen P have been back-transformed from the log form which was analysed. Numbers with letter subscripts in common are not different (α=0.05) based on Fisher's protected LSD.

Table 3.36 Equations for fitted lines for Caucasian clover shoot yield and Olsen P for each soil in Figure 5.8 (a, c and e) in the thesis.

Soil	Equation type	Equation	R^2	P value
WT	Hyperbola, Single Rectangular I, 3 Parameter	y= -1495.195+1504.508*x/(0.033+x)	0.91	NAN
AR	Polynomial, Quadratic.	y= 15.145-0.352*x+0.004*x^2	1.00	NAN
PK	Polynomial, Quadratic.	y = -1.671+0.522*x-0.007 *x^2	1.00	NAN
MO	Hyperbola, Single Rectangular I, 3 Parameter	y= -2250.126+2266.622*x/(0.045+x)	0.96	NAN
GM	Polynomial, Quadratic.	y= 5.995+0.368*x-0.004 *x^2	1.00	NAN
BD	Hyperbola, Single Rectangular II, 3 Parameter	y = 29.433*x/(11.702+x)-0.194 *x	1.00	NAN
OM	Hyperbola, Single Rectangular I, 3 Parameter	y= -5930.436+5950.075*x/(0.011+x)	0.90	NAN
LP	Polynomial, Quadratic.	y= 11.622+0.416*x-0.010 *x^	1.00	NAN
GF	Hyperbola, Single Rectangular I, 3 Parameter	y= -2607.948+2629.236*x/(0.053+x)	0.98	NAN
MG	Polynomial, Quadratic.	y= 7.321+0.399*x-0.005 *x^2	1.00	NAN

NAN means not a number.

Table 3.37 Equations for fitted lines for lucerne shoot yield and Olsen P each soil in Figure 5.8b, d and f in the thesis.

Soil	Equation type	Equation	R^2	P value
WT	Hyperbola, Single Rectangular I, 3 Parameter	y= -646.544+654.139*x/(0.082+x)	0.99	NAN
AR	Hyperbola, Single Rectangular I, 3 Parameter	y = -2864.514+2884.479*x/(0.133+x)	0.90	NAN
PK	Polynomial, Quadratic.	y= 3.581+0.123*x-0.002*x^2	1.00	NAN
MO	Polynomial, Quadratic.	y= 0.132+0.129*x-0.001 *x^2	1.00	NAN
GM	Hyperbola, Single Rectangular I, 3 Parameter	y= 11.859-2.048e+8*x/(-1.468e+9+x)	0.17	NAN
BD	Hyperbola, Single Rectangular I, 3 Parameter	y = -7424.609+7441.700*x/(0.013+x)	0.75	NAN
OM	Hyperbola, Single Rectangular I, 3 Parameter	y = -4107.359+4129.285*x/(0.034+x)	0.88	NAN
LP	Hyperbola, Single Rectangular I, 3 Parameter	y = -781.812+811.503*x/(0.101+x)	0.99	NAN
GF	Exponential Rise to Maximum, Single, 3 Parameter	y= -1.858e+6+1.858e+6*(1-exp(-0.885*x))	0.99	NAN
MG	Hyperbola, Single Rectangular I, 3 Parameter	y = -3513.085+3536.227*x/(0.023+x)	0.85	NAN

NAN means not a number.

3.7 Equation for the lines fitted for covariate graphs for the P dataset

Table 3.38 Linear trend lines and R^2 values for the relationship between lucerne shoot yield and log soil Al for the P dataset presented in Figure 5.9a in the thesis.

Soil	Equation for fitted linear trendline	R^2
WT	y = -65.589x + 40.575	0.99
AR	y = -5.8974x + 15.201	0.01
PK	y = 11.757x - 4.256	0.98
MO	y = 1.8625x + 0.759	0.04
GM	y = 94.35x - 74.608	0.75
BD	y = 24.86x - 1.5582	0.78
OM	y = 34.117x - 9.7366	0.95
LP	y = 46.321x + 28.939	0.82
GF	y = 27.975x - 17.768	1.00
MG	y = 52.25x - 12.738	0.87

Table 3.39 Linear trend lines and R^2 values for the relationship between lucerne shoot yield and K% for the P dataset presented in Figure 5.9b in the thesis.

Soil	Equation for fitted linear trendline	R^2
WT	y = -3.923x + 9.1164	0.96
AR	y = -9.9885x + 19.866	1.00
PK	y = -2.0586x + 7.669	1.00
MO	y = 2x - 2	5E-14
GM	y = -15.733x + 28.555	1.00
BD	y = -13.633x + 32.437	0.93
OM	y = -13.7x + 26.598	0.98
LP	y = -11.85x + 38.1	0.99
GF	y = -8.8795x + 21.304	0.96
MG	y = -7.9875x + 30.173	0.98

Table 3.40 Linear trend lines and R^2 values for the relationship between lucerne shoot yield and B (mg kg^{-1}) for the P dataset presented in Figure 5.9c in the thesis.

Soil	Equation for fitted linear trendline	R^2
WT	y = -0.2592x + 11.609	0.52
AR	y = -1.1507x + 42.021	0.99
PK	y = -0.1405x + 9.5091	0.97
MO	y = 0.0975x - 1.291	0.99
GM	y = -1.205x + 40.309	1.00
BD	y = -0.6423x + 34.024	1.00
OM	y = -0.5514x + 29.868	1.00
LP	y = -0.618x + 49.988	0.99
GF	y = -0.6072x + 22.323	0.97
MG	y = -0.6817x + 35.208	1.00

Table 3.41 Linear trend lines and R^2 values for the relationship between lucerne shoot yield and soil pH for the P dataset presented in Figure 5.9d in the thesis.

Soil	Equation for fitted linear trendline	R^2
WT	y = 43.829x - 231.51	0.90
AR	y = -18.276x + 105.18	0.69
PK	y = 1.2113x - 0.9791	0.19
MO	y = 4.9786x - 23.806	0.90
GM	y = -62.933x + 327.49	1.00
BD	y = -29.557x + 168.65	0.98
OM	y = -45.698x + 252.97	0.65
LP	y = -16.27x + 113.63	0.33
GF	y = -27.41x + 144.1	0.93
MG	y = -55.29x + 298.27	0.91

4 Appendix D Rhizobox Experiment

4.1 Root Physiology

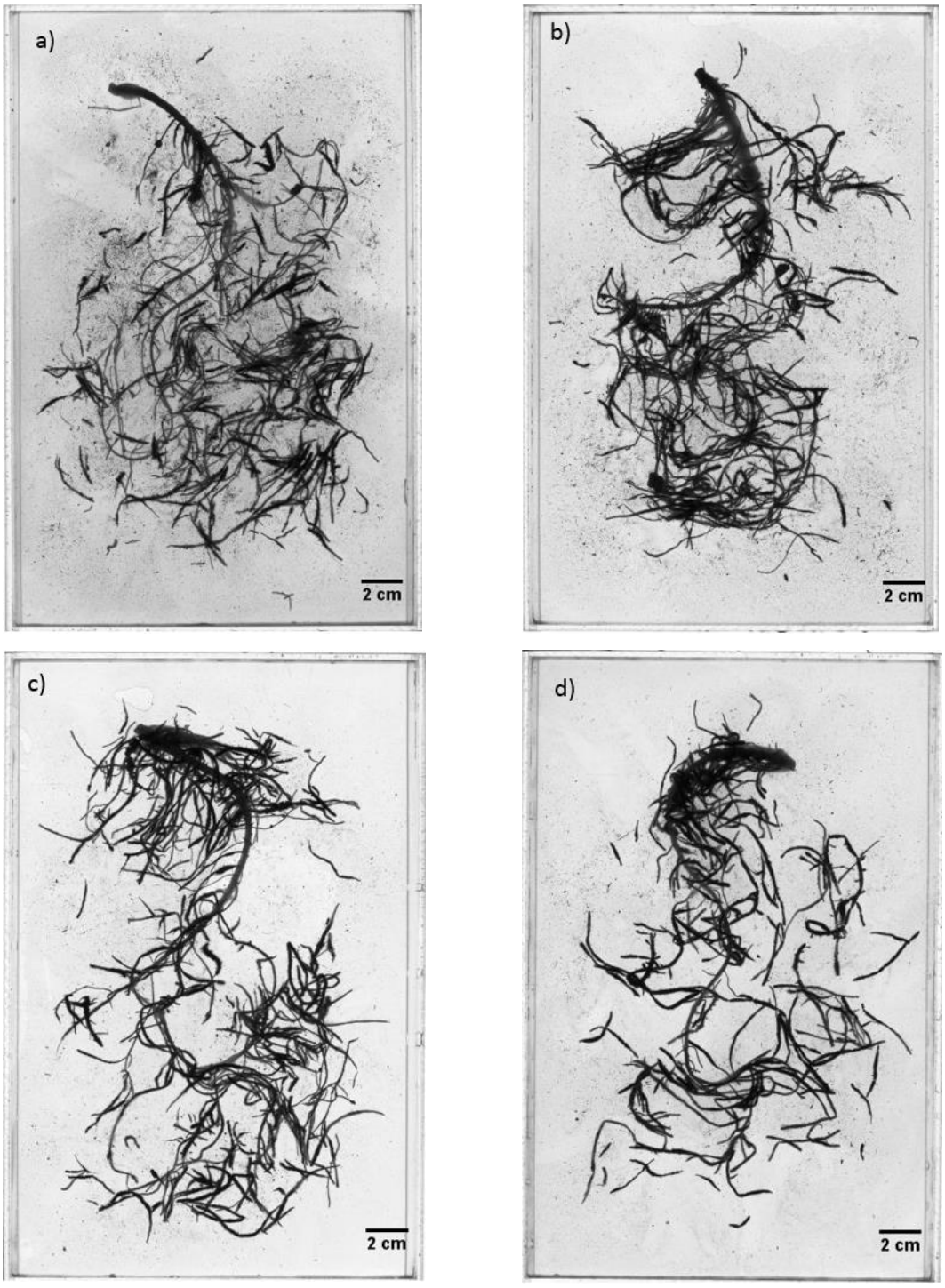
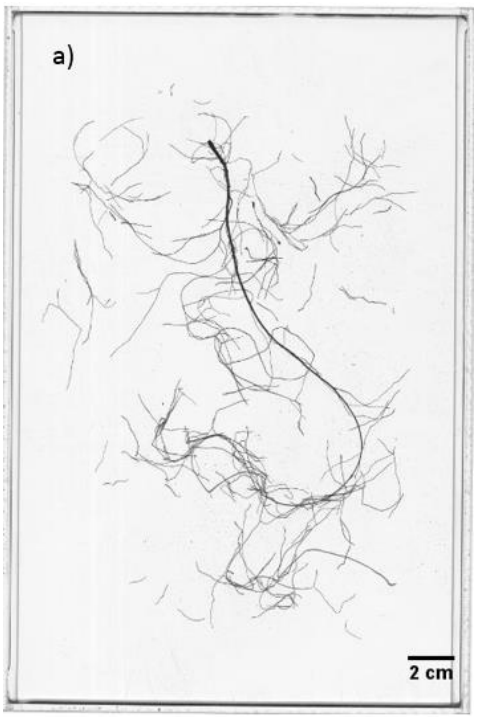


Plate 4.1 LP-1 (a), LP-2 (b), LP-4 (c) and LP-4- (d) root scans.


a)
2 cm


b)
2 cm


c)
2 cm


d)
2 cm

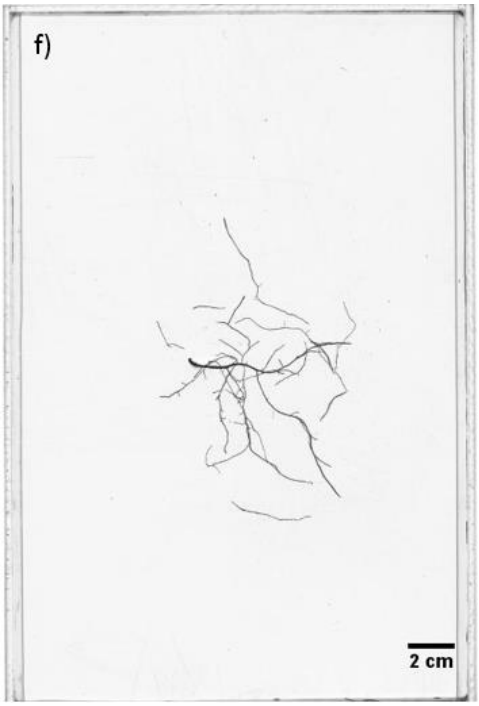
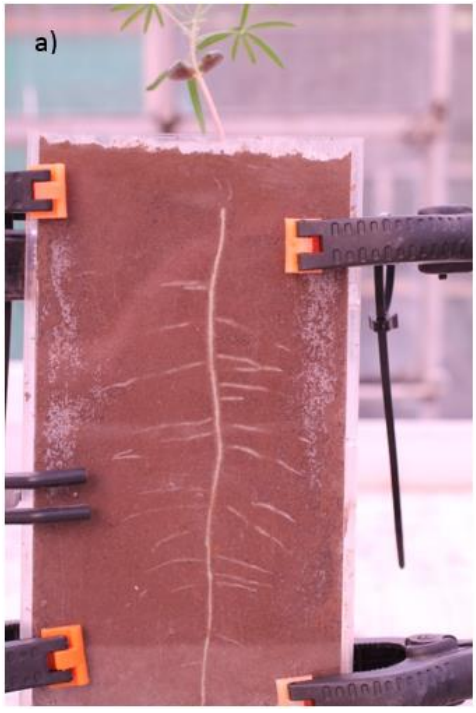


Plate 4.2 LU- 0.5 (a), LU-1 (b), LU-2 (c), LU-4 (d), LU-4- (e) and LU-8 (f) root scans.



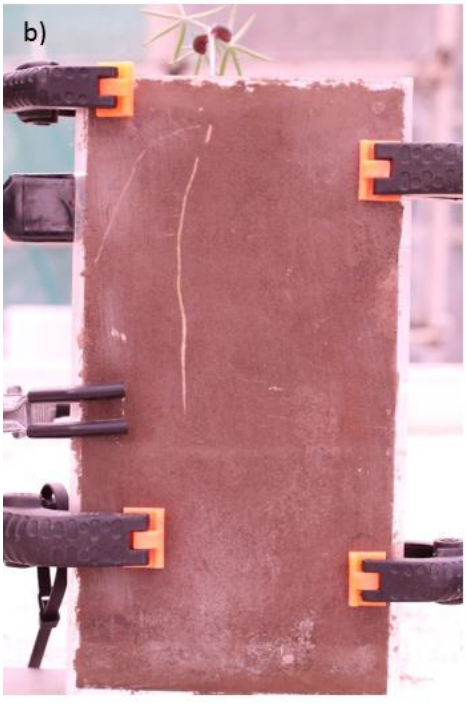


Plate 4.3 Root growth of LP-0.5 (a), LP-8 (b) and LU-0.5 (c) on the 10th August 2015.

4.2 Root Nodules

Table 4.1 Nodule characteristics including number, position on the root, colour, size and overall nodule score for plants grown in the rhizobox experiment.

Rhizobox treatment	Number	Position	Colour	Size	Nodule score
LP-0.5	2	2	1	1	6
LP -1	1	1	1	1	4
LP -2	2	2	1	1	6
LP -4	2	2	1	1	6
LP -4-	2	2	1	1	6
LP - 8	1	2	1	0	4
LU-0.5	-	-	-	-	-
LU-1	-	-	-	-	-
LU-2	1	2	2	0	5
LU-4	1	2	0	0	3
LU- 4-	-	-	-	-	-
LU-8	-	-	-	-	-

Note: - represents plants with no nodules on the roots. The numbers in the above table refers to classes and are codes for the specific number, position, colour and size, which are presented in Table 5.7, Chapter 5 Materials and methods.

4.3 Root-Soil Interface

4.3.1 Differences in Al and Mn Flux across the gel

Tables 6.5 and 6.6 in the thesis contain results showing the distribution of Al and Mn (flux over the 24 hour deployment period) as taken up by the DGT gel for the lupin treatments in Figures 4.1a and b and 4.2 a and b.

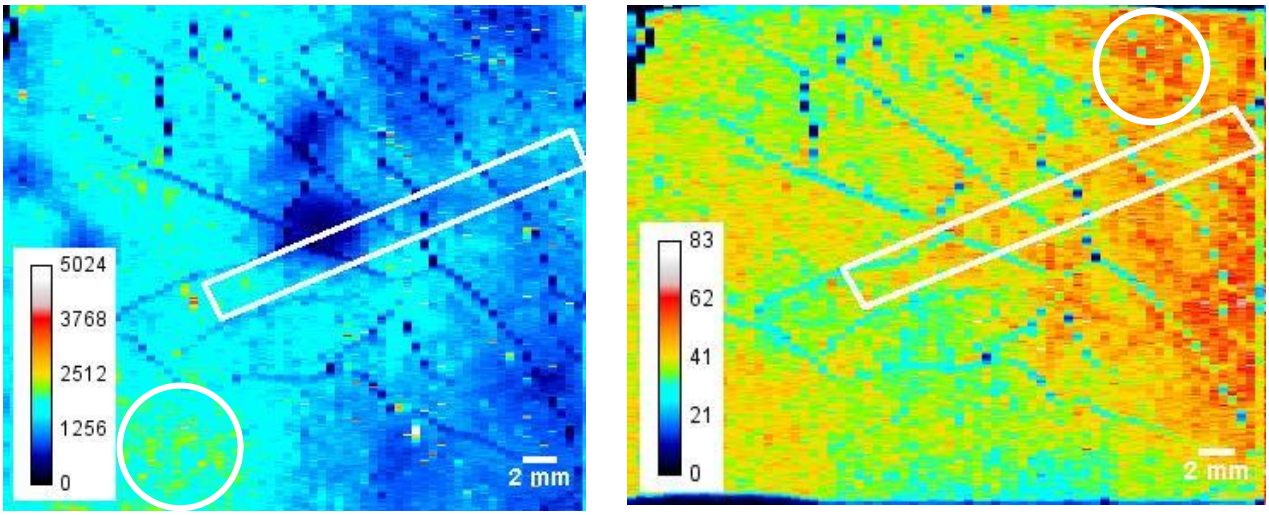


Figure 4.1 LP-1 Al (a) and Mn (b) metal flux (ng cm^{-2} day^{-1}) onto the DGT gels. White rectangles are aligned along the root axis and indicate the locations used for root analysis. White circle denotes the general location where an Al or Mn hotspot was identified.

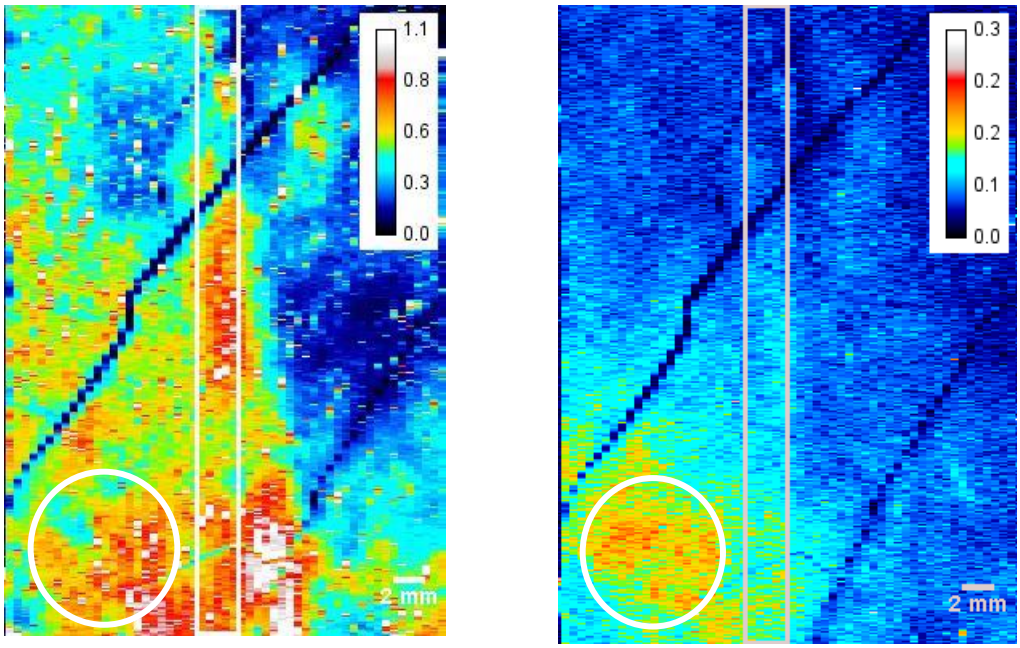


Figure 4.2 LP- 4- Al (a) and Mn (b) metal flux (ng cm^{-2} day^{-1}) onto the DGT gels. White rectangles are aligned along the root axis and indicate the locations used for root analysis. White circle denotes the general location where an Al or Mn hotspot was identified. The black line running through the image is a crack in the gel caused by the prolonged dry conditions in the laser ablation chamber.

5 Appendix E Laboratory Investigation

5.1 Preliminary testing

Preliminary testing was carried out using the standard operating procedure for 0.02 M $CaCl_2$ extractable Al supplied by the Analytical Research Laboratories Ltd (ARL) in New Zealand (Analytical Research Laboratory, 2014). This was to determine if, by using the same method, our results would be within the same Al concentration range as the ARL results for the same soils. The 11 soils (three replicates), internal quality control soils and blanks were run at the 1:4 soil to extractant ratio of 5 g of soil and 20 mL of 0.02 M $CaCl_2$. Samples were placed on the end-over-end shaker for 60 minutes and filtered through Whatman 1 filter paper (110 mm; filter pore size 11 µm; Sigma Aldrich, St Louis, Missouri, USA). The solution was analysed by Inductively Coupled Plasma Optical Emission Spectrophotometry (Varian 720-ES ICP-OES; Varian Pty Ltd, Melbourne, Australia). During this process, the Whatman 1 filter paper was tested to determine its suitability for the test. The Whatman 1 filter paper used is an equivalent filter paper to the Ahlstrom N°1 filter papers (filter pore size 10 µm) used by ARL (Analytical Research Laboratory, 2014). The results for the 11 soils from our 1:4 extraction were compared to the concentrations from ARL for the same soils (Figure 5.1). The results showed that by carrying out the same methods as ARL at Lincoln University, the extractable Al_{CaCl2} concentrations were very close to those obtained by the commercial laboratory, with a strong linear relationship across the range of soils (R^2=0.99). However, at an extractable Al_{CaCl2} concentration of <5 mg kg^{-1}, the concentrations were more similar between the two labs. An increase in the extractable Al_{CaCl2} above 5 mg kg^{-1} lead to an increase in the variation and our values appear to be higher than those obtained by ARL.

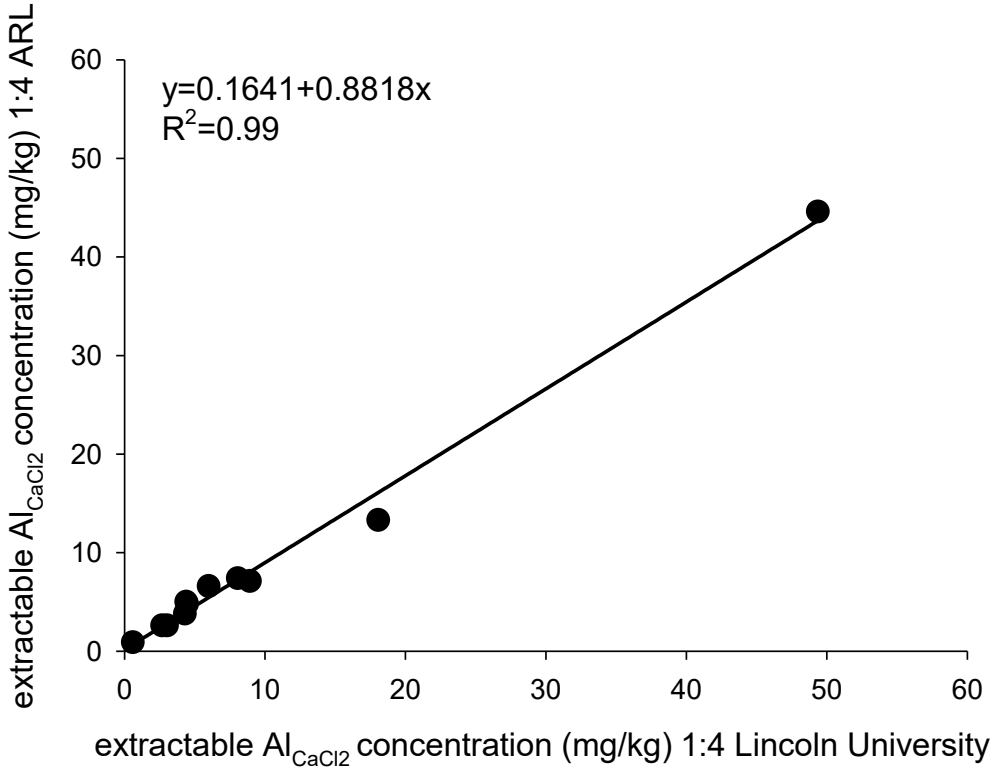


Figure 5.1 Soil extractable Al_{CaCl2} concentrations extracted by the 0.02 M $CaCl_2$ extraction with a 60 minute extraction time and at a soil to extractant ratio of 1:4, Lincoln University laboratory results compared to ARL for 11 soils.

5.2 Soil: extractant ratio

The standard operating procedure used by ARL is a modified 0.02 M $CaCl_2$ test for Al, with a ratio of 1:4 soil to extractant, compared to the standard test for 0.02 M $CaCl_2$ Al stated in the literature as 1:2 (Hoyt & Nyborg, 1971; Hoyt & Nyborg, 1972). Internal testing by ARL found that using a 1:4 ratio yielded the same results as a ratio of 1:2 for aluminium (Venter, 2016a). This modification was prompted by the highly absorptive characteristics of some New Zealand soils (e.g. peats), which resulted in insufficient filtrate for analysis by the reference method. Acceptance of the modification to the standard method was validated by work undertaken on a number of soils of differing generic soil types from New Zealand and Australia (Analytical Research Laboratory, 2014).

The 1:4 ratio was selected for all of the other soil Al tests on Ashburton lakes, glasshouse and rhizobox soils for consistency with those soils in the study that had been previously analysed at ARL. In order to directly compare the Al concentrations to the literature for this laboratory investigation, a 1:2 soil to extractant ratio was used.

5.3 Standard deviations (SD) for mean Al concentrations

5.3.1 $CaCl_2$ extraction

Table 5.1 Back-transformed mean Al_{CaCl2} concentrations (mg kg^{-1}) extracted by $CaCl_2$ for individual soils, WT, AR, MO, OM and GF with increasing molarity (3 levels of molarity; ranging from 0.01-0.05 M) and increasing extraction time (3 levels; ranging from 20-180 minutes). The SD (upper, lower) for each mean are reported for each soil in relation to each mean and back-transformed. Soil acronyms are described in Table 5.1 in the thesis.

		Mean Al_{CaCl2} (mg kg^{-1})				
	Soil Type	WT	AR	MO	OM	GF
	Grand mean	4.3	6.5	14.7	5.8	10.5
molarity (M)	0.01	1.7 (2.20, 1.34) $_c$	3.6 (4.22, 3.01) $_c$	8.8 (10.16, 7.68)	1.8 (2.04, 1.53) $_b$	4.8 (5.19, 4.42) $_c$
(mean of the three shake times, *n* = 6, upper and lower standard deviation for the mean)	0.02	4.0 (4.97, 3.25) $_b$	6.4 (7.84, 5.27) $_b$	21.0 (24.29 , 18.14)	7.6 (16.07, 3.55) $_b$	10.7 (22.37, 10.15) $_b$
	0.05	11.8 (12.68, 10.93) $_a$	12.3 (14.29, 10.54) $_a$	†17.1 (114.29 ,2.57)	14.7(20.23, 10.67) $_a$	22.5 (34.75, 14.55) $_a$
extraction time	20	5.2 (11.38, 2.34) $_a$	6.6 (12.11, 3.62)	8.1 (36.31, 1.81)	5.0 (11.61, 2.11)	11.6 (24.43, 5.50)
(min)	60	4.4 (10.30, 1.92) $_b$	6.6 (11.78, 3.74)	21.0 (45.19, 9.75)	7.0 (21.63 , 2.30)	9.3 (17.99, 4.84)
(mean of the three molarities, *n* = 6, upper and lower standard deviation for the mean)	180	3.5 (9.27, 1.35) $_c$	6.4 (11.25, 3.66)	18.7 (41.88, 8.36)	5.6 (18.75, 1.68)	10.7 (23.50, 4.84)

Note: This table relates to Table 7.3 in the thesis. † The standard deviation was large for the replicates of the 0.05 M $CaCl_2$ MO soil extraction. This is not an error.

5.3.2 KCl extraction

Table 5.2 Back-transformed mean Al_{KCl} concentrations ($cmol_c/kg$) extracted by KCl for individual soils, WT, AR, MO, OM and GF with increasing molarity (4 levels of molarity; ranging from 0.2-2 M) and increasing extraction time (3 levels; ranging from 5-60 minutes). The SD (upper, lower) for each mean are reported for each soil in relation to each mean and back-transformed. Soil acronyms are described in Table 5.1 in the thesis.

		Mean Al_{KCl} ($cmol_c/kg$)				
	Soil Type	WT	AR	MO	OM	GF
	Grand mean	1.0	0.4	2.2	0.7	1.9
molarity (M)	0.2	0.5 (0.66, 0.38) $_c$	0.2 (0.24, 0.13) $_c$	1.5 (1.83, 1.17) $_c$	0.4 (0.49, 0.26) $_c$	1.5 (2.44, 0.97) $_c$
(mean of the three shake times, *n* = 6, upper and lower standard deviation for the mean)	0.5	0.9 (1.30, 0.56) $_b$	0.3 (0.44 , 0.22) $_b$	2.0 (2.81, 1.40) $_b$	0.6 (0.85, 0.47) $_b$	1.8 (2.22, 1.50) $_b$
	1.0	1.5 (1.76, 1.32) $_a$	0.7 (0.82, 0.57) $_a$	2.5 (3.72, 1.72) $_{ab}$	1.1 (1.95, 0.64) $_a$	2.0 (2.33, 1.71) $_b$
	2.0	1.6 (1.77, 1.40) $_a$	0.6 (0.67, 0.56) $_a$	3.0 (3.39, 2.58) $_a$	1.0 (1.24, 0.86) $_a$	2.5 (3.05, 2.06) $_a$
extraction time (min)	5	1.1 (1.81, 0.69) $_a$	0.4 (0.82, 0.19)	2.3 (3.16, 1.61)	0.6 (1.10, 0.38)	1.6 (2.41, 1.09) $_b$
(mean of the four molarities, *n* = 8 upper and lower standard deviation for the mean)	30	1.0 (1.87, 0.53) $_{ab}$	0.4 (0.76, 0.21)	2.0 (3.08, 1.30)	0.8 (1.69, 0.38)	2.0 (2.55, 1.63) $_a$
	60	0.9 (1.61, 0.52) $_b$	0.4 (0.63, 0.21)	2.2 (3.39, 1.47)	0.7 (1.13, 0.44)	2.2 (2.83, 1.72) $_a$

Note: This table relates to Table 7.5 in the thesis.