Liming Effects on Legume Production and Phosphorus Availability in Acid South Island Hill and High Country Soils

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by

Daniel Leslie Martin-Hendrie

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

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There is large potential for widespread development and increased production in South Island hill and high country by increasing legume production. Despite regular past applications of superphosphate fertiliser, current low P and S fertility levels, along with soil acidity and Al toxicity, limit legume production and persistence in these environments.

In a survey of 19 farmed South Island hill and high country soils pH_{H2O} ranged from 4.3 to 5.5, and soil exchangeable Al ranged from 0.5 to 24.5 mg/kg in 0-7.5 cm deep soil. Sulphur (S) concentrations were generally very low, and S deficiency is therefore likely to be a major limitation to legume production and biological nitrogen (N) fixation across the hill and high country. In contrast, a considerable amount of P has accumulated in these soils, with total P ranging from 587 mg P/kg up to 1570 mg P/kg. However, plant available P only accounted for 7.2±0.45% of total soil P, or 78±7.9 mg P/kg across all of the soils. The greatest amount of P has accumulated in moderately labile organic (39.1±1.50% of total P) and inorganic (13.3±0.91% of total P) P fractions.

To investigate if liming would invoke a 'P-sparing effect', four soils were treated with rates of 0, 1, 2, and 4 t/ha of lime, with and without additional P, and exhaustively cropped in a glasshouse experiment. Russell lupins (*Lupinus polyphyllus*), and lotus (*Lotus pedunculatus*) were used as bio-indicators of soil P availability in four high country soils. Liming increased plant growth ($P<0.001$), shoot P concentrations ($P<0.001$), plant P uptake ($P<0.001$), and the utilisation of available soil P ($P<0.001$) for both species up to certain optimal pH ranges. For Russell lupins this optimal pH_{H2O} range was 5.5-6.5, and for lotus it was 6.2-6.8. When soil pH rose above these levels plant growth, shoot P concentration and P uptake rapidly declined. As the optimal pH levels for each species was different, the results indicate that liming effects up to optimal pH levels were due to plant physiological responses and increased plant ability to access and utilise existing soil P, rather than increased soil P availability due to a 'P-sparing effect'.

Liming effects on soil P availability were also investigated in a field experiment at Mt Grand Station. The annual application of 1 and 3 t lime/ha alone had no effect on pasture growth (P=0.583) and clover content (P=0.187), and did not increase soil P availability. Instead, liming was shown to promote the accumulation of plant unavailable Ca-P secondary minerals in the soil, especially in plots treated with additional P fertiliser (P=0.016). The initial exchangeable Al concentration at the site was 1.9 mg/kg, so it is suspected that greater clover growth responses to liming would have occurred if the initial exchangeable Al concentration had exceeded the general 3 mg/kg toxicity threshold. Instead, clover growth (P<0.001) and content in the sward (P<0.001) responded greatest to the application of S, and the suppression of grass competition by the application of a selective grass herbicide (P<0.001).

Subsoil acidity and Al toxicity can also restrict the growth of deep rooting legumes, such as lucerne (*Medicago sativa*). Another aspect of this project was to implement and test a prototype machine for directly injecting lime deep into acid subsoils to alleviate acidity and Al toxicity. This was successfully achieved at Omarama Station, where soil pH_{H2O} was increased to >5.5 (P=0.010) from 5.0-5.2, and exchangeable Al was reduced to <3 mg/kg (P<0.005) from 4.6-7.5 mg/kg, at 20-30 cm depth by the deep application of lime. This resulted in lucerne yield being increased from 980±140 kg DM/ha to a maximum of 2890±330 kg DM/ha (P<0.001) in plots treated with 2 t/ha of deep placed lime in the fourth spring of the experiment. However, lucerne was out-yielded by Russell lupins, which were not affected by deep liming at any of the four sites.

From this project it can be concluded that liming acid South Island hill and high country soils will not cause 'P-sparing effects' and increase the quantity of plant available P in soil. But by alleviating Al toxicity, improving micronutrient availability, and many other beneficial effects, legume production and the ability of plants to utilise existing soil available P will be increased by liming.

Keywords: soil acidity, aluminium toxicity, phosphorus, sulphur, lime, superphosphate, subsoil acidity, deep liming, hill country, high country, Russell lupin, *Lupinus polyphyllus*, lucerne, *Medicago sativa*, lotus, *lotus pedunculatus*, annual clovers, phosphorus fractionation.

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Publications

Prior to the submission of this thesis the following journal paper arising from Chapter 6 of this thesis has been accepted for publication.

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Chapter 1

General Introduction

1.1 Background

Lowland farmland in New Zealand has rapidly intensified in recent years with the widespread conversion of sheep and beef farms to more lucrative dairy, and is now reaching peak production capacity and environmental sustainability limits imposed by government and society (Robertson 2010). However, in August 2012 the New Zealand government announced its ambition for the value of New Zealand's primary industry annual export earnings to be doubled from $32 billion to $64 billion by 2025. In doing so, this would increase the country's total annual export earnings from 30% of GDP to 40% (Tarrant 2012). Therefore, to increase agricultural export earnings NZ needs to add value to products, which is difficult to do given that prices attained are dictated by global commodity prices, and/or increase production from less intensive areas, such as the hill and high country.

Export earnings and production from hill and high country has decreased in recent years due to increased production costs and lower returns for meat and wool. The NZ sheep flock has decreased from a peak of 70.3 million sheep in 1982 to just 27.3 million in 2018 (Burtt et al. 2018). However, despite the more than 55% decline in sheep numbers since 1990, total national lamb meat production only decreased by 12% from 1990 to 2018 (Beef+Lamb 2018; Burtt et al. 2018). This increase in per animal productivity can be attributed to improved farm management, genetic gain of stock, higher quality pastures, and uptake of innovative knowledge and technology by farmers (Moot et al. 2009; Robertson 2010). Despite a decrease in beef cattle numbers over the same period, beef production has also increased (MacLeod & Moller 2006; Beef+Lamb 2018). There is potential for further production gains by increasing stock numbers through more intensive farming in what are currently lowly intensive, low stocked areas. Historically, the main source of revenue for hill and high country farmers has been wool (60–70% of farm income) but recent low wool prices have resulted in a wide spread shift to store lamb and cattle production for greater returns (Moot et al. 2009).

Due to the intensification of lowland areas, sheep and beef production is becoming restricted to hill and high country (Gillingham et al. 2003). Therefore, to maintain and increase production and export earnings there is a need to increase hill and high country pasture production to support more livestock. However, there is limited ability of high country farming systems to sustainably expand and with the processes of tenure review and urban development the area available to pastoral production is decreasing (Stevens et

al. 2014). Therefore, the only way to increase pastoral production will be through intensification (Moot et al. 2009).

The major limiting nutrient in hill and high country soils is nitrogen (N) (Lambert et al. 2003; Stevens et al. 2014). The cost of N fertiliser application is high, so most high country farmers rely on leguminous plants that fix N from the atmosphere as their only source of N input to their farming system. As well as increased N fertility and cycling, growing more leguminous plants, such as clovers, lotus, lupins, and lucerne, is also an effective way to increase production as they are a high yielding, high quality, and palatable feed for livestock (Scott 2003; Maxwell et al. 2013). The growth of leguminous plants is strongly dependent on phosphorus (P), sulphur (S) and molybdenum (Mo) availability (Caradus 1980; Haynes & Williams 1993a; Moir et al. 2000), and soil pH and soil exchangeable aluminium (Al) content (Moir & Moot 2010). The success of specific legume species in the high country depends on their ability to tolerate the climatic, edaphic, and grazing management conditions of this extensive pastoral area.

Growing conditions for legumes and other pastures species varies in the hill and high country and depends on numerous uncontrollable and controllable variables (Keoghan et al. 1995; Scott 2003). Uncontrollable factors which have the greatest effect on pasture production on both small and large scales are variations in climate and soil moisture holding capacity. However, agronomy and grazing management and seed and fertiliser inputs are controllable by farmers' based on economics and the interaction with uncontrollable factors.

Climate is a principle factor governing farming type and pasture production in the high country which is characterised by long cold winters and hot, dry summers (Keoghan et al. 1995). Climate and soil type (as determined by climate and parent material during pedogenesis) varies with changes in slope, altitude, aspect and region, causing large variations in potential pasture production across landscapes. High country soils are generally slow to warm up in spring and then quickly dry out in summer due to high evapotranspiration and low moisture holding capacity in summer. Therefore farmers face a limited growing season of 3-5 months which restricts their production and stocking regimes. In flat areas with access to water, high country farmers use irrigation to intensify small areas of their farms which now produce a large proportion of the total farm dry matter. However, small gains in production, and regaining production steadily lost over time, on the vast areas of largely unimproved tussock hill country could further increase farm carrying capacity.

To operate an efficient farming system high country farmers need to be able to effectively manage and utilise their pasture production to utilise growth and maintain quality. Subdivision on hill country is often the limiting factor governing the ability of farmers to effectively manage pasture and maintain quality (Parker & McCall 1986; Squire 1986). Other factors affecting pasture management and the ability of land

to be subdivided include steepness, accessibility, pasture species, stocking rate and type, access to stock water, grazing management, nutrient transfer, and weeds.

Soil fertility and acidity also affect legume establishment, growth and persistence, and can be manipulated by farmers. Legumes require P and S at higher concentrations than grasses for production and persistence amongst competition from grasses (Caradus 1980; Haynes & Williams 1993a; Moir & Moot 2010). High country soils are generally low in P, S and have low pH and corresponding high levels of toxic exchangeable Al (Edmeades et al. 1984a; Moir et al. 2000; Moir & Moot 2010; Whitley et al. 2016). Traditionally farmers applied single superphosphate (SSP) fertiliser as a source of P and S to encourage pasture production using topdressing planes. However, the application of SSP in the high country has decreased since the mid-1980's due to increased application costs, lower returns, and a reduction in response to P and S application (Ledgard & Brier 1993). Long term grazing studies by both Lambert et al. (1990) and O'Connor et al. (1990) found that withholding P fertiliser off North Island hill country for 4-7 years reduced pasture production by up to 30% and reduced the proportion of clover and sown ryegrass in the pasture sward, which reverted back to poor quality browntop and moss. This deterioration in pasture species composition may affect the response of pastures to future application of P fertilisers (Edmeades et al. 1984a). In contrast, Ledgard and Brier (1993) showed that within two years of recommencing SSP inputs pasture production returned to 95% of the original production levels, after having withheld P fertiliser application for seven years. However, this response could have been benefited by the more intensive grazing management during the withholding period and a larger rate of P fertiliser application, compared with the study by Edmeades et al. (1984a).

Soil acidity, and associated high levels of exchangeable Al, will restrict the establishment and growth of legumes (Haynes & Williams 1993a; Moir et al. 2000; Moir et al. 2018; Morton & Moir 2018). Soil acidity affects nutrient availability, especially molybdenum, and plant root function and at toxic levels exchangeable Al affects root function and morphology and rooting depth which substantially decreases the ability of plants to access soil water and nutrients (Foy et al. 1978; Adams 1981). Aluminium becomes toxic to plants, especially legumes, at a concentration of 3 mg Al/kg soil (Edmeades et al. 1983; Moir et al. 2016a). However, studies have revealed different levels of tolerance exist among different legume species (Edmeades et al. 1991a; Wheeler et al. 1992; Moir et al. 2015; Moir et al. 2016a). Approximately 500,000 hectares of farmed high country in New Zealand has low soil pH and possibly high exchangeable Al (Moir & Moot 2010). To alleviate soil acidity and Al toxicity levels lime needs to be applied to soil. However, the cost of application in the high country, especially on steep slopes, is uneconomic due to the high rates of lime required and the high cost of aerial application (Craighead 2005; Moir & Moot 2010). Few studies on the response of high country soils to liming exist (Moir & Moot 2010; Berenji et al. 2018), however, a study by Stevens et al. (2014) showed no direct increase in pasture production to lime application alone. In

contrast Edmeades et al. (1984a) proposed that economic increases in pasture production could be achieved at lime application rates as low as 1.25 t lime/ha, relative to that point in time.

There have been few studies conducted on P and S in the high country (Scott & Covacevich 1987; Scott 1998a, 2008) and there is little understanding of the extent to which applied fertiliser P is immobilized and made unavailable to plants as a result of low soil pH in high country soils (Wheeler & Edmeades 1995; McDowell et al. 2003; Devau et al. 2009) but it is likely to be substantial. The use of P and S fertilisers in the high country used to be extensive and most farms have regular fertiliser application histories, but fertiliser application has now become limited due to economics (Maxwell et al. 2013) and widespread reductions in pasture production response to P and S application is believed to be occurring. This may be a result of low soil pH which has become even lower over decades of farming practices, plant growth and N fixation (Bolan et al. 1991). Low soil pH can be alleviated by liming but lime inputs to the high country have been limited and pH has continued to decline (Moir & Moot 2014). It is unknown how much release of locked up P due to low pH would occur following liming, but a substantial increase in Olsen P following liming was reported in a study by Berenji et al. (2018) at Glenmore Station near Tekapo. Further study is required to investigate this phenomenon.

It is important that if high country farms are to sustainably become more intensive and profitable that a greater proportion of legumes persist in the pasture sward, for both improved N fixation and feed quality. Investigating ways in which P and S can be made more available to legumes in an economic and sustainable manner will provide important information to enhance legume growth and persistence, and the sustainable management of high country farming in New Zealand. Apart from the study by Berenji et al. (2018), quantifiable P release as a result of liming has not been documented in the literature for acidic hill and high country soils. Investigations are required to determine whether soil pH is causing applied P and S to be transformed into non-available forms in soil making it less available to legumes, or if low soil pH and associated high levels of exchangeable Al restrict the ability of legume roots to function and access the P and S in the soil. Increasing the availability of P and S to legumes will aid in improving hill and high country pasture production and this PhD project aims to investigate methods by which P and S availability can be increased to selected legume species by modification of the soil chemical environment, particularly through the application of lime.

One way to increase legume production and persistence in acidic high country soils is to identify legume species that are tolerant of low soil pH, high exchangeable Al, and are able to effectively utilise the P and S already held in those soils. Plants that thrive in low P and S fertility soils and respond to minimal P and S inputs would also be advantageous. For this project, commonly sown legume species of varying tolerances to low P fertility, low soil pH, and high exchangeable Al will be selected for comparison in response of applied treatments between species. Measurement of species response to applied

treatments will allow for greater understanding of their potential for use in acidic hill and high country soils and of the optimal soil conditions for maximum production. The growth and persistence of naturalised adventive clover species will be compared to that of sown species at Mt Grand Station, Lake Hawea, to determine their adaptation ability for certain edaphic conditions. Russell lupin (*Lupinus polyphyllus*) is one legume species that has been identified as tolerant of low soil pH and high exchangeable Al and are currently being investigated for widespread use in high country farming systems (Miller 1989; Scott 1989; Moot & Pollock 2014; Scott 2014; Berenji et al. 2018). Understanding how Russell lupins are able to persist and access P in these soils compared with more sensitive legume species, such as lucerne, may lead to their greater use in high country pasture systems.

A second way to increase legume production and persistence in the hill and high country is to modify the soil environment to create favourable soil conditions for selected species. The application of lime to acidic high country soils is an effective way to alleviate acidity and Al toxicity issues. However, it is uncertain, and not quantified, what effects the application of lime may have on the availability of soil P and S to legumes in acidic hill and high country soils. This research will investigate whether the application of lime to acidic high country soils with regular fertiliser application histories will result in a release of plant available P from P sorbed in soil from previous fertiliser applications due to such low soil pH. It is uncertain whether the release of P will be substantial, short-lived, or an effective means of increasing plant available P for an extended period. As P is a finite resource (Cordell et al. 2009) careful management of fertiliser P is required, so by making resident P more available to plants in the high country would be an effective way to utilise past P inputs.

When applied to the soil surface, it takes a considerable time for lime to be absorbed into soil due to limited rainfall and large particle size affecting its transport down the soil profile (Moir & Moot 2010, 2014). Consequently, soil remains acidic along with possible high levels of exchangeable Al at depth. Surface application of lime may improve legume rooting depth to the depth of lime penetration, but beyond that root growth of sensitive plants will still be impeded. In cultivatable areas of the high country legumes, such as lucerne and Russell lupins, have the potential to be highly productive crops due to their leguminous qualities and deep taproots that are able to access water from deep down in the soil profile unlike other pasture species. However, lucerne is highly susceptible to Al toxicity which restricts its ability to root deep into the soil (Moot & Pollock 2014). Lime can be cultivated into soil by conventional cultivation but general farming practices now revolve around minimal tillage systems to conserve soil organic matter, water, reduce erosion risk and reduce time spent cultivating. Therefore, the third main aspect of this PhD study will be to use a novel, minimum till method of applying lime at depth in the soil which is to rip the lime deeper into the soil profile where the greatest soil pH and exchangeable Al barrier is to legume roots using a custom designed and built lime applying ripper built by Flexiseeder Limited. This

machine pneumatically applies fine particle pelletised lime at depths of up to 30 cm into soil to alleviate low soil pH and high exchangeable Al at depth, with less disturbance of soil than conventional cultivation. The machine will be commissioned in this PhD study to evaluate the effectiveness of applying lime to improve the rooting depth, access to water and potential P released from acidic soil, and consequent increase in production of legumes such as lucerne and lupins in hill and high country systems.

Throughout the study soil chemical analysis will be used to measure changes in soil pH, exchangeable Al, P, and S content and plant availability resulting from applied treatments. And legumes, including lucerne, Russell lupins, sown clovers, and adventive annual clovers as bio-indicators of treatment effects. With using plants as bio-indicators only measuring differences in dry matter production between treatments may not be an effective measure of treatment effect as other variables such as climate, water, and N availability, may restrict growth. Therefore, P uptake by plants will also be measured as a means of measuring changes in P availability in soil as plants take up P from soil whether they can synthesise it into dry matter or not (luxury P uptake) (Mengel & Kirkby 2001).

1.2 Objectives and thesis structure

The overall objective of this PhD research is to quantify and understand the soil available P resources and the extent of pH induced lock-up of hill and high country soils with regular historical SSP inputs under differing soil pH and liming regimes, and to examine the ways in which the availability of these P resources can be modified by liming to increase legume production and persistence. The principal hypothesis is that low soil pH limits soil P availability, legume establishment and persistence in high country soils with typical historical SSP inputs, but low pH and high exchangeable Al effects are reduced by targeted inputs of P, S, and lime, and species selection. This hypothesis is based on the expected release of labile P from soil by liming. This release of P, increase in soil pH, and reduction in exchangeable Al can be expected to lead to improved legume growth and N fixation. Increased soil pH and N cycling will promote soil microbial activity and breakdown of organic carbon, further releasing organic and labile P from organic matter and sorbed P, creating an overall substantial release of P and improved nutrient cycling and legume production and persistence.

To conduct this study and meet the overall objective a series objectives and experiments (Figure 1.1) have be devised:

Objective 1: *To investigate soil P and S availability in unlimed, acid high country soils varying in fertiliser application history*. To meet this objective a range of field soils varying in fertiliser and lime history, aspect and slope will be sampled from commercial high country farms and analysed for basic soil chemistry and

P fractionation to investigate the current status of P and S resources in these soils and to examine the relationships between P and S availability and soil acidity (Chapter 3).

Objective 2: *To quantify P availability and extraction by legumes in response to liming acid high country soils*. To meet this objective four soils relating to the field experiments (Chapters 5 and 6) will be treated with different rates of lime and P and exhaustively cropped in a glasshouse pot experiment to investigate how liming may affect the availability of P in these soils and how much P plants can extract from them (Chapter 4).

Objective 3: *To investigate the effects of liming, P and S fertilisation, and herbicide application on soil chemistry, and the abundance and growth of sown and naturalised clover species at Mt Grand Station*. Following on from the findings in Chapters 3 and 4, different rates of lime and superphosphate fertiliser, as well as a herbicide treatment, will be applied to determine the optimal combination for maximising clover growth to meet the objective of this experiment (Chapter 5).

4. *To determine the effect of deep lime placement on soil pH and aluminium, P availability and plant growth*. To meet this objective the Flexiseeder® lime ripper will be used to deliver different rates of pelletized lime at depth in the soil profile at three high country stations. Selected deep rooting Al sensitive and Al tolerant legume species will be used as bio-indicators to examine the effectiveness of this deep placed lime for mitigating subsoil acidity and Al toxicity (Chapter 6).

By meeting these objectives the project aims to provide recommendations and technology to hill and high country farmers for mitigating soil acidity and Al toxicity issues, and managing fertiliser and lime inputs for increased legume production and returns.

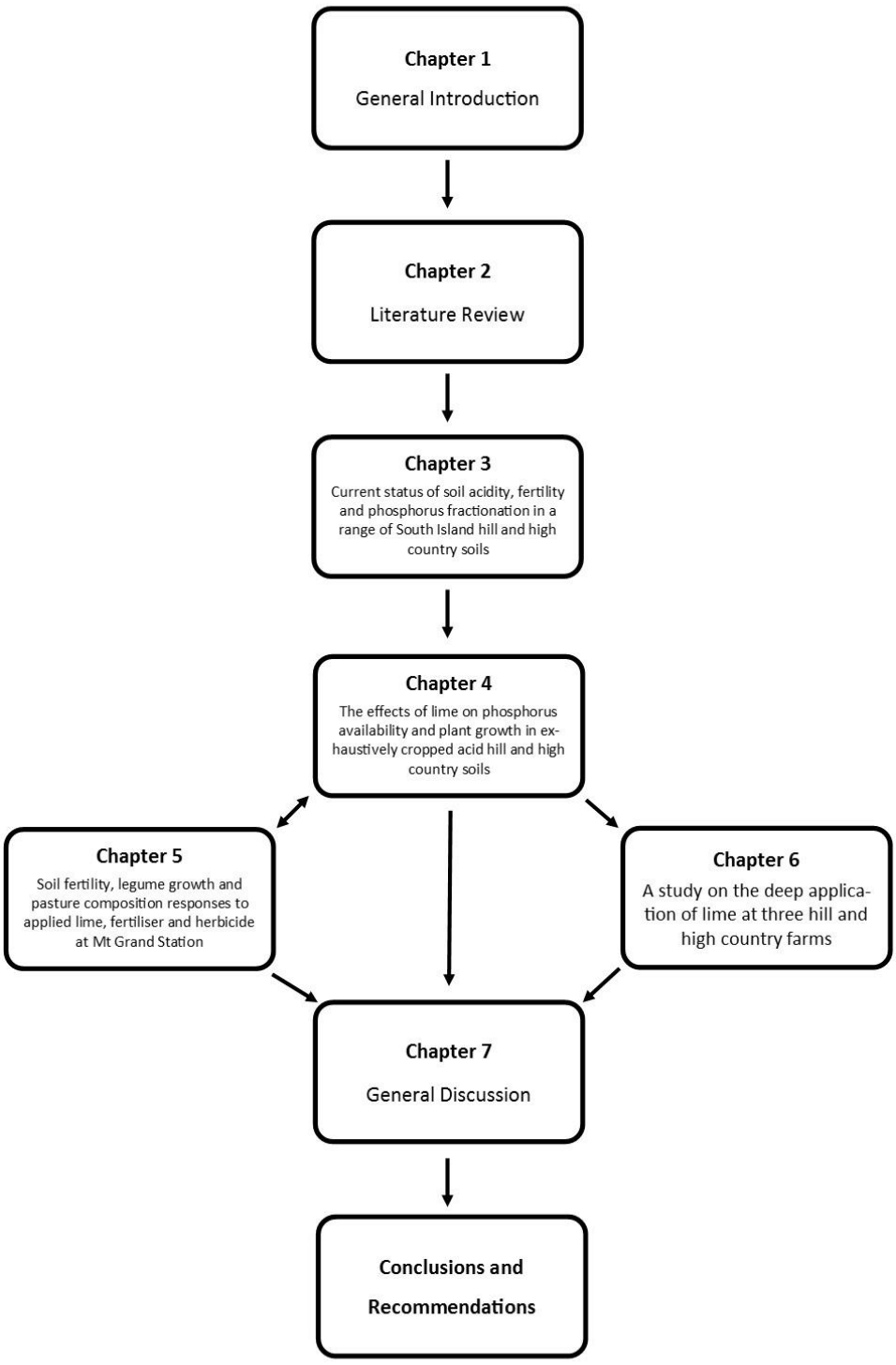

Figure 1.1 Thesis structure

Chapter 2

Literature Review

2.1 Introduction

The South Island high country comprises of 6 million hectares, of which 2.5 million is farmed with the remainder being held as conservation land by the Crown (Moot et al. 2009a). Of the farmed high country land an estimated 500,000 ha has been developed into improved pasture, and the remaining 2 million hectares are undeveloped and extensively grazed, typically at <1 SU/ha (Hodgson et al. 2005; Moot et al. 2009a).

Farming in the high country is climate limited with long, cold winters and hot dry summers (Scott et al. 1995). Legumes, such as annual clovers, are relied on to make use of the short spring growing season and to provide a good quality feed for nursing stock (Boswell et al. 2003b; Maxwell et al. 2010). Deep rooting legumes, such as lucerne and Russell lupins, are grown to persist in these dry environments where other pasture plants fail.

N is the most limiting nutrient in the high country and legumes are mostly relied upon as the sole source of N through the process of biological N fixation. Legumes require higher levels of soil fertility, particularly P, S, and Mo than grasses (Caradus 1980; Haynes & Williams 1993b), and vary in sensitivity to soil acidity and exchangeable Al concentrations, which generally becomes toxic to legumes at >3 mg/kg ($CaCl_2$ extractable) (Edmeades et al. 1983; Moir & Moot 2010; Moot & Pollock 2014).

The soils of the high country are generally acidic, have high levels of toxic exchangeable Al, and are low in P and S (Haynes & Ludecke 1981b, a; Moot et al. 2009a; Moir & Moot 2010). Soil acidity and associated Al toxicity is a major limiting factor to plant production on an estimated 500,000 ha (Moir & Moot 2010). It is estimated that 50 percent of arable land worldwide is acidic (Liu et al. 2014). Soil acidity and Al toxicity can be alleviated with the application of lime. However, the financial implication of broadcasting large quantities of lime at high application rates onto broad acre hill slopes by fixed-wing aircraft is a major limitation to lime application in the high country and most areas are unlimed (Scott & Mills 1981; Edmeades et al. 1985; Craighead 2005; Moir & Moot 2010). In contrast, superphosphate fertiliser has been broadly aerially applied to high country for over 50 years (Gillingham et al. 1999). Plant-soil-P-lime interactions in acid soils have been broadly studied in global literature with no clear consensus on the nature of these interactions reached (Haynes 1982). Due to a lack of research it is unsure how acid soils in the South Island high country will react to liming in terms of P availability, given that they have generally been well fertilised in the past.

2.2 Soil Acidity

Soil acidity, as measured on by the pH scale, is a measure of the concentration of hydronium (H_3O^+, typically represented as H^+) in soil solution. The optimum pH range for pasture production on mineral soils is pH 5.8-6.3, and no more than pH 5.0 for peat soils (Edmeades et al. 1984a; Edmeades et al. 1984b; Edmeades et al. 1985; O'Connor et al. 2007). Below this pH range soil acidity can be detrimental to the growth of plants and soil organisms, resulting in the inhibition of the formation of legume-rhizobia symbioses and biological N fixation, and the decomposition of OM and nutrient mineralisation (Coventry et al. 1985; Robson & Abbott 1989; Runge & Rode 1991; Bolan et al. 2003; Dodd & Sheath 2003; Ferguson et al. 2013; Berenji et al. 2017). Low soil pH also affects the availability of both non-essential and essential plant nutrients in soil through its effects on the physical, chemical and biological characteristics of soil (Bolan et al. 2003), and these are suggested to be the main factors that reduce plant growth in acid soils, rather than H^+ concentration (Foy 1992). These factors are discussed further below. Most high country soils have a pH of 4.5-5.5 (Edmeades et al. 1984a; Moot et al. 2009a). Despite the defined optimum pH ranges for pasture production, in a study of 12 pasture legumes grown in an acidic high country soil amended with lime, Moir et al. (2016a) found that the minimum pH required to maximise plant growth was species dependent and ranged from 5.4 (Gland clover and Persian clover) to 6.0 (Arrow leaf clover) for the range of legumes examined. Edmeades *et al.* (2016) reported that due to the increased cost of aerially applying lime to hill country, the economic optimal pH for clover dominant pastures is 5.5-5.6, and below pH 5.3 liming is essential to improve pasture production.

2.2.1 Causes of soil acidification

The acidification of soil is an ongoing process caused by natural processes and anthropogenic factors in both direct and indirect manners. The rate of acidification is dependent on the pH buffering capacity of the soil, climatic factors, and plant growth, and has been shown to be greater under leguminous crops than grass pastures and natural ecosystems (Haynes 1983; Bolan et al. 1991). Shallow soils low in organic matter (OM) and cation exchange capacity (CEC), which is typical of South Island high country soils, have low buffering capacities and are easily acidified (Haynes 1983). In poorly buffered soils that are slightly acid (pH 5.5-6.0), even small amounts of acidification can lead to large losses in productivity (Haynes 1983; Sumner et al. 1991).

Natural factors that acidify soil include, and are not limited to; the leaching and plant uptake of base cations from soil solution and cation exchange sites (Haynes & Swift 1986; Helyar et al. 1988). Cations are then replaced on cation exchange sites and in solution with acidic H^+ and Al^{3+}. Plants will also exude H^+ and organic acids from their roots into the rhizosphere. They do so to increase the availability of nutrients

in the surrounding rhizosphere soil, to maintain plant-soil-microorganism symbioses, and to maintain the ionic balance within them (Richardson et al. 2009; Carvalhais et al. 2011; Lambers et al. 2013; Dissanayaka et al. 2017; Imai et al. 2019). This Al^{3+} can be considered an acidic cation as it is a hydrated ion that dissociates H^+ as it hydrolyses in soil as pH increases (Adams 1981; Haynes 1984; Lindsay & Walthall 1989). The sequence of dissociation reactions in acid soils (pH<7.0), excluding the waters of hydration surrounding the Al^{3+} ion, is shown in Equation 2.1, and the solubility of each Al species product is shown in Figure 2.1.

Eq 2.1

$$Al^{3+} + H_2O \leftrightarrow Al(OH)^{2+} + H^+$$

$$Al(OH)^{2+} + H_2O \leftrightarrow Al(OH)_2^{+} + H^+$$

$$Al(OH)_2^{+} + H_2O \leftrightarrow Al(OH)_3 + H^+$$

Finally, the mineralisation of organic C, N, P and S, followed by removal of these nutrients from the system, particularly by plant uptake and assimilation, and anion leaching, results in a net excess of H^+ (Haynes 1983; De Vries & Breeuwsma 1987; Bolan et al. 1991). The most acidifying of these processes is the nitrification and assimilation of ammonium (NH_4^+), in which 1 mole of H^+ is released per mole of NH_4^+ nitrified or assimilated by plant roots (Bolan et al. 1991). Associated with this process is respiration by soil microbes (and also plant roots) which produces carbon dioxide that can react with soil water to form carbonic acid and with organic compounds to form carboxylic acids (Bolan et al. 1991; Campbell 1998).

In managed farmland there are two main anthropic practises that directly result in soil acidification; the introduction of legumes to the system and the application of acidifying fertilisers. Legumes form symbiotic relationships with rhizobia to biologically fix N_2 from the atmosphere and in this process create NH_4^+ and release H^+ ions back in to soil solution, the created NH_4^+ (Haynes 1983; Liu et al. 1989; Bolan et al. 1991; Bolan et al. 2003). However, this relationship and biological process can be restricted by severe soil acidity (Lapinskas 2007; Ferguson et al. 2013; Ferreira et al. 2016). The application of fertilisers is necessary for the development of soil fertility and improving plant production (Boswell & Floate 1992). However, some fertilisers, particularly N and S based fertilisers, directly acidify soil. The acidifying effect of fertilisers can be quantified by their 'acidity equivalent' (Table 2.1.), which is the amount of pure lime (kg) required to neutralise the acidifying effect per 100 kg of applied fertiliser. A negative acidity equivalent indicates a liming effect.

Table 2.1 The acidity equivalent of a range of fertilisers

Fertiliser Product	Acidity Equivalent (kg $CaCO_3$/100 kg)
Nitrogen Fertilisers	
Anhydrous ammonia	148[1]
Sulphate of ammonia	113[1]
Urea	83[1]
Ammonium nitrate	61[1]
Monoammonium phosphate	55[2]
Diammonium phosphate	74[2]
Phosphorus/sulphur fertilisers	
Single superphosphate	8[2]
Triple superphosphate	15[2]
Elemental sulphur	310[2]
Gypsum	-12.4[3]
Phosphate rock	-47 to -56[5]
Potassium fertilisers	
Potassium chloride	0[1]
Potassium sulphate	-64[2]
Magnesium fertilisers	
Dolomite	-109[4]

[1]Kealey (1992); [2]Bolan et al. (2003); [3]Bolan et al. (1991); [4]Brady (1974); [5]Acidity equivalents of a range of phosphate rocks measured by Bolan (1995).

N fertilisers directly contribute to soil acidification in two ways; by providing NH_4^+ for nitrification, and by supplying N for nitrate leaching (Bolan et al. 2003). Elemental S is a very acidifying fertiliser, but when used in small quantities soil buffering capacity is usually able to restrict this. In soil elemental S is first oxidised to sulphuric acid, which then dissociates into H^+ and SO_4^{2-} ions, the latter contributes to base cation leaching (Bolan et al. 2003). As an anion, S is poorly retained in soils and regular S fertiliser applications are required to maintain plant growth. Due to this S is often deficient, especially in hill and high country soils, where it is applied in single superphosphate or elemental S fortified superphosphate (Craighead et al. 1990; Edmeades et al. 2005; Craighead & Metherell 2006; Maxwell et al. 2012).

N fertilisers are very rarely applied to hill and high country pastures (Stevens et al. 2014), instead P and S fertilisers are applied to promote legume growth and biological N fixation, which indirectly leads to more acidification (Bolan et al. 1991; Bolan et al. 2003; Scott 2003; Schefe et al. 2015). Other indirect effects of

applying fertilisers and adding legumes to farming systems that acidify soil result from increased plant growth and nutrient uptake, and the accumulation and mineralisation of organic matter in soil.

2.3 The effects of soil acidity on toxic metal and nutrient availability

2.3.1 Aluminium

One of the major consequences of soil acidification, especially for legumes, is the mobilisation of exchangeable aluminium (Al^{3+}, simply abbreviated to Al) due to the hydrolysis of monomeric Al-hydroxy species (Figure 2.1) (Haynes 1984; Powell et al. 1997).

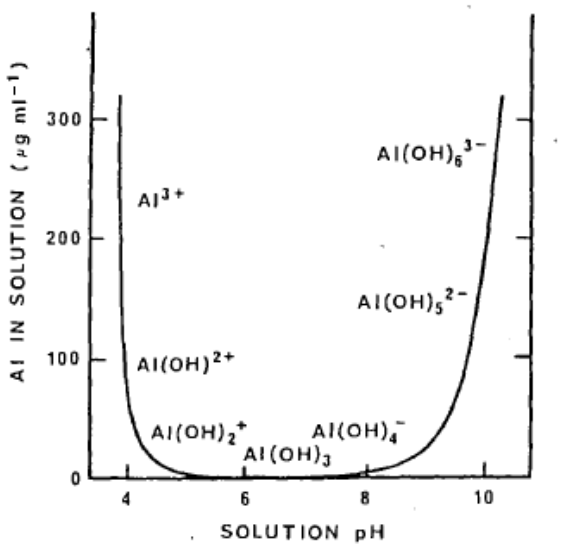

Figure 2.1 The effect of pH on the hydrolysis and speciation of Al in soil solution (Haynes 1984), adapted from McLean (1976).

In toxic concentrations, Al inhibits root growth, function and rooting depth by damaging root hairs and growing tips, preventing rhizobia from nodulating roots, and inhibiting root nutrient uptake, particularly P (Foy et al. 1978; Adams 1981; Haynes & Ludecke 1981a; Edwards 1991; Brockwell et al. 1995; Dodd & Sheath 2003; Ferguson et al. 2013; Berenji et al. 2017). Moir and Moot (2010) estimated that as much as 500,000 ha of South Island hill and high country soils may potentially contain toxic concentrations of Al. Al generally becomes toxic to plants at concentrations of >3 mg/kg ($CaCl_2$ extractable) which usually occurs at a pH of <5.5, and its concentration in soil increases exponentially as pH decreases (Figure 2.2) (Edmeades et al. 1983; Moir & Moot 2010; Moot & Pollock 2014; Whitley et al. 2016; Berenji et al. 2018). Despite the generalised 3 mg/kg toxicity threshold, other studies have revealed different levels of tolerance exist among different legume species (Edmeades et al. 1991a; Wheeler et al. 1992; Moir et al. 2015; Moir et al. 2016a).

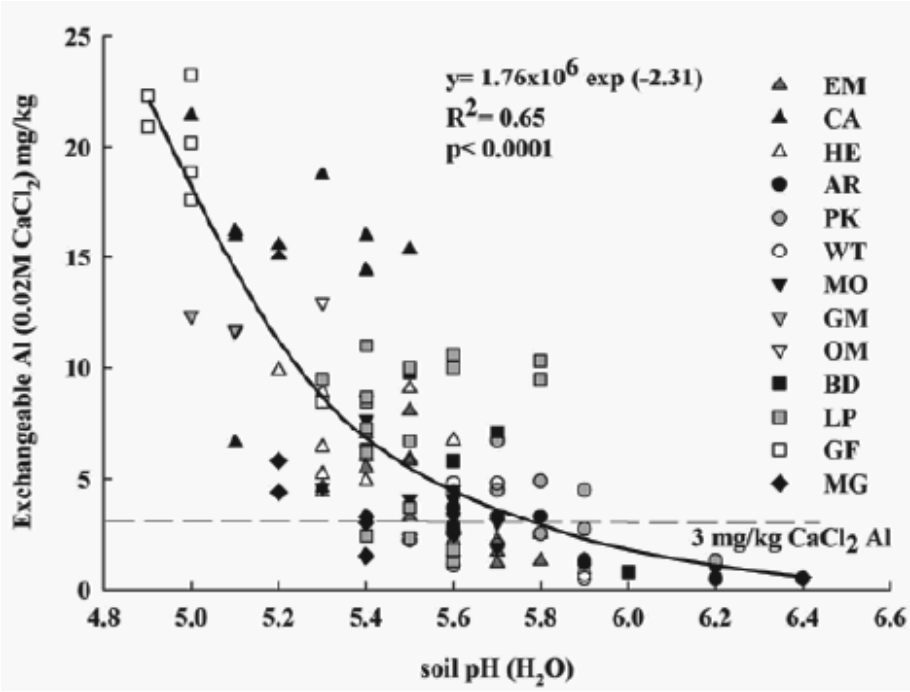

Figure 2.2 The relationship between increasing soil Al with decreasing soil pH for 116 soil samples across 13 hill and high country farms in New Zealand (Whitley et al. 2016).

2.3.2 Manganese

As with Al, the concentration of plant available Mn (Mn^{2+}) increases in soil as pH decreases, but unlike Al, Mn is an essential nutrient in plants. In plants, Mn is used as an activator and co-factor of multiple enzymatic reactions (Burnell 1988). However, as soil acidifies and Mn availability increases, the concentrations of Mn taken up by plants can become toxic and inhibitive to their growth by being directly toxic to plants, and by indirectly inducing Fe deficiencies (Hewitt 1963; Truong et al. 1971; Bromfield et al. 1983; Smith et al. 1983; Bolan et al. 2003). This usually occurs at concentrations of 340 mg Mn/kg DM in lucerne, 570 mg Mn/kg DM in white clover, and 1110 mg Mn/kg DM in perennial ryegrass tissue (Smith & Edmeades 1983; Smith et al. 1983; Wheeler & O'Connor 1998). Waterlogging of soil and the reducing conditions it induces can also cause Mn concentrations to reach toxic levels (Sparrow & Uren 2014). Compared to the extent of Al toxicity, findings by Smith and Edmeades (1983) suggest that Mn toxicity is unlikely to be a major issue in the South Island hill and high country given that in the 371 pasture samples they took from across the South Island the Mn content averaged only 191±94 mg Mn/kg DM, and of the 3411 samples taken across the whole country Mn concentrations only exceeded the 570 mg Mn/kg DM toxicity threshold for white clover in 6 individual samples. However, Mn concentrations were not compared to soil pH in this study (Smith and Edmeades 1983).

2.3.3 Phosphorus

Hill and high country soils are naturally low in P and require P fertilisation in order to increase plant production (Edmeades et al. 1984a; Dodd & Sheath 2003), especially for growing clovers (Caradus 1980;

Haynes & Williams 1993b). A study by Chen et al. (2003) showed that grassland soils in New Zealand generally contain between 375 and 2600 mg P/kg soil, and on average 34.7% of this P is inorganic P (Pi) and 59.3% is organic P (Po), with the remainder being contained in primary P minerals. Only a very small quantity of the P contained in soil is in solution phase (0.01-0.24% in the soils studied by Chen et al. (2003), and available for plant uptake at any one time, and therefore requires constant replenishment from other Pi and Po pools (Figure 2.3.). However, soil acidity can strongly influence the speciation and processes that release P into solution in soil.

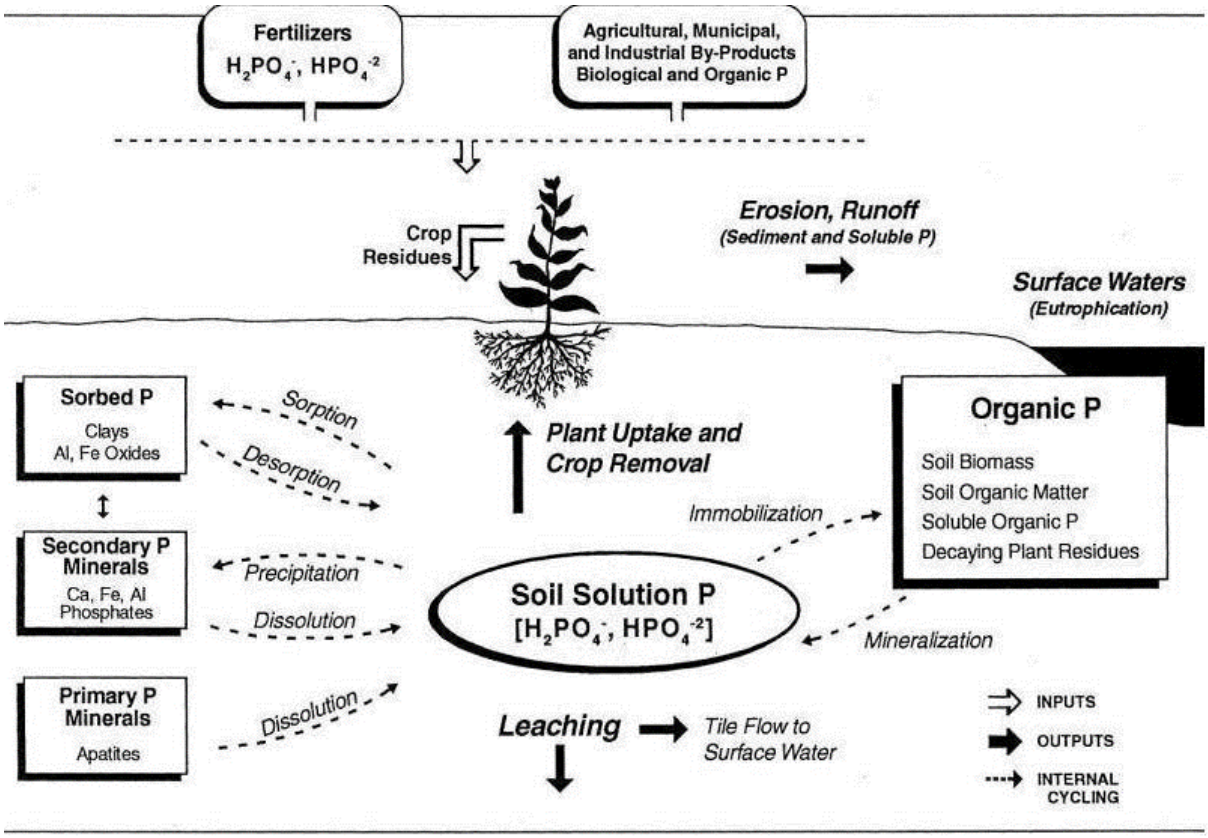

Figure 2.3 A simple diagram of the phosphorus cycle in soil (Pierzynki et al. 2005).

When applied to acid soil, soluble fertiliser P is quickly adsorbed by Al and Fe hydroxides on the surfaces of clays and OM through the exchange of phosphate ions and hydroxyl groups in the process of ligand exchange (Parfitt 1978; Haynes 1984; Parfitt et al. 1989; Frossard et al. 2000; McDowell & Condron 2001; Pierzynki et al. 2005; McLaughlin et al. 2011; Redel et al. 2016). This process governs the availability of P in acid soils (McDowell et al. 2003), and the affinity of adsorption is dependent on the degree of soil acidity (Figure 2.4) (Sanchez & Uehara 1980; Hartono et al. 2005; McDowell 2005; Arai & Sparks 2007; Shen et al. 2011), and is greatest at pH <4.0 (Haynes 1984). This is due to the increase in reactivity of Al, Fe and Mn (minor) as they hydrolyse and become more soluble as pH decreases. Adsorption of P with soluble Al, Fe, and Mn (secondary P minerals, Figure 2.3) results in the formation of insoluble precipitates, rendering the P unavailable for plant uptake (McLean 1976; Foy et al. 1978; Haynes 1984; Parfitt et al. 1989; Edwards 1991; Sloan et al. 1995; Hartono et al. 2005; Arai & Sparks 2007). In higher pH soils, P tends to precipitate

more with calcium (Ca) than Al and Fe, but even in acid soils, considerable amounts of P bound to Ca can be found (Edwards 1991; Chen et al. 2003; McDowell 2005; McLaughlin et al. 2011). When applied to soil, localised temporary acidification due to the dissolution of superphosphate granules in soil can cause Al, Fe, and Mn oxides to hydrolyse into soluble forms and precipitate some of this newly applied P (Haynes 1984; Bolan et al. 2003; Hedley & McLaughlin 2005; McLaughlin et al. 2011). Advantages of soil acidity is the potential for using lowly soluble P fertilisers such as the direct application of phosphate rock where rainfall is adequate (Bolan et al. 1990; McLaughlin et al. 2011), and the increased mineralisation of primary P minerals (McLaughlin et al. 2011).

Conversely, this precipitation reduces the concentrations of toxic soluble Al in solution (Coleman et al. 1960; Adams 1981; Bache & Crooke 1981; Manoharan et al. 1995; Sloan et al. 1995). Manoharan et al. (1996; 2007) also report the reduction of Al toxicity following P fertiliser addition due to the complexation of Al with fluorine (F), which is a contaminant in these fertilisers. Although Al-P precipitation in soil has been shown to reduce toxic Al concentrations, such Al-P precipitation reactions can also occur in the cortex of plant roots, preventing plants from being able to utilise P that they have taken up, invoking P deficiency (Foy et al. 1978; Edwards 1991; Foy 1992).

Over time, adsorbed P can diffuse into soil aggregates or become occluded on them by coatings of Al or Fe oxides (Barrow 1984; Parfitt et al. 1989; Arai & Sparks 2007; Shen et al. 2011). Both of these processes renders the P completely plant unavailable.

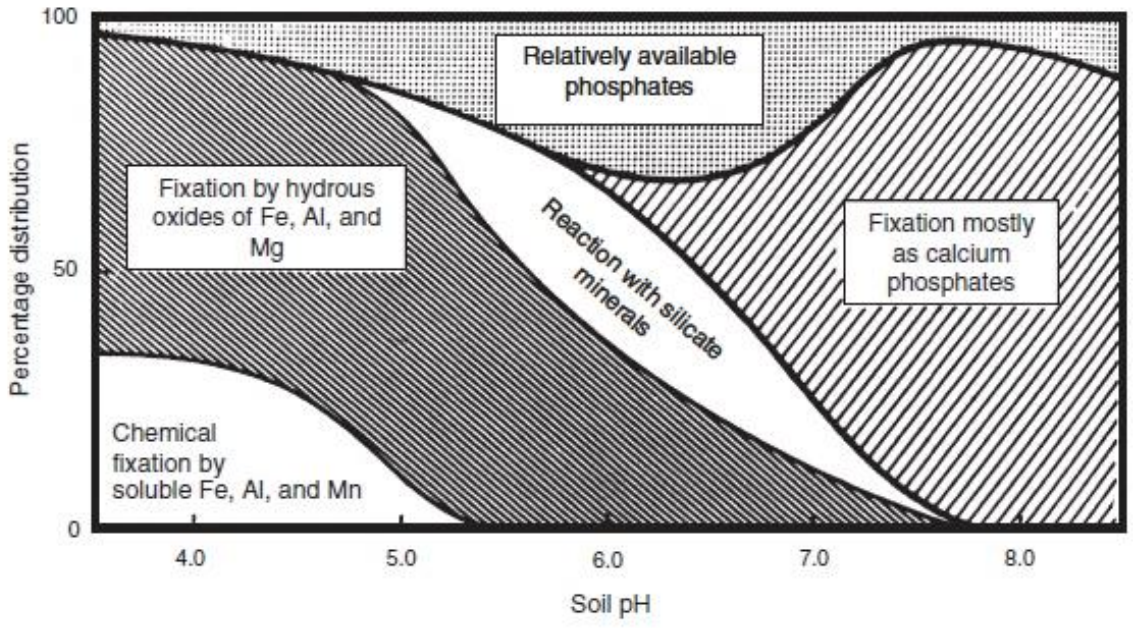

Figure 2.4 The availability and adsorption (fixation) of inorganic P to soil compounds in relation to soil pH (Brady 1974; McDowell 2005).

Turner and Blackwell (2013) concluded that soil pH has little direct effect on the total amount of Po in soil. However, acidification of soil is known to reduce OM decomposition and mineralisation of Po into plant available forms due to its inhibition of soil microbial activity and phosphatase enzyme production (at pH <5.0) (Halstead et al. 1963; Eivazi & Tabatabai 1977; Haynes 1984; Gerke & Meyer 1995; Chen et al. 2003; Condron et al. 2005; Shen et al. 2011; Turner & Blackwell 2013).

As well as the effects of soil acidity on the conversion of fertiliser P to plant unavailable forms, the effects that soil acidity can have on plant growth also limit the utilisation of applied fertiliser P. This leads to the accumulation of significant amounts of fertiliser derived P in plant unavailable forms in soil. Few studies exist on investigating the extent of which this fertiliser P is adsorbed, precipitated and immobilised in relation to soil acidity, especially in acid New Zealand soils. Understanding these processes and the fate of applied fertiliser P is important to consider when designing fertiliser input strategies.

The most thorough method for measuring the quantity and speciation of P in soil is the method of P fractionation which was first developed by Hedley et al. (1982), and has since been further developed (Olsen & Sommers 1982; Condron et al. 1996; Condron & Newman 2011). This method makes use of sequential extraction of soil using stronger and stronger reagents to extract P of decreasing lability and availability to plants, and separates extracted P into organic and inorganic forms. The extracted P can be classified into soil solution P, plant available P, moderately labile P, non-labile and residual P fractions. The fractionation of P using this method has not been extensively measured in South Island hill and high country soils, but may show the effect soil acidity, and other chemical factors, have on the accumulation of applied fertiliser P in non-plant available forms and further research is required to do this.

At the Winchmore long term fertiliser trial, annual inputs of fertiliser P have been shown to be accumulating predominantly in inorganic forms, especially as moderately labile inorganic P (NaOH Pi I fraction) (Tian et al. 2017). However, McDowell and Condron (2012), and Condron and Goh (1989) showed from data collected at the site, prior to the application of 4 t lime/ha lime in 1972 an initial rapid accumulation of organic P occurred in the high fertiliser treatment before the rate of accumulation decreased to a steady state, beyond which P primarily accumulated as inorganic P. This result was also shown in early work by Jackman (1964), and overseas by Herlihy and McGrath (2007). Other overseas studies have shown the accumulation of fertiliser P in acid soils as predominantly being in inorganic forms (McLaughlin et al. 2011; Annaheim et al. 2015; McLaren et al. 2015; Redel et al. 2016; Boitt et al. 2018c).

2.3.4 Sulphur

In soils that aren't regularly fertilised with S, mineralisation of the organic S (Org-S) pool becomes the main supplier of sulphate-S (SO_4-S) for plant uptake, and as with Po, above, this process can be inhibited by soil acidity (Bolan 1995). Being an anion, SO_4-S can also become adsorbed and precipitated by Al and

Fe (Martini & Mutters 1984; Marsh et al. 1987; Bolan et al. 2003), but this usually only counts for a small quantity (<10%) of the total S in soil (Perrott & Sarathchandra 1987; Watkinson & Kear 1996). However, such adsorption of SO_4-S can be beneficial in acid subsoils to prevent the leaching and complete loss of SO_4-S from soil systems (Bolan et al. 2003).

2.3.5 Micronutrients

Soil acidification adversely affects the availability of some micronutrients, such as selenium, and of particular importance to legumes; molybdenum (Mo) (Bolan et al. 2003; Kaiser et al. 2005; Rutkowska et al. 2017). Plants take up Mo as the molybdate ion ($HMoO_4^-$, MoO_4^{2-}), and as an anion molybdate is adsorbed by Al, Fe, and Mn oxides in acid soil (Rutkowska et al. 2017), with maximum adsorption occurring at pH <5.0 (Xie et al. 1993; Xu et al. 2013). In contrast, the acidification of soils can increase the availability of other micronutrients, even to the point which they become toxic to plant growth, including Mn (as described above), boron (Bo), copper (Cu), iron (Fe), zinc (Zn), and nickel (Ni) (Davis et al. 1978; Foy et al. 1978; Sims 1986).

2.4 Liming hill and high country soils

The most common and easily accessible liming material in New Zealand for increasing soil pH and alleviating Al and Mn toxicity is crushed calcium carbonate ($CaCO_3$), simply known as lime. Other, less common liming materials include burnt lime (CaO), slaked lime ($Ca(OH)_2$), dolomite ($CaMg(CO_3)_2$), and of less liming value; unreactive phosphate rock (Brady & Weil 1999; Bolan et al. 2003). In soil, lime reacts with H ions to form water and carbon dioxide (Eq.2.21), and its reactivity is dependent on the fineness to which it is ground, but this can affect its spreadability.

Eq. 2.2 $$CaCO_{3(s)} + 2H^+_{(aq)} \rightarrow Ca^{2+}_{(aq)} + H_2O_{(l)} + CO_{2(g)}$$

The Ca applied in lime aids to displace H, and Al ions, from soil cation exchange sites to solution which can then be neutralised. Increasing soil pH causes Al ions to precipitate as insoluble polymeric hydroxyl-Al amorphous cations in the process of polymerisation (Haynes 1982, 1984). These newly formed amorphous Al polymers are highly reactive in soil and can precipitate free anions (especially phosphate, see below), adsorb to clay particles, complex with OM, or form crystalline hydroxide structures. Without the formation of crystalline structures, the hydroxyl-Al can remain amorphous and reactive in soil, and subject to hydrolisation if pH were to decrease again (Haynes 1984). The process of crystallisation appears to be dependent on the drying of soil post-liming and the formation of pure Al structures (gibbsite) is very rare (Haynes 1982). As with Al, Fe will precipitate as hydroxyl-Fe polymers as soil pH is increased, and Mn will oxidise. These three can co-precipitate and crystallise together (Haynes 1984).

As well as increasing soil pH, and reducing Al and Mn toxicity, liming soil has also been shown to increase plant growth through improved soil chemical, biological, and physical conditions in numerous ways (Haynes & Swift 1988; Bolan et al. 2003). Increases in the availability and plant uptake of nutrients due to pH dependent soil chemical processes and increases in OM mineralisation, due to increased microbial activity, resulting from liming acid soils have been well documented in studies on New Zealand soils, and abroad, for N (Edmeades et al. 1981; Edmeades et al. 1986; Zhou et al. 1993; Stevens & Laughlin 1996; Wheeler et al. 1997; Wheeler & O'Connor 1998), S (Martini & Mutters 1984; Marsh et al. 1987; Bolan et al. 1988; Haynes & Naidu 1991; Valeur et al. 2000), Mo (Xie et al. 1993; Zhou et al. 1993; Wheeler & O'Connor 1998; Rutkowska et al. 2017), and for many other nutrients (Bolan et al. 2003). However, care is also required not to over lime as this can reduce the availability of some micronutrients. Biological N fixation has also been shown to be improved in response to liming due to increases in legume growth and root function, increases in rhizobia populations and function, and greater nodulation (Coventry et al. 1985; Peoples et al. 1995; Unkovich et al. 1996; Wheeler et al. 1997; Ferguson et al. 2013; Berenji et al. 2017). Soil physical conditions have been shown to be improved by liming through the accumulation of organic matter, increased soil fauna (Hirth et al. 2009), improved root penetration and biomass, flocculation of clays due to Ca addition, and stabilisation of soil aggregates (Haynes & Swift 1988; Haynes & Naidu 1998; Bolan et al. 2003).

Although the effects of liming on the availability of N, S and Mo in soil are well known the effects liming has on P availability are less clear. It is often proposed that a reason for liming acid soil is to increase P availability for legumes (Sanchez & Uehara 1980; Haynes 1982; Edmeades et al. 1989). However, many studies have found conflicting results in which P availability is either reduced, increased, or not affected by liming. The findings of these studies have been summarised in critical reviews by Haynes (1982, 1984) and Bolan et al. (2003). As with other nutrients the two main processes in which liming can increase P availability is desorption of P from Al and Fe oxides, and increased mineralisation of organic P due to increased microbial activity (Robertson et al. 1954; Haynes 1982; Barrow 1984; Haynes 1984; Edmeades et al. 1989; Naidu et al. 1990; Haynes & Naidu 1991; Condron et al. 1993; Wheeler & O'Connor 1998; Bolan et al. 2003; McDowell et al. 2003; O'Connor et al. 2007). But liming soil also adds Ca and increases the precipitation of Ca-P minerals (Figure 2.4) (Naidu et al. 1990; Richardson et al. 2009), and creates new, highly reactive, surfaces that have strong affinity for binding any released P (Haynes 1982, 1984). These new surfaces are the hydroxy-Al amorphous cations ($Al(OH)^{2+}$ and $Al(OH)_2^{+}$) that are formed when soils are limed (Figure 2.1, Eq 2.1). Their concentration is dependent on initial Al concentrations and the amount of lime applied. Without a repeated wetting-drying phase of the soil these newly formed reactive surfaces can precipitate any released and newly applied soil P in completely insoluble forms. For this reason Haynes (1982, 1984), following on from the findings in Haynes and Ludecke (1981a), believes that the main P response to liming acid soils is the alleviation of Al toxicity where this was initially a limitation

to P uptake and utilisation by plants. Due to the potential for P to be precipitated by hydroxyl-Al cations it is not recommended to apply P fertiliser soon before, with, or after recent liming on soils that have high concentrations of Al.

The collective term for the quantifiable increase in plant available P, or 'release of P' from plant unavailable fractions, resulting from liming is the 'P-sparing effect'. Few studies have investigated and found occurrences of the P-sparing effect in New Zealand (Tillman & Syers 1982; Mansell et al. 1984; Edmeades et al. 1989; O'Connor et al. 2007). In a study of North Island volcanic and sedimentary soils Mansell et al. (1984) recorded a P-sparing effect on 11 out of 25 soils studied. However, in only four of these soils was the P-sparing effect sufficient to be of practical significance, and were equivalent of fertiliser P inputs of 10-20 kg P/ha. In another study on Northland clay soils O'Conner et al. (2007) recorded large P-sparing effects up to the equivalent of 50-60 kg P/ha/yr which reoccurred for three years after lime application of 5 t/ha on a soil that already had an initial pH of 5.8. Mansell et al. (1984), O'Conner et al. (2007), and Edmeades et al. (1989) all concluded that occurrences and sizes of P-sparing effects are indeterminate and unpredictable. However, these recorded P-sparing effects mostly appear to occur on soils that already have high concentrations of plant available P and have previously been well fertilised. At the Winchmore long term fertiliser trial in the South Island, Metherell et al. (1997) and McDowell and Condron (2012) reported small quantities of Po mineralisation occurring in response to liming. However, the effect was only short-lived and any released P quickly reverted back to being plant unavailable. No such studies have recorded or investigated the potential of P-sparing effects occurring on South Island hill and high county, although Berenji et al. (2018) recorded an increase in Olsen P in response to applied lime at Glenmore Station near Lake Tekapo. Due to this lack of investigation it is uncertain if liming previously unlimed hill and high country soils will invoke a P-sparing effect, and if so, it is unknown if the release of P will be substantial, and sustained.

2.4.1 Subsoil acidity and liming

Despite the surface application or shallow incorporation of lime by cultivation, acidity in the subsoil, and associated Al toxicity, can still limit root growth and plant production. The passive movement of lime down the soil profile has been shown to be a slow process, especially in dry environments, such as the hill and high country of the South Island (Farina & Channon 1988; Helyar et al. 1988; Conyers & Scott 1989; Kirchhof et al. 1995; Moir & Moot 2010, 2014). In order to more efficiently reduce sub soil acidity and alleviate Al toxicity a range of studies have investigated methods and outcomes of directly injected lime into acid sub soils (Wojta et al. 1955; Anderson & Hendrick 1983; Sumner et al. 1986; Farina & Channon 1988; Coventry 1991; Jayawardane et al. 1995; Kirchhof et al. 1995; Nelson et al. 2012; Hendrie et al. 2018). By building a variety of machines to either directly inject lime, or to cut slots through the soil for lime to be incorporated in, these studies generally resulted in positive, but expensive, reductions of sub

soil acidity and Al toxicity. In the studies that investigated the effects of subsoil liming, Sumner et al. (1986) showed that lucerne was able to extract much greater volumes from water from deep limed soil than non-limed soil due to eliminated subsoil Al toxicity in southeast USA. This resulted in a 50% greater yield in the limed soil (18 t lime/ha) than in the control treatment, and 25% greater yield than in a 10 t/ha surface applied gypsum treatment, over a four year period. Farina and Channon (1988) measured maize crop yield increases of 400 kg DM/ha in a wet year to 1400 kg DM/ha in a dry year over three years post-liming when they directly injected lime at 14 t/ha to a depth of 70 cm deep, this was with 90 cm spacing's between the lime coulters. This study was followed up by Farina et al. (2000) that showed that over a 10 year period post-liming the average yield increase was 600 kg DM/ha and results were very season dependant.

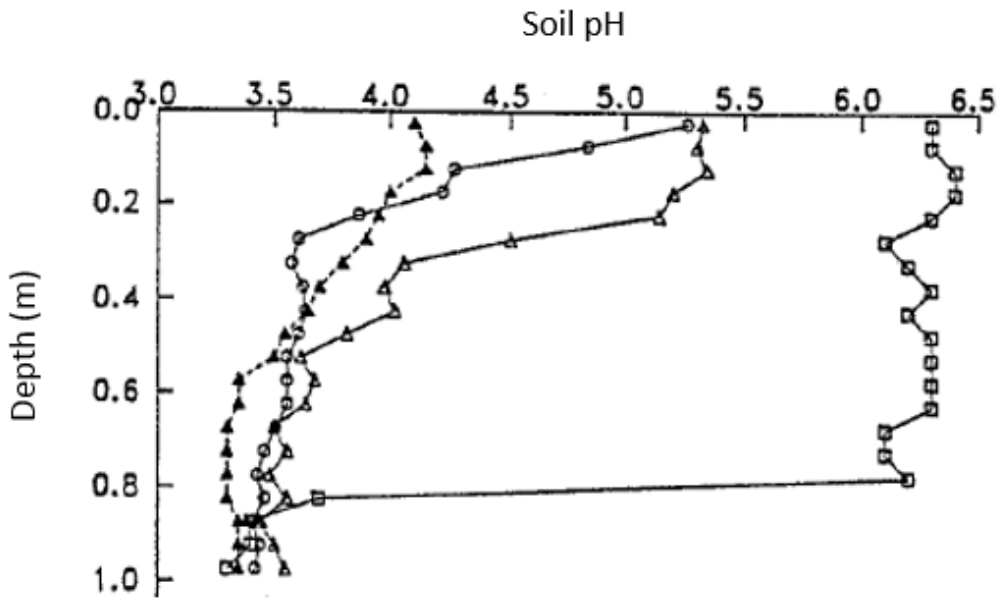

Figure 2.5 The effects of incorporating surface applied lime at 20 t lime/ha by deep ploughing (○) and deep ripping (Δ), and at 20 t lime/ha using specialised deep lime incorporating machinery (□) on soil pH compared to unlimed soil (▲) (Kirchhof et al. 1995).

Jayawardane et al. (1995), following on from the study by Kirchhof et al. (1995), measured a yield increase from 700 kg DM/ha to 2700 kg DM/ha in a medic crop in Australia where lime was incorporated using specialist machinery at 20 t/ha into 15 cm wide, 80 cm deep slots (Figure 2.5). These studies also investigated completely incorporating 113 t lime/ha throughout the soil profile to 80 cm deep using repacked soil cores. This further increased yield to 5900 kg DM/ha, which shows what the potential crop yield is when subsoil acidity is not a limitation. A substantial amount of other machine development and plant yield response work has been carried out in Australia in recent years (2010-present), but has yet to be published.

2.5 Legumes for hill and high country

The importance of growing legumes as the primary source of N and for producing quality animal feed in hill and high country has been well studied (Adams 1964; Nordmeyer & Davis 1977; White 1995; Brown & Green 2003; Scott 2003, 2008; Moot 2012). As has the impacts that soil acidity and aluminium toxicity has on their growth, access to soil nutrients, and persistence as previously discussed. However, climatic and soil physical properties remain problematic for establishing and maintaining the growth of legumes in hill and high country. Summer drought, coupled with low soil water holding capacity, can be a major limitation for legume establishment and persistence, especially for traditionally sown clover species such as white clover (*Trifolium repens*) (Nordmeyer & Davis 1977; White 1995; Knowles et al. 2003; Scott 2003; Moot 2012). Failure to ensure the survival and productivity of legumes in hill and high country can lead to the invasion of low quality grasses and invasive weeds, especially heracium (*Pilosella officinarum*) and sorrel (*Rumex acetosella*) (Harris & Fan 1996; Gillespie et al. 2006; Steer & Norton 2013; Nichols et al. 2016). The key to successfully establishing and optimising the growth of legumes as monocultures, or in new or existing pastures, is to select those with characteristics that will allow them to exploit ecological niches and persist in the given environment matched with the grazing pressure they will be subjected to (Scott et al. 1985; Dodd & Sheath 2003; Scott 2003).

In cultivatable areas, deep rooting perennial legumes are useful for utilising soil moisture deeper in the soil profile, making them productive in dry conditions and persistent through droughts. Lucerne (*Medicago sativa*) has been shown to be the most important of these legumes for exploiting this niche and for animal production in dryland areas (Scott 2003; Moot 2012; Anderson et al. 2014). However, lucerne requires a high level of P and S fertility (Scott 2003), and is particularly sensitive to soil acidity and Al toxicity (Moir & Moot 2010; Moir et al. 2016a; Berenji et al. 2018; Hendrie et al. 2018). Where lucerne is not suitable other deep rooting legumes that are more suited to low fertility soil and high concentrations of Al have been identified for use. Russell lupins (*Lupinus polyphyllus*) have relatively recently been identified for growth as animal feed and their agronomic potential is still being explored (Nordmeyer & Davis 1977; Davis 1981; Scott & Covacevich 1987; Scott 1989; White 1995; Scott 2001, 2003; Black et al. 2014b; Moot & Pollock 2014; Ryan-Salter et al. 2014; Scott 2014), but they have been shown to thrive in very low fertility soils with high concentrations of Al that would otherwise severely inhibit the growth of lucerne. Although not yet studied in Russell lupins, the ability of other lupin species to thrive in low P and high Al soils is probably due to their ability to exude low molecular weight organic acids, such as carboxylates, into their rhizosphere to dissolve soil P and bind Al (Hue et al. 1986; Lambers et al. 2013; Dissanayaka et al. 2017; Imai et al. 2019). However, perceived palatability issues of Russell lupins, due to their alkaloid content, and lower performance of stock grazing them has been a limitation to their uptake

as a pasture legume, but recent studies show promising results and further research is required (Scott et al. 1994; White 1995; Black et al. 2014b; Scott 2014).

Of greater palatability than Russell lupins is Lotus (*Lotus pedunculatus*), which is another perennial legume that is able to tolerate very acid soils with high concentrations of Al and low P fertility compared to other legumes (Nordmeyer & Davis 1977; Davis 1981; Morton 1981; Scott & Mills 1981; Sheath 1981; Floate et al. 1985; Lowther et al. 1987; Lowther 1991; White 1995; Trolove et al. 1996; Widdup et al. 2004; Berenji et al. 2018). Of particular importance is the tetraploid cultivar 'Grasslands Maku' (Armstrong 1974). Lotus has the additional benefit of being able to be oversown on to hill country in addition to conventional sowing (Lucas et al. 1981). However, its main disadvantage that contributes to it not being widely grown is that it is intolerant of set stocking and fails to persist if overgrazed (White 1995). Other perennial species that have been shown to be more suited and productive in South Island hill and high country than white clover, but are seldom sown, include Birdsfoot trefoil (*Lotus corniculatus*) (Davis 1981; Scott & Charlton 1983; Lowther et al. 1987; Bologna et al. 1996; Widdup et al. 2004), Caucasian clover (*Trifolium ambiguum*) (Bryant 1974; Lucas et al. 1981; White 1995; Scott 1998b; Black & Lucas 2000; Stevens & McCorkindale 2002; Boswell et al. 2003b; Black et al. 2014a; Mills et al. 2015; Berenji et al. 2017), and red clover (*Trifolium pratense*) which is often included in pasture mixes (Scott et al. 1994; Brown et al. 2003; Moot 2012).

Annual legumes play an important role in uncultivatable hill and high country as their growth pattern closely matches that of feed demand for sheep (Taylor et al. 1979; Ledgard et al. 1987). They are also able to exploit the availability of moisture as hard seeds will only germinate if sufficient soil moisture is available, and they can complete their lifecycle and produce large amounts of seed before summer drought conditions set in, that perennial legumes would otherwise struggle in (Boswell et al. 2003b). Of the annual clovers sub clover (*Trifolium subterranean*) is considered to be the most important for hill and high country farming and is the most widely used (Boswell et al. 2003b; Smetham 2003; Lucas et al. 2015; Monk et al. 2016). It is most often conventionally sown in pasture mixes (Lucas et al. 2015), but can also be oversown on to uncultivatable hill country, although its success when oversown is often limited compared to other annual legumes due to its relative large seed size (Awan et al. 1994). Top flowering annual clovers have been shown to be more prolific seeders and more persistent in uncultivatable hill and high country (Awan et al. 1994; Boswell et al. 2003b), and also more suited to low fertility soils (Scott 2003). They are generally low yielding but have been shown to produce large yields when favourable conditions occur (Williams et al. 1980; Nori et al. 2015), especially if grass competition is reduced (Hepp et al. 2003). Of the top flowering annual clovers, Balansa clover (*Trifolium michelianum*) has been shown to be the most productive (Nori et al. 2015). Although it is currently the most commonly oversown species of top flowering annual clover on uncultivatable hill country (Monk et al. 2016) its use still remains low

(MacFarlane et al. 2015). Since being first introduced a range of approximately 20 top flowering annual clovers have become naturalised in the New Zealand hill and high country (Boswell et al. 2003b; Scott 2003). The most common of these in South Island hill and high country are striated clover (*Trifolium striatum*), cluster clover (*Trifolium glomeratum*), suckling clover (*Trifolium dubium*), and haresfoot clover (*Trifolium arvense*) (Boswell et al. 2003a; Boswell et al. 2003b; Power et al. 2006; Maxwell et al. 2010). These clovers are usually small and low yielding (Scott 2003), but are tolerant of harsh climatic conditions, acid soil and low P and S fertility and are able to persist by producing large quantities of seed (Boswell et al. 2003b; Maxwell et al. 2012; Maxwell et al. 2016). However, as with all annual legumes they require careful grazing management, including spelling from grazing, to ensure seed set for their persistence (Monks et al. 2008; Maxwell et al. 2016).

The range of legume species available, and the traits that make them thrive and persist in dryland hill and high country farming situations have been well documented. There is much potential to extend the use of legumes in hill and high country, however their use is mostly restricted by the current lack of availability of seed, excluding the common white, red and sub clover cultivars, and lucerne (Maxwell et al. 2016; Monk et al. 2016; Nichols et al. 2016). The availability of rhizobia for inoculation, especially for the legumes that require specific rhizobia to nodulate, is also limited (Scott 2003). Beyond improving soil pH and fertility to meet the fertility requirements of available species, future research is required to continue to identify and promote legume species that are able to exploit ecological niches in New Zealand and be productive and persistent in grazing systems without the need for major intervention.

2.6 Conclusions

The soils of the South Island hill and high country are generally acidic and Al toxicity is a widespread issue. With ongoing pasture growth and inputs of fertilisers to maintain production these soils can be expected to continue to slowly acidify. This ongoing acidification has shown to lead to reductions in P, S and micronutrient availability to plants due to adsorption, and at lower pH precipitation reactions, and reduced mineralisation of organic matter. There is little understanding of the current availability of P and S in South Island hill and high country soils, and the extent to which applied fertiliser P has been made unavailable to plants due to these soil pH conditions and acidification. Research is required to investigate and quantify the current availability of, and relationships between, these nutrients and soil acidity to determine the requirements for fertilisation and liming to increase production and profitably from hill and high country farming.

Broad scale aerial application of lime will be required in the South Island hill and high country to reduce soil acidity and alleviate Al toxicity to improve the productivity and persistence of legumes. But first research is required to determine how these soils and legumes will respond to liming in order to develop

strategies to manage liming and maximise the economic and productive outcomes. It is also unknown how these soils will respond to liming in terms of whether a P-sparing effect will occur, or whether alleviating Al toxicity will simply allow plant roots to greater access available soil P, or both. Further research is required to investigate how liming hill and high country soil may affect P availability and the utilisation of applied P, and the growth of legumes, in these soils. As well as being an issue in top soil, acidity and Al toxicity in the subsoil can be a major limitation for root growth and function of deep rooting legumes such as lucerne. Research is required as to how this subsoil acidity and Al toxicity can be alleviated and managed to increase legume growth in these soils. Liming soil with high concentrations of soluble Al leads to the precipitation of this Al, creating highly reactive hydroxy-Al cations which have high affinities for binding P, so it is recommended not to apply P fertiliser soon after liming soils with initial high concentrations of soluble Al. Research is required to determine if this is will be an issue when liming hill and high country soils.

Chapter 3

Current status of soil acidity, fertility and phosphorus fractionation in a range of South Island hill and high country soils

3.1 Introduction

Since the arrival of aerial topdressing in 1949 (Gillingham et al. 1999) hill and high country soils in the South Island of New Zealand were generally regularly well fertilised with superphosphate. But following the removal of economic farm subsidies (EFS) in the mid-1980's the use of fertiliser in South Island hill and high country has substantially reduced (Ledgard et al. 1991; Ledgard & Brier 1993; Dodd & Ledgard 1999). The labile soil P status of hill and high country soils is generally low and response to added fertiliser is variable, mostly dependant on topography (Gillingham & During 1973; Lambert et al. 1983; Gillingham et al. 2003). The soils of the high country are generally acidic, high in exchangeable Al (>3 mg/kg) and have not been limed. When P is applied to acid soils much of it is rapidly absorbed by soil compounds containing Fe and Al hydroxides, fixing P into inorganic and organic forms and making it plant unavailable to various degrees (Parfitt et al. 1989), resulting in low immediate utilisation of applied P (Lambert et al. 1998; Roberts & Johnston 2015; McLaren et al. 2016). This immobilised P can slowly become plant available over time, but completely withholding superphosphate application has been shown to be detrimental to farm productivity. Withholding superphosphate applications can cause significant reductions in Olsen P, annual pasture production, desirable pasture species abundance, such as clovers, and conversely increased abundance of weed species in pastures, including; heracium, browntop and other low producing grasses (Clark et al. 1990; Gillingham et al. 1990; Lambert et al. 1990; O'Connor et al. 1990; Dodd & Ledgard 1999). Therefore, it is clearly important that regular fertiliser applications be maintained. Liming soils has the potential to reduce P sorption and improve P availability to plants, but negative results, by means of reductions in P availability, have also been observed (Haynes 1982; Sorn-Srivichai et al. 1984; Edmeades et al. 1989). However, due to the high economic cost of applying lime by aerial topdressing the high country of New Zealand has mostly gone unlimed (Scott & Mills 1981; Edmeades et al. 1985; Craighead 2005). It is uncertain how these soils will react to liming and how the availability of P within them may be affected.

Few studies have investigated the accumulation of applied P in different P fractions in New Zealand (McDowell & Condron 2012; Mackay & Costall 2016; Tian et al. 2017), and no such studies have been performed on acid soils of the South Island hill and high country. Molloy and Blakemore (1974), and Tate and Newman (1982) examined basic P fractions in a climate driven sequence of undeveloped South Island tussock grassland soils, as did Chen et al. (2003) in a range of grassland soils, but no inference was made

to any potential P fertiliser additions or relationships with soil acidity. The majority of the research on P fertiliser additions to hill and high country soils has been performed in the North Island of New Zealand. This research has predominantly only focused on changes of immediately available P (Olsen P), as driven by fertiliser P inputs, and has not looked at changes in soil P fractions or investigated the degree of P immobilisation and P mineralisation in these soils.

Studies of long term fertiliser trials have shown that applied P that is not immediately utilised by plants initially accumulates in soil in organic forms up to a plateau, beyond which applied P then predominantly accumulates in sparingly soluble inorganic forms (McDowell & Condron 2012; McLaren et al. 2015; Schefe et al. 2015). Recent studies on P fractions in fertilised and unfertilised acid soils overseas have shown that the soil compounds with the highest affinity for P sorption are oxalate extractable Al>clay>organic carbon>oxalate extractable iron (Herlihy & McGrath 2007; Redel et al. 2016; Velasquez et al. 2016). In these soils, residual P was mostly correlated with oxalate extractable iron, which is most likely associated with P occlusion on soil particles.

Understanding the fate of applied P fertiliser to hill and high country is important for future designing and managing of fertiliser and liming strategies, in order to optimise fertiliser P use and economic return. To meet the objective of this experiment; "*To investigate soil P and S availability in unlimed, acid hill and high country soils varying in fertiliser application history*." detailed chemical analysis, including P fractionation, was performed on a collection of 19 farmed South Island high country soils to investigate the P and S resources they contain and to determine if the speciation and plant availability of these can be determined by soil acidity related factors. Understanding of the speciation of P and S resources in these soils is required to envision the potential changes in plant availability that liming these soils may invoke.

3.2 Methods

3.2.1 Soil Sample Collection and Preparation

At each of 19 field sites, 30 soil cores (15 cm deep, 2.5 cm diameter) where taken and then divided into 0-7.5 cm and 7.5-15 cm sampling depths. Site specific details of each soil are presented in Table 3.1. The collected soil was then air dried at 30˚C for one week and passed through a 2 mm sieve. A 60 mL subsample of each soil was then oven dried at 65˚C for a further 24 hours for use in P fractionation analysis.

Table 3.1 Site specific details of the collected soils.

Collection Site	Soil ID	Location	Mean Annual Rainfall (mm)[5]	Allevation (masl)	Soil Order
Molesworth Station[1]	MO	Marlborough	720	915	Brown[1]
Glenmore Station[1]	GM	Tekapo	620	765	Brown[1]
Omarama Station[1]	OM	Omarama	490	490	Recent[1]
Ben Dhu Station[1]	BD	Omarama	610	545	Brown[1]
Lindis Peaks Station[1]	LP	Tarras	700	530	Pallic[1]
Glenfoyle Station[1]	GF	Hawea	580	785	Brown[1]
Mt Grand Station[1]	MG	Hawea	570	565	Brown[1]
Sawdon Station 1[2]	SW1	Tekapo	460	755	Recent[4]
Sawdon Station 2[2]	SW2	Tekapo	460	760	Recent[4]
Simon's Hill Station 1[2]	SH1	MacKenzie	660	510	Pallic[4]
Simon's Hill Station 2[2]	SH2	MacKenzie	660	510	Pallic[4]
Waikora Station[2]	WK	Hakataramea	550	580	Pallic[4]
Ida Valley Station 1[2]	IV1	Poolburn Range	550	820	Pallic
Ida Valley Station 2[2]	IV2	Poolburn Range	550	800	Pallic
Glenmore Ripper Site[3]	GMR	Tekapo	620	740	Brown
Omarama Ripper Site[3]	OMR	Omarama	490	475	Brown[4]
The Dasher[2]	TD	Oamaru	850	570	Brown[4]
The Dasher 'Paradise'[3]	TDP	Oamaru	850	540	Brown[4]
The Dasher 'PH Lane'[3]	TDPH	Oamaru	850	570	Brown[4]

[1]Soil and soil data collected by Whitley et al. (2016). [2]Soil collected June 2017. [3]Soil collected from deep placed lime experiment (Chapter 6). [4]Soil data sourced from S-Map Online (Manaaki Whenua Landcare Research 2018). [5]Mean annual rainfall data sourced from NIWA Cli-Flo database (NIWA 2019) using the nearest weather recording station, excluding GM, GMR, OM, OMR, MG, TDP, and TDPH sites where rainfall data has been recorded on site during this study.

The MO, GM, OM, BD, LP, GF and MG soils were all collected by Whitley et al. (2016) for a study investigating the relationship between soil pH and Al in high country environments and are ideal soils to further analyse for P fractions for use in this study. Where soils collected as part of this study were selected from the same farm (SW1 and SW2, TD, IV1 and IV2,) each was selected based on knowing that in the past one site had received inputs of P fertiliser and/or lime, and one had not been developed as intensively (or at all). By doing so, the effects of applying lime and P fertiliser on soil chemistry and P fractions were investigated. Alternatively, for the SH1 and SH2 soils the SH1 sample is from a south facing slope and the SH2 sample from a north facing slope so any effects of soil aspect could be investigated. Analysis of the

soils from each of the sites used in the deep placed lime experiment (GMR, OMR, TDP, and TDPH, Chapter 6) add both to the depth of this experiment and provides useful information for the deep place lime experiment. The TD soil, which has been developed into pasture with both lime and P fertiliser, is directly adjacent to where the completely undeveloped TDPH soil was collected from, so may provide useful information on how inputs of lime and P fertiliser can affect soil chemical characteristics and P fractionation.

3.2.2 Soil Chemical analysis

pH_{H2O}

Soil pH in water was measured at a 2.5:1 DI water to soil ratio following 30 minutes on an end-over-end shaker then a 4 hour rest (Blakemore et al. 1987).

pH_{CaCl2}

Soil pH in calcium chloride ($CaCl_2$) was measured at a 2.5:1 0.01M $CaCl_2$ to soil ratio following 30 minutes on an end-over-end shaker then a 4 hour rest (Blakemore et al. 1987).

Exchangeable Aluminium

Soil was extracted with 0.02 M $CaCl_2$ at a 4:1 $CaCl_2$ to soil ratio by being shaken on an end-over-end shaker for one hour then filtered (Whatman 1) with the supernatant analysed by ICP-OES (Varian 720-ES ICP-OES; Varian Inc., Victoria, Australia) (Edmeades et al. 1983).

Olsen P

Soil Olsen P was measured by the extraction of 1 g of soil in 20 mL 0.5 M $NaHCO_3$ at pH 8.5 by being shaken for 30 minutes on an end-over-end shaker then filtered (Whatman 42) (Olsen et al. 1954). The filtered supernatant was then colourmetrically analysed according to the method of Murphy and Riley (1962) using a UV-Vis spectrophotometer (Varian Cary 50, Varian Inc., Victoria Australia) with the wavelength set to 882 nm (see Appendix A.2).

Sulphate-S and Organic-S

Soil was extracted with 0.02 M KH_2PO_4 at a 10:1 KH_2PO_4 to soil by being shaken on an end-over-end shaker for 30 minutes then filtered (Whatman 41). Sulphate-S in the supernatant was then determined using high pressure ion chromatography (HPIC) (Dionex ICS-5000+, Thermo-Fisher Scientific Inc., Whaltham, MA USA) according to the method of Watkinson and Kear (1994) using NaOH as the eluent.

The total amount of sulphur extracted by the KH_2PO_4 (TES) was measured by ICP-OES and extractable organic-S was determined as the difference between TES and Sulphate-S (Watkinson & Kear 1996).

Total Carbon and Nitrogen

Using an Elementar Vario-Max Cube Analyser CN (Elementar Analysensystane GmbH, Hanau, Germany) soil was combusted at 900˚C in a pure stream of oxygen. All carbon was fully oxidised to CO_2 and all nitrogen to N_2 gas and both were then measured by thermal conductivity detector to determined total C and N.

CEC, Exchangeable Cations and Base Saturation

Soil CEC and exchangeable cations (Ca, Mg, K, Na) were measured by the extraction of soil at a 1:50 soil to 0.01 M Silver thiourea ratio by being shaken on an end-over-end shaker for 16 hours, then centrifuged at 3000 rpm for 10 minutes before being filtered (Whatman 40). Concentration of each cation in the supernatant (me/100g) was then determined by ICP-OES (Varian 720-ES ICP-OES; Varian Inc., Victoria, Australia). CEC was measured as the total concentration of the base cations along with Al and Mn in the supernatant (Blakemore et al. 1987; Rayment & Lyons 2011).

Base saturation was determined as the combined concentration of base cations (Ca, Mg, K, Na) within the cation exchange capacity (%).

Anion Storage Capacity (ASC)

Soil ASC was measured by shaking soil at a 1:5 soil to 1000 mg P/l KH_2PO_4 solution at pH 4.6 on an end over end shaker for 16 hours before being filtered (Whatman 42). The P left remaining in solution is then measured colourmetrically according to the method of Murphy and Riley (1962) using a UV-Vis spectrophotometer (Varian Cary 50, Varian Inc., Victoria Australia) with the wavelength set to 882 nm (see Appendix A.2). The difference in initial P in the KH_2PO_4 solution and the final P in the extracted supernatant determines the amount of P (%) retained by the soil (Saunders 1965; Blakemore et al. 1987).

P Fractionation

Soil P fractionation was carried out according to the method of Boitt et al. (2018a); developed from the methods of Hedley et al. (1982), Condron and Goh (1989), Condron et al. (1996), Olsen and Sommers (1982), and Condron and Newman (2011).

For this analysis 0.5 g of oven dried soil was sequentially extracted to quantify forms of inorganic P (Pi) and organic P (Po) with decreasing lability using the following extractants: 10 mL 1 M NH_4Cl (NH_4Cl Pi), 10 mL 0.5 M $NaHCO_3$ at pH 8.5 ($NaHCO_3$ Pi and $NaHCO_3$ Po); 10 mL 0.1 M NaOH (NaOH I Pi and NaOH I Po); 10 mL 1M HCl (HCl Pi), 10 mL 0.1 NaOH for a second time (NaOH II Pi and NaOH II Po); and finally digestion with concentrated H_2SO_4 + H_2O_2 (Residual P). For specific method details see Appendix A.

Cross and Schlesinger (1995) and Boitt et al. (2018a) describe the P form in these extracts as soil solution P (NH_4Cl), labile Pi and Po ($NaHCO_3$), moderately labile Pi and Po (NaOH-I and NaOH-II), calcium-bound Pi (HCl), and highly stable P or 'residual P' (conc. H_2SO_4 + H_2O_2).

For the acidic extractions (NH_4Cl and HCl) the method of Murphy and Riley (1962) was used to determine the amount of Pi in the extracted supernatant with a UV-Vis spectrophotometer at a wavelength of 882 nm (Appendix A.2). For the alkali extractions ($NaHCO_3$, NaOH-I and NaOH-II) the method of Dick and Tabatabai (1977), with the procedure modification by He and Honeycutt (2005), was used to determine the amount of Pi in the extracted supernatant with a UV-Vis spectrophotometer at a wavelength of 850 nm (Appendix A.3), and auto-clave digestion using the USEPA (1983) method incorporating the recommendations of do Nascimento et al. (2015). This was followed by determination of total P (P_T) contained in the supernatant by way of the Murphy and Riley (1962) method. With Po being the difference between Pi and P_T.

3.2.3 Statistical Analysis

Differences in means of soil pH and Al data and P fractions between the 0-7.5 cm and 7.5-15 cm sampling depths were analysed for significance ($P \leq 0.05$) by one-way ANOVA in Genstat 16. All measured soil chemical data and P fractions for the more intensively chemically analysed 0-7.5 cm sampling depth were then analysed by Pearson Correlation (Genstat 16) and Principal Component Analysis (PCA) (R Graphics version 3.3.3).

3.3 Results

3.3.1 Soil Chemical Characteristics

Soil from the 7.5 cm deep core samples collected at each sampling site were extensively chemically analysed and the results are presented in Table 3.2.

Mean soil pH_{H2O} in the 0-7.5 cm depth range across all of the sites was very low (pH_{H2O} 5.2), and pH_{CaCl2} was on average 0.7±0.03 units lower than pH_{H20}. The soil at the MO and TDPH sites had the most extreme acidity with pH_{H20} of 4.6 and 4.3 respectively, and pH_{CaCl2} of <4.0 for both. Despite a mean Al concentration of 4.1±1.44 mg/kg in these soils, Al only exceeded the 3 mg/kg general toxicity threshold at 7 out of the 19 sites at the 0-7.5 cm sampling depth. Due to the exponential nature of the relationship between Al and soil pH (Figures 3.1 and 3.2), where Al did exceed the toxicity threshold it generally greatly exceeded. This was particularly the case at the MO and TDPH sites where pH_{H2O} was <5.0 and pH_{CaCl2} was <4.0. The very high concentrations of Al at these sites skews the overall mean Al concentration to above the 3 mg/kg toxicity threshold for across all of the sites. The TD site is directly adjacent to the TDPH site and has been limed in the past. Despite the soil pH still being very low at the TD site (pH_{H2O} of 5.35), soil pH_{H2O} was 1.0

unit higher than the TDPH site due to past development, and as a consequence the Al concentration has been greatly reduced to 2.06 mg/kg at TD, compared to 24.5 mg/kg at TDPH.

Table 3.2 Basic chemical characteristics of collected soils at 0-7.5 cm depth.

Site	pH_{H2O}	pH_{CaCl2}	Al (mg/kg)	C (%)	N (%)	C:N	Olsen P (µg/ml)	Total P* (mg/kg)	SO_4-S (mg/kg)	Org-S (mg/kg)	ASC (%)
MO	4.6	3.9	17.0	5.59	0.42	13.4	20	1180	5	8	47
GM	4.8	4.3	6.6	9.04	0.71	12.7	21	1570	14	12	34
OM	5.1	4.3	3.2	2.56	0.19	13.3	7	587	<1	<1	20
BD	5.5	4.5	1.5	4.24	0.29	14.6	12	807	2	1	33
LP	5.3	5.0	0.6	2.47	0.23	10.8	13	752	16	9	21
GF	4.9	4.1	7.4	4.65	0.35	13.3	12	1010	3	4	33
MG	5.1	4.4	2.3	4.92	0.44	11.1	11	998	4	4	20
SW1	5.5	4.7	0.6	3.69	0.30	12.3	34	1400	8	3	18
SW2	5.3	4.4	1.9	2.93	0.20	14.4	58	1340	13	3	22
SH1	5.5	4.8	0.9	4.08	0.39	10.5	13	1280	4	3	19
SH2	5.3	4.5	1.1	4.21	0.37	11.5	11	1220	4	3	21
WK	5.5	4.7	0.5	2.58	0.22	11.8	17	723	2	3	13
IV1	5.0	4.3	3.6	4.58	0.41	11.3	16	1010	10	6	23
IV2	5.5	4.8	0.9	4.59	0.38	11.9	16	1030	6	4	17
GMR	4.7	4.2	5.9	5.53	0.44	12.5	32	1200	26	8	30
OMR	5.3	4.6	1.5	2.88	0.24	11.8	16	764	7	4	19
TD	5.4	4.8	2.1	6.91	0.51	13.6	12	915	6	7	33
TDP	5.1	4.7	1.9	7.43	0.58	12.9	14	916	8	15	35
TDPH	4.3	3.7	24.5	7.51	0.46	16.2	9	813	15	12	59

* From P fractionation

Soil carbon (C %) and nitrogen (N %) content was greatest at the GM site. Compared to the other sites the C % and N % was also elevated (>6.9%, and >0.46% respectively) in the three soils collected from The Dasher (TD, TDP, and TDPH). Other than these four sites, soil C % was only in the range of 2.47-5.59% at the other 15 sites. N % was generally very low across the other 15 sites, ranging from 0.19% at OM to 0.44% at the MG and GMR sites. At the recently fertilised SW1 and SW2 sites, as indicated by elevated Olsen P and SO_4-S levels, N % was still very low (0.2 and 0.3% respectively) indicating that very little inputs of N from legume biological N fixation has occurred despite the fertilisation. C:N ratio was generally quite consistent across the sampled sites, apart from at the TDPH site where is was much higher (16.2 compared to a mean of 12.6±0.33).

Soil Olsen P and SO_4-S show to be good measures of recent fertiliser history across the sites with most of the soils having low levels of each, compared to some soils where known recent fertiliser application is evident by elevated Olsen P and SO_4-S levels. This is especially the case for the GM, GMR, and the recently developed SW1 and SW2 soils. In contrast it is obvious the OM soil has received very little to no fertiliser in the past, with an Olsen P of only 7 µg/ml and severely deficient SO_4-S and Org-S levels, both <1 mg/kg. Also low is the BD soil with an Olsen P of 12 µg/ml and SO_4-S and Org-S levels of only 2 mg/kg and 1 mg/kg respectively. At LP a low Olsen P level, but higher SO_4-S and Org-S levels, indicates the possible use of straight elemental-S fertiliser, or a very highly element-S fortified superphosphate use. For the TDP and TDPH soils the greater levels of S, particularly Org-S, is mostly likely to have arisen from atmospheric inputs due to the vicinity and exposure of the site to the sea. Generally SO_4-S and Org-S levels are very low across these soils and this is very likely to be the major limiting nutritional factor in these soils.

Across the sampled soils ASC, or P retention, was generally relatively consistent, within the range of 17 to 35%, aside from the MO and TDPH soils where it was 47% and 59% respectively.

Of the available chemical measures, only soil pH and Al concentrations were measured in the 7.5-15 cm layer (Table 3.3). Comparing between the 0-7.5 and 7.5-15 cm layers, the average soil pH was relatively consistent, with only the average pH_{CaCl2} being slightly lower (0.12 pH units) in the 7.5-15 cm layer than the 0-7.5 cm layer (P=0.205). This indicates a possible greater amount of reserve acidity in this deeper layer. Soil Al was also slightly greater in the 7.5-15 cm soil layer (6.23±1.98) compared to the 0-7.5 cm layer (4.08±1.44) (P=0.330). Compared to the 0-7.5 cm soil layer where 7 out of the 19 sampled soils had Al concentrations greater than the 3 mg/kg toxicity threshold, at 7.5-15 cm 10 soils exceeded this threshold. Al concentration was especially high in this layer at TDPH (37.2 mg/kg) and the less developed SW2 site (16.7 mg/kg). Interestingly, Al concentration at MO was very much lower at 7.5-15 cm depth (0.51 mg/kg) compared to the 0-7.5 cm soil (17.0 mg/kg). Soil pH and Al was particularly extreme at the TDPH site, especially in the 7.5-15 cm layer, but in the adjacent TD site that has previously been limed and developed into permanent pasture both pH and Al were much less extreme.

Table 3.3 Soil pH and exchangeable Al concentrations of collected soils at 7.5-15 cm depth.

Site	pH_{H2O}	pH_{CaCl2}	Al (mg/kg)
MO	5.4	4.6	0.5
GM	5.3	4.5	2.0
OM	5.6	4.8	0.5
BD	5.4	4.6	0.5
LP	5.4	4.7	1.9
GF	5.7	4.7	0.3
MG	4.9	4.1	7.3
SW1	5.4	4.6	0.9
SW2	4.8	4.1	16.7
SH1	5.0	4.3	6.0
SH2	5.4	4.1	6.2
WK	5.3	4.4	1.4
IV1	4.8	4.0	11.5
IV2	5.1	4.4	2.2
GMR	4.9	4.3	6.3
OMR	5.0	4.2	8.0
TD	5.2	4.3	4.0
TDP	4.8	4.2	5.1
TDPH	4.3	3.7	37.2

Soil Al concentration increased exponentially in relation to lower soil pH across the 19 sampled soils. Soil Al concentrations exceeded the 3 mg/kg toxicity threshold at pH_{H20} of 5.1 (Figure 3.1), and at pH_{CaCl2} of 4.3 (Figure 3.2), at both sampling depths (0-7.5 and 7.5-15 cm).

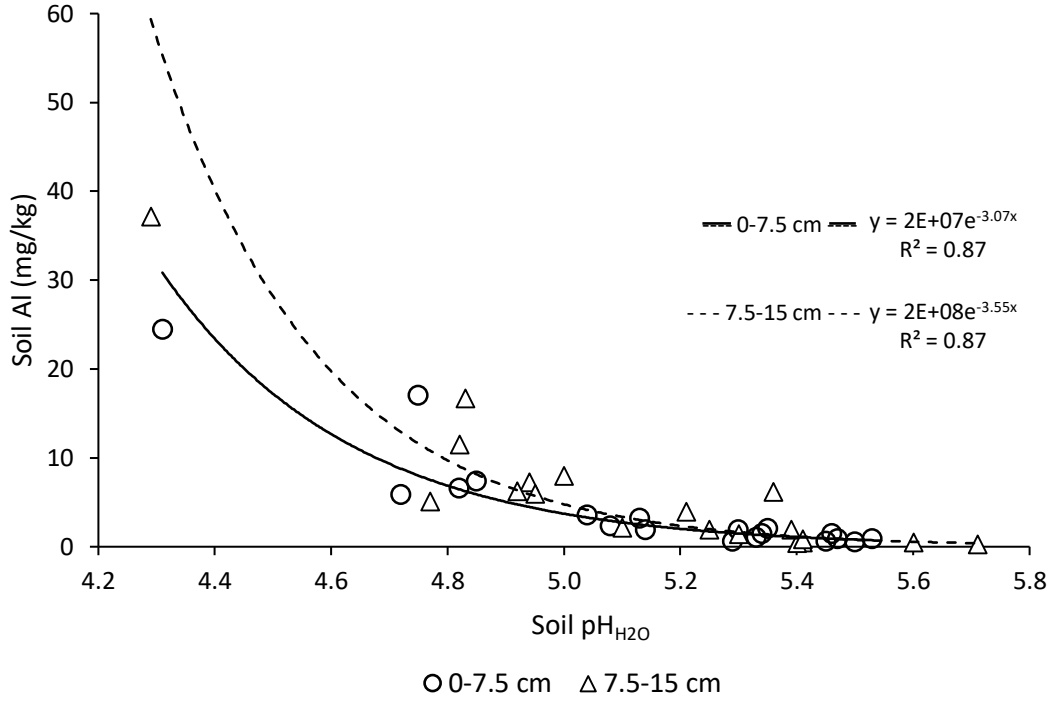

Figure 3.1 The relationship between soil pH$_{H2O}$ and Al across all sampled sites at depths of 0-7.5 cm and 7.5-15 cm.

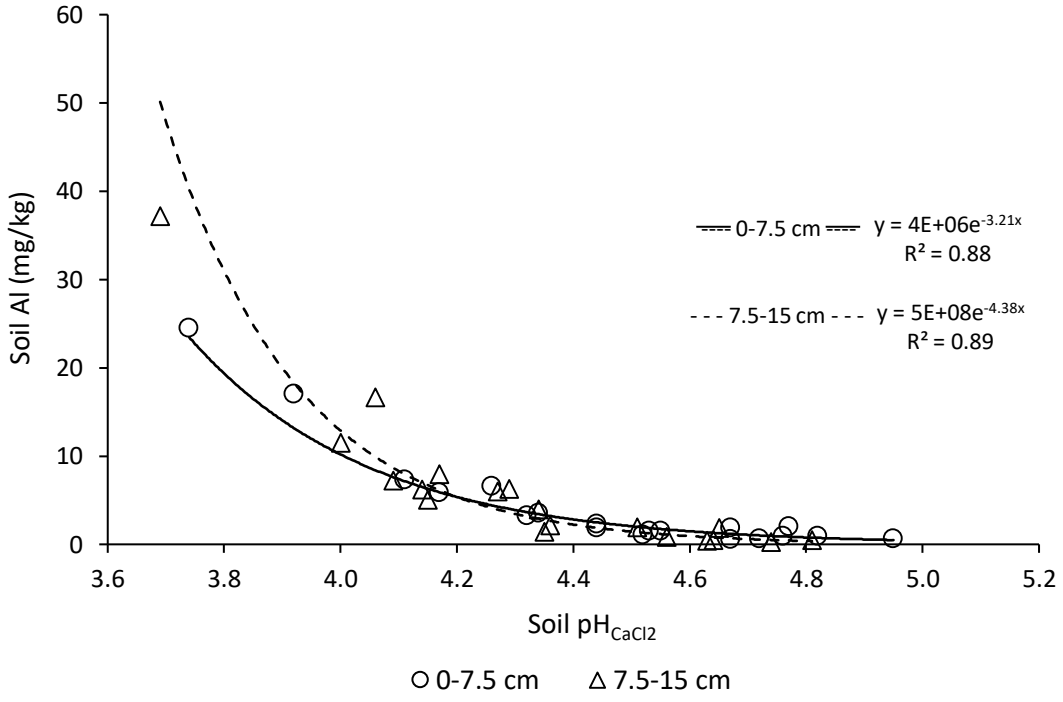

Figure 3.2 The relationship between soil pH$_{CaCl2}$ and Al across all sampled sites at depths of 0-7.5 cm and 7.5-15 cm.

The exponential relationship between pH$_{H2O}$ and Al concentrations was highly correlated (R^2 of 0.87 and 0.81 for the 0-7.5 and 7.5-15 cm layers respectively), and the relationship between pH$_{CaCl2}$ and Al was even more highly correlated (R^2 of 0.88 and 0.89 for the 0-7.5 and 7.5-15 cm layers respectively).

3.3.2 Soil P fractionation

The quantity and distribution of P contained in each fraction, and additionally total plant available P, across the 19 sampled soils at both sampling depths is presented in Figure 3.3. This figure (as well as Figures 3.4 and 3.5) shows the median P concentration with upper and lower quartiles of the data, with the mean presented as a light grey bar where different from the mean. The whiskers of the boxes show the absolute maximum and minimum P concentration measured in each fraction from within the 19 soils. A full tabulated description of all P fraction data for each soil at both sampling depths is presented in Appendix B.

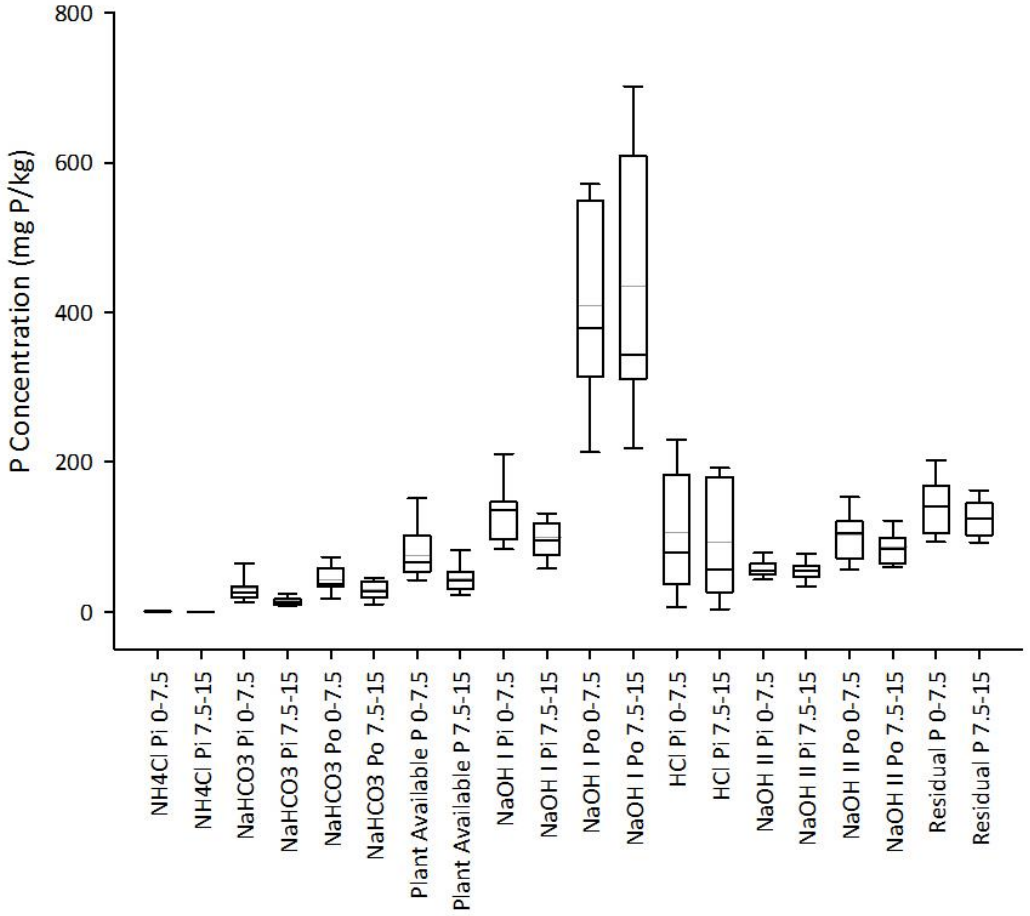

Figure 3.3 The concentration and distribution of P contained in the 19 sampled high country soils within each of the nine P fractions, as well as total plant available P, at sampling depths of 0-7.5 cm and 7.5-15 cm.

P concentrations within the first four of the nine sequentially extracted fractions (NH_4Cl, $NaHCO_3$ Pi and Po, and NaOH I Pi) were significantly greater in the 0-7.5 cm soil layer than the 7.5-15 cm soil ($P<0.001$, $P=0.001$, $P=0.009$, and $P=0.024$ for the four fractions respectively). Corresponding to these results total available P (NH_4Cl, $NaHCO_3$ Pi and $NaHCO_3$ Po combined) was also greater in the 0-7.5 cm soil layer (78.1±7.91 mg P/kg soil) compared to the 7.5-15 cm layer (44.4±4.20 mg P/kg soil) ($P<0.001$).

Within the 0-7.5 cm soil layer, soil solution P (NH_4Cl extractable P) was the smallest fraction, averaging just 0.69±0.10 mg/kg. Soil solution P was greatest in the recently fertilised SW1 and SW2 soils at 1.48 and 1.44 mg/kg for the two respectively. $NaHCO_3$ Pi was also greatest at these two sites at 64.3 and 93.1 mg/kg respectively. In contrast to the SW1 and SW2 sites, $NaHCO_3$ Pi was only 12.7 mg/kg at the OM site, and only 13.1 mg/kg at the TDPH site. As with the $NaHCO_3$ Pi, $NaHCO_3$ Po was lowest at the OM at just 7.30 mg/kg. At the GM site $NaHCO_3$ Po was outstandingly greater than the other soils at 113.2 mg/kg, compared to an average of 43.7±5.47 mg/kg across all 19 sites. The high $NaHCO_3$ Pi at the SW2 site, and the high $NaHCO_3$ Po at the GM site contributed to these sites having the greatest amounts of total plant available P at 151.5 mg/kg and 152.0 mg/kg respectively. Compared to these sites total plant available P was just 20.1 mg/kg at the OM site which was the lowest of all the sites. Across all the sites total plant available P averaged just 7.18±0.45% of the total amount of P contained in these soils.

In both sampling layers, the NaOH I fraction contained the greatest amount of Pi and Po compared to all the other fractions, with the NaOH I Po fraction by far containing the greatest amount of P overall. In the 0-7.5 cm layer, NaOH I Pi ranged from 71.6 mg/kg (TDPH soil) up to 299.5 mg/kg (SW2 soil). NaOH I Po ranged from 176.5 mg/kg (OM soil) up to 729.7 mg/kg (GM soil). The range of P contained in HCl fraction across the sampled soils was also considerably large, ranging from a very low 3.30 mg/kg (TDPH soil) up to 280.8 mg/kg (SW1 soil). This indicates a large range in the amount of P bound to Ca in the sampled soils.

For the second NaOH extractable fraction (NaOH II) there was much less P stored in this fraction than in the NaOH I fraction. The range in Pi and Po contained in this NaOH II fraction was also much narrower, ranging from 33.8 mg/kg (TDPH soil) to 81.1 mg/kg (IV1 soil) for Pi, and from 54.5 mg/kg (SW2 soil) to 167.8 mg/kg (GM soil) for Po. Both of these are for the 0-7.5 cm sampling depth. Residual P was also relatively consistent between the sampled soils, averaging 142.0±8.44 mg/kg in the 0-7.5 cm sampling depth.

In comparing the total amounts of Pi and Po contained at the 0-7.5 cm and 7.5-15 cm sampling depths across all of the soils (Figure 3.4) only the total amount of Pi was greater ($P=0.042$) in the 0-7.5 cm soil layer (347±33.3 mg/kg) compared to the 7.5-15 cm layer (265±25.7 mg/kg). Total Po ($P=0.549$) and overall total P ($P=0.145$) were no different between the two sampling layers, averaging 553±30.4 mg/kg for total Po and 983±42.2 mg/kg for overall total P respectively.

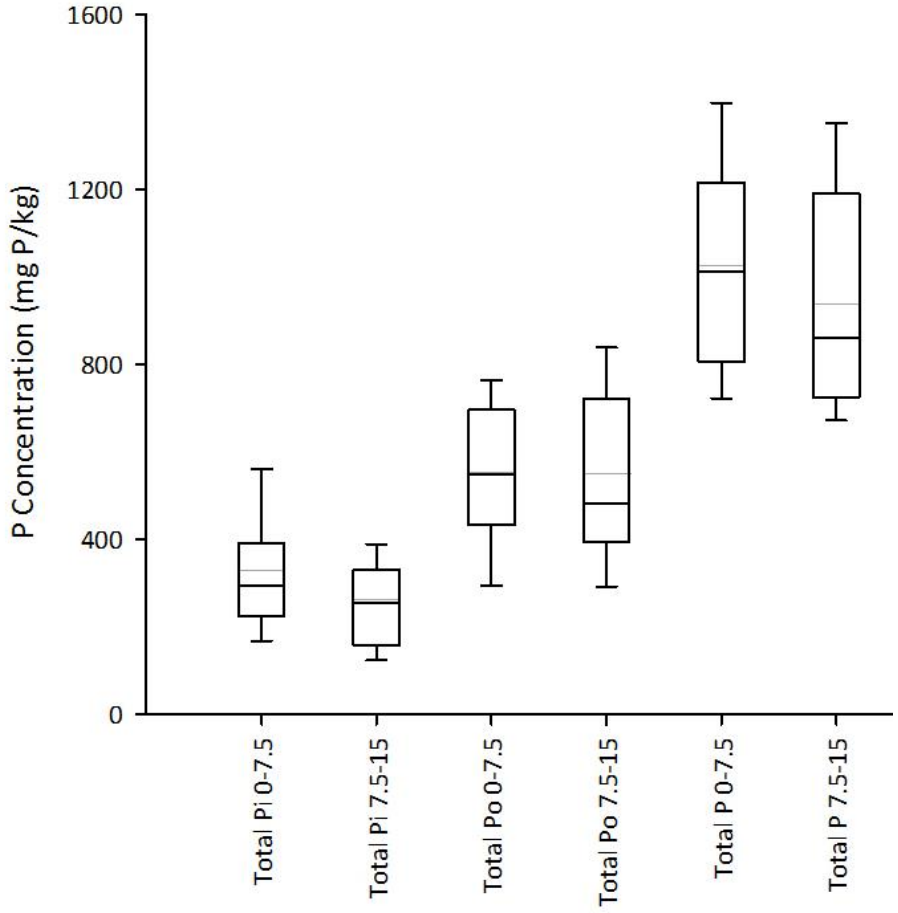

Figure 3.4 The distribution of the total amounts of Pi and Po, and overall total P at sampling depths of 0-7.5 cm and 7.5-15 cm, in the 19 sampled soils.

The quantity of P contained in each P fraction can also be expressed in terms of its proportion within the total amount of P in soil (Figure 3.5). As with the total concentrations of plant available P (Figure 3.3.), when expressed as a proportion of total P the total plant available P was greater in the 0-7.5 cm sampled soil than the 7.5-15 cm soil ($P<0.001$). This is due to the proportion of NH_4Cl Pi ($P<0.001$), although only a very small proportion of total P (<0.1%), and $NaHCO_3$ Pi ($P<0.001$) being greater in the 0-7.5 cm soil than the 7.5-15 cm soil. $NaHCO_3$ Po was however not different at either depth ($P=0.093$). Overall, plant available P (NH_4Cl, $NaHCO_3$ Pi and $NaHCO_3$ Po combined) was on average only 7.18±0.45 % and 4.83±0.36 % of total soil P at 0-7.5 cm and 7.5-15 cm depth respectively. NaOH I Pi was only slightly greater ($P=0.050$) as a proportion of total P in the 0-7.5 cm layer (13.3±0.91%) that the 7.5-15 cm layer (10.9±0.75%). Compared to P concentration, when expressed as a proportion of total P the P contained in NaOH I Po fraction was greater ($P=0.013$) in the 7.5-15 cm deep soil (45.2±1.74%) than the 0-7.5 cm deep soil (39.1±1.50%). Beyond the NaOH I Po fraction, there were no differences in the proportion of total P contained in the remaining fractions.

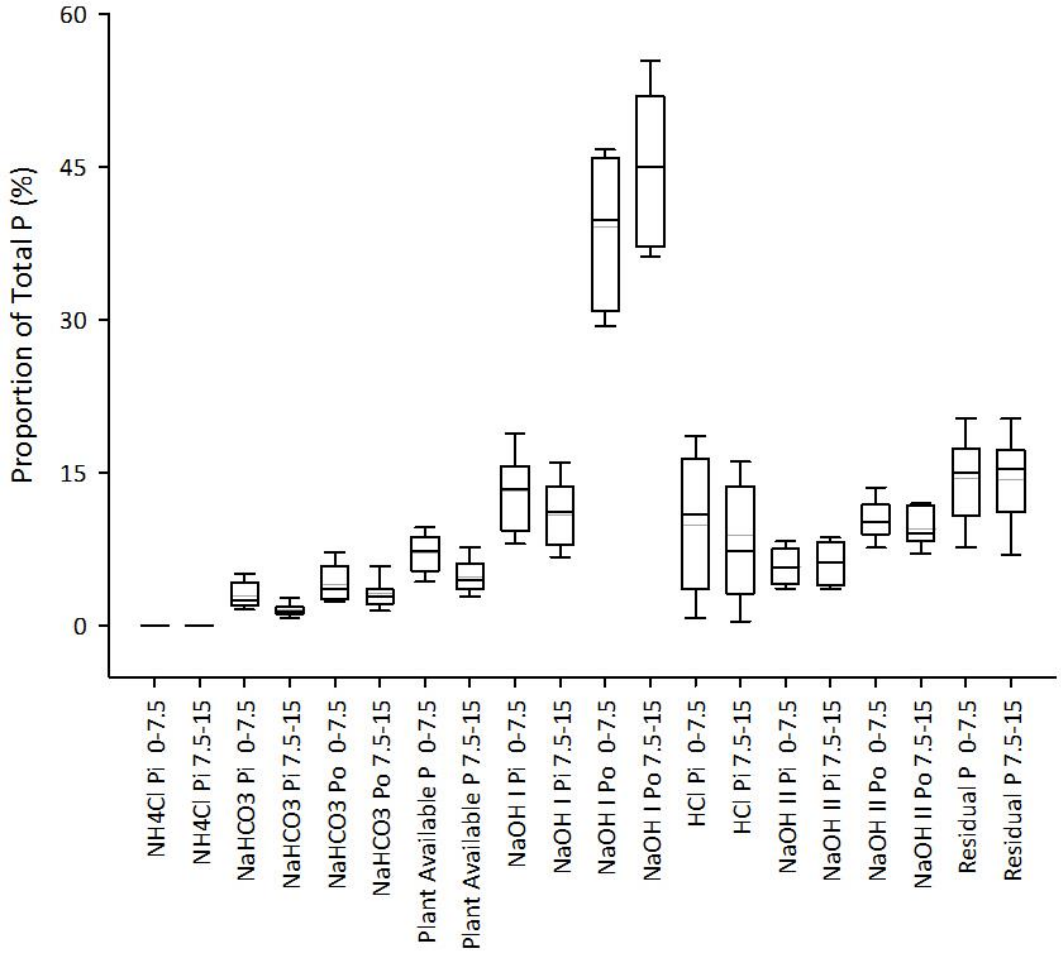

Figure 3.5 The concentration and distribution of P contained in the 19 sampled high country soils expressed as the proportion of total soil P (%) within each of the nine P fractions, as well as total plant available P, at sampling depths of 0-7.5 cm and 7.5-15 cm.

3.3.3 Pearson's correlations between soil P fractions and chemical characteristics

Aside from the strong correlation between soil pH and Al (Figures 3.1 and 3.2), analysis of all soil chemical characteristics and P fractionation results in the 0-7.5 cm sampling range against one another by Pearson's correlation showed very few other strong correlations.

Interestingly, within the P fractions themselves, the Pi NaOH II fraction was not at all correlated to any of the other nine fractions ($-0.15<R^2<0.11$) or to the total amount of P in each soil ($R^2=0.08$). The residual P fraction was also not well correlated to the other fractions ($-0.45<R^2<0.55$).

Soil pH (H_2O and $CaCl_2$) and exchangeable Al were not correlated to the either the amount or proportion of P contained in each of the P fractions. Correlation coefficients between soil pH and each of the P fractions, including plant available P, total Pi and Po, and overall total P, were all within the range of $-0.54<R^2<0.37$ for pH_{H2O}, and $-0.40<R^2<0.28$ for pH_{CaCl2}. And for Al each was within the range of $-0.46<R^2<0.43$.

Each of the P fractions were also not strongly correlated to ASC (-0.47<R^2<0.46). However, ASC was strongly negatively correlated to soil pH (R^2=-0.80 and -0.73 for pH_{H2O} and pH_{CaCl2} respectively), strongly correlated to Al (R^2=0.88), and moderately related to soil C content (R^2=0.70).

Olsen P was strongly correlated to NH_4Cl Pi (R^2=0.74), $NaHCO_3$ Pi (R^2=0.98), and NaOH I Pi (R^2=0.93). However, in contrast to the two plant available Pi fractions, NH_4Cl Pi and $NaHCO_3$ Pi, Olsen P was poorly correlated to $NaHCO_3$ Po (R^2=0.25).

There was a slight correlation between SO_4-S and each of the plant available P fractions, giving an R^2 of 0.57 for the total amount of plant available P, and Olsen P (R^2=0.45). The amount of N in soil (N%) was slightly correlated to Org-S (R^2=0.59) (and not Olsen P (R^2=-0.17)), which indicates biological N fixation is more closely related to S availability than P availability in these high country environments.

At the 7.5-15 cm sampling depth slightly stronger positive correlations of each of the Pi fractions and soil pH (H_2O and $CaCl_2$), and to a lesser extent Al than in the 0-7.5 cm sampling depth. Correlations of P fractions against soil chemical data were generally weaker when P was expressed as a proportion of the total P in soil rather than absolute concentrations at both sampling depths.

For the full tabulation of correlation coefficients see Appendix B.

3.3.4 Statistical analysis of all soil chemical and P fractionation data in 0-7.5 cm sampling depth by principal component analysis

Ordination of all the chemical and P fractionation data from all of the sampled soils within the 0-7.5 cm sampling depth by PCA revealed three main principal components (PC's) within the data (Figure 3.6). These PC's primarily relate to soil organic chemistry, including organic P (PC1); soil pH, Al and exchangeable cations (PC2); and then the plant available and moderately labile P fractions, including Olsen P, and SO_4-S (which possibly indicates a relationship to fertiliser history) (PC3). Together these three PC's account for 31.8%, 24.7% and 20.5% of the variance within the total dataset respectively, cumulatively totalling 77.0%. All further PC's diminishingly explained >7.50% of the remaining variance each and were deemed to have minor importance in comparison to the first three PC's.

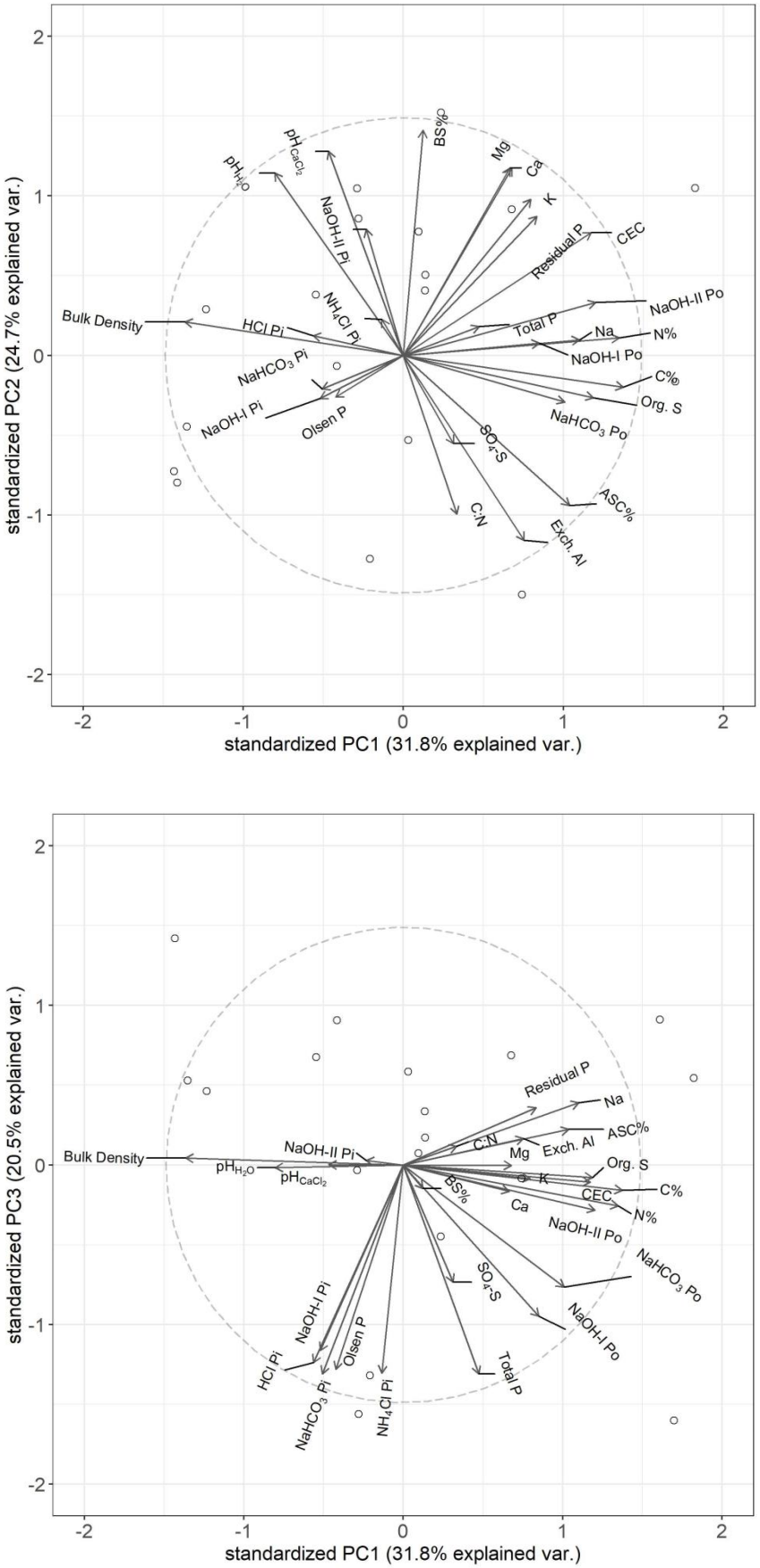

Figure 3.6 Graphical representation of the first principal component (PC1) against the second (PC2) (top), and PC1 against the third principal component (PC3) (bottom).

In general, the soil P fractions were not specifically related to any other chemical factors when comparing the principal components against each other, as indicated by the size and orientation of the vector loadings (Fig 3.6). Soil pH was also not consistently related to other factors but was shown to be inversely related to Al in both graphs, as already shown in Figures 3.1 and 3.2. However, the NaOH II Pi fraction was shown to be related to soil pH in both PCA graphs, although they were concurrently poorly correlated in the analysis by Pearson's coefficients (R^2= 0.09 for pH_{H2O}).

3.4 Discussion

In general, Olsen P concentrations across the sampled sites were reasonably adequate for hill and high country pastoral farming. Although most were below proposed biological optimum levels of 20-30 µg/ml (Edmeades et al. 2016), all but two sites had an Olsen P of >10 µg/ml which is likely to be well within the economic optimal range, given the soil and climatic limitations to production, at these sites (Gillingham et al. 1984; Gillingham et al. 2003). However, S concentrations were found to be very low (Edmeades et al. 2005) at the majority of the sites and are likely to be the main nutrient limitation to clover production and N fixation at these sites.

It has been stated that P fertilisation and availability is the main driver of hill and high country pasture production (Edmeades et al. 1984a; Edmeades et al. 2016), but given these findings it is likely that S, rather than P, is the main limiting nutrient to clover production in South Island hill and high country pastures. Of the 19 sampled sites only two (GM and TDPH) contained concentrations of both SO_4-S and Org-S greater than the recommended 10 mg/kg critical minimal concentration for both (Edmeades 2005; Watkinson & Kerr 1996). Given that S is an inexpensive nutrient to apply, relative to P (Scott 1998a; Edmeades et al. 2016), is it therefore likely that applying S will produce the greatest clover growth and economical response in these soils.

Scott (1998a) reports that in a high country environment, pasture yield, response to fertiliser, and stock carrying capacity was more dependent on the amount and frequency of S fertiliser applied over that of P. And both Craighead et al. (1990), and Craighead and Metherell (2006), reported that pasture clover content and soil N content (%) were dependant on S inputs when developing a South Island high country site that had an Olsen P of 19 µg/ml. In this survey soil N% was not found to be as strongly correlated to soil S concentrations (R^2=0.59 for Org-S), but was more so that Olsen P (R^2=-0.17). Boswell et al. (2003a) reported that without S fertilisation clover growth, and hence biological N fixation, was not existent without the addition of S fertiliser at a South Island high country site.

The low concentrations of S in the sampled soils, the results of other past studies, and given the propensity of S to leach from soil, highlights the importance of regularly applying S fertiliser to sustain clover growth

and biological N inputs to South Island hill and high country (Edmeades et al. 2005; Craighead & Metherell 2006). Superphosphate is most commonly applied fertiliser to hill and high country, and has the benefit of containing both P and S. In these instances where applying S is of high importance superphosphate can be fortified with additional elemental S and this would be recommended for these sampled soils, possibly with the exception of the GM (and GMR) soil where both Olsen P and S concentrations are already adequate. Other forms of S based fertiliser are available, including elemental S pressed into prills so that S can be solely applied (Boswell & Swanney 1986; Boswell 1994). When applying elemental S consideration has to be made to the slight acidifying effects it has and the lime requirement to counteract this (De Vries & Breeuwsma 1987; Boswell 1994; Bolan 1995). Given the acidity of these soils, the development of a lime-sulphur based fertiliser has the potential to be useful combination for hill and high country soils where Olsen P levels are above economic optimal concentrations. An example of such a situation is at the SW1 and SW2 sites where recent fertilisation has led to very high Olsen P concentrations (>30 µg/ml), yet sulphur concentrations remain below optimal (Edmeades et al. 2005) and soil Al is above the >3 mg/kg general toxicity threshold (Edmeades et al. 1983). A lime-sulphur fertiliser product would also be beneficial for soils where P sparing effects occur, especially if the quantity of P released from soil is enough to sustain P availability to plants.

The low N content (%) of the sampled soils, and corresponding high C:N ratios (especially the soils where C:N exceeds 13), relative to more intensely farmed soils elsewhere in New Zealand (Steele et al. 1981; Sparling & Schipper 2004; Schon et al. 2011), indicates low inputs of biologically fixed N occurring in these soils and potentially low clover abundance and growth. As well as this potentially being due to S deficiency, it is also likely to be related to soil acidity and factors relating to such acidity, namely Al toxicity and pH effects on nutrient availability (Bolan et al. 2003; Moir et al. 2016a). Across all of the 19 sampled sites, soils were very acidic at both sampling depths and pH_{H2O} was well below the biological optimum for pasture production of 5.8-6.0 (Edmeades et al. 1984a; Edmeades et al. 1985; O'Connor et al. 2007). The soils were even more acidic when considering the 'exchangeable' acidity (pH_{CaCl2}), which takes the acidic ions bound to soil cation exchange sites into account (Minasny et al. 2011).

Soil Al was generally lower than the general 3 mg/kg toxicity threshold and only exceeded this at 7 out of the 19 sampled sites at the 0-7.5 cm sampling depth. However, the exponential nature of increasing soil Al concentration as pH declines shows the potential for Al to very rapidly become toxic with ongoing soil acidification. This is especially a risk for the soils nearing pH_{H2O} of 5.1, below which soil Al exceeds 3 mg/kg in these sampled soils at 0-7.5 cm depth, if these soils aren't limed soon. Compared to other studies on South Island hill and high country soils the pH_{H2O} at which soil Al exceeded the 3 mg/kg toxicity threshold was lower in this experiment (pH_{H2O} of 5.1) than at the pH_{H2O} values reported by Berenji et al. (2018) (pH_{H2O} of 5.4), Moir and Moot (2010) (pH_{H2O} of 5.5), Moir and Moot (2014) (pH_{H2O} of 5.4 to 5.7 over three

sites), and by Whitley et al. (2016) who was much higher at pH_{H2O} 5.8. This indicates that the Al concentrations measured in the soils of this experiment were generally lower than those in these other studies.

Across the 19 sampled sites, Al toxicity (> 3 mg/kg) was more prominent at the 7.5-15 cm depth, and the rate at which soil Al concentration increased as soil pH declined was greater in this layer than the 0-7.5 cm layer. This emulates the recent findings of Whitley et al. (2018). This highlights the importance of testing for soil Al beyond just the standard 0-7.5 cm sampling depth when planning to grow Al sensitive species in acid soils. Soil pH and Al were particularly extreme at the TDPH site, especially in this 7.5-15 cm layer. In comparison, at the adjacent TD site both pH and Al were much less extreme as the result of past liming. This TD site was the only site of the 19 known to have previously been limed and developed into permanent pasture. However, despite this past liming soil Al still exceeds the toxicity threshold in the 7.5-15 cm sampling layer there and this could still be a limitation to the growth of deep rooting legumes at this site. This illustrates the slow movement of lime down the profile and highlights the ineffectiveness of surface applied lime for reducing soil acidity and Al toxicity deeper in the soil profile (Farina & Channon 1988; Kirchhof et al. 1995; Moir & Moot 2010, 2014; Hendrie et al. 2018).

Although not tested for in this experiment, it would be expected that the availability of micronutrients whose availability is pH dependent, especially Mo, will be low in these sampled soils, even if these micronutrients have been supplemented in past fertiliser applications, and are therefore likely to be restrictive to clover production (Kaiser et al. 2005; Rutkowska et al. 2017).

It was hypothesized in this experiment that soil pH would be a driver of P speciation within the nine fractions, and the extent at which applied P is sorbed and fixed in high country soils. However, soil pH and associated exchangeable Al were shown not to be correlated to the amounts or speciation of P contained in these sampled soils. Therefore, the null hypothesis must be accepted. Along with this, ASC was also found not to be a driver of P speciation or fixation either, despite being strongly correlated to pH (R^2=-0.80 for pH_{H2O}) and Al (R^2=0.88). Herlihy and McGrath (2007), and Redel et al. (2016) found P sorption in soils to be strongly correlated to soil oxalate extractable Al and iron (Fe), clay content and organic carbon, but apart from the latter these attributes were not measured in the sampled soils in this study.

The results of the P fractionation analysis showed that plant available P was only a very small proportion of the total amount of P contained in the sampled soils (7.2±0.45%), but these soils contained large amounts of moderately labile P (NaOH I Pi and Po) that has the potential to become plant available. Compared to studies at the Winchmore long-term P fertiliser trial in Canterbury, New Zealand, where inputs of zero, 188 kg/ha and 376 kg/ha of superphosphate have been applied annually since 1952 (Condron & Goh 1989; Metherell et al. 1997; McDowell & Condron 2000; Tian et al. 2017; Boitt et al.

2018b), the soils in this study contained much less plant available P at depths of 0-7.5 cm and 7.5-15 cm. However, they contained a lot more moderately labile P, in terms of both the total amount of P and the proportion of total P in the soils at these depths. Due to the greater accumulation of applied P in plant available forms at Winchmore, compared to the soils in this study, the results of this study suggest that hill and high country soils of the South Island may naturally contain more P than the lowland soil at Winchmore. And that there is less movement of applied P down the profile at these dryland hill and high country sites, and there are other chemical and biological factors relating to these soils that cause more of the applied P to become less plant available when applied to hill and high country soils. Compared to other New Zealand soils, the amounts of P contained in the soils of this study were consistent with other South Island soils (Chen et al. 2003), but generally contained much less P than the volcanic soils of the North Island (McDowell & Condron 2000; Chen et al. 2003).

Plant available P as measured by soil fractionation was much greater in the sampled soils compared to the corresponding values for the standard Olsen P test. Olsen P was shown to be well correlated to the concentrations of NH_4Cl Pi (R^2=0.74) and $NaHCO_3$ Pi (R^2=0.98), but not to $NaHCO_3$ Po (R^2=0.25), which in these soils accounted for a 57.5 and 66.5 % of total plant available P at the 0-7.5 and 7.5-15 cm sampling depths respectively. This indicates that the Olsen P test may not be well taking this Po into consideration. Also in comparison to P fractionation the Olsen P test does not account for the potential of moderately labile NaOH I Pi and Po to become plant available.

Aside from the discrepancy between plant available P and Olsen P, analysing soil for P fractionation at the two sampling depths was shown to be a useful indicator of P fertiliser history given that the accumulation of P beyond plant available forms is able to be determined. Concentrations of plant available P were greater in the 0-7.5 cm layer, potentially driven by fertiliser P inputs and return of organic P (plant residues and animal wastes) to soil surface (Redel et al. 2016). Recent fertiliser P inputs were evident when considering the correlations between the concentrations of P in each of the plant available P fractions, and total plant available P (R^2=0.57), Olsen P (R^2=0.45), and SO_4-S in the 0-7.5 cm layer. This suggests that approximately half of the plant available P in these soils is fertiliser derived, presuming that the majority of the SO_4-S in these soils is superphosphate fertiliser derived. From the P fractionation results recent fertilisation is evident at the SW1 and SW2 sites where total P is much greater in the 0-7.5 cm layer than the 7.5-15 cm layer. Most of this applied P is shown to have accumulated in the moderately labile Pi fraction, and some in plant available $NaHCO_3$ Pi fraction. Other P fractionation results show P in the 0-7.5 cm layer becoming depleted compared to 7.5-15 cm layer (OM, BD), indicating a lack of recent P inputs. Whereas some soils are shown to just be generally low in P content altogether in both layers, indicating poor past fertiliser history (LP, WK, OMR). At the south facing SH1 site there is large amounts of P in 0-7.5 cm layer and equally large amounts in the 7.5-15 cm layer. However, in the opposing north facing SH2 site

the large amounts of P in 0-7.5 cm are not emulated in the deeper 7.5-15 cm layer. The difference between these sites in relation to their aspect could relate to soil depth, moisture, P movement through the soil profile, and P losses through sediment erosion (Lambert et al. 1985).

Across all of the sampled soils concentrations of total Pi were greater in 0-7.5 cm layer than in the 7.5-15 cm layer, whereas Po was no different. This indicates that applied P is accumulating as Pi in the upper layer of these soils, mostly in the NaOH I Pi fraction rather than the plant available $NaHCO_3$ Pi fraction. This suggests that applied P is not being well utilised by plants when applied and is instead becoming plant unavailable, likely due to being bound to Al and Fe hydroxides in the moderately labile NaOH I Pi fraction. Other recent studies have also shown the accumulation of $NaHCO_3$ and NaOH I Pi in soil to be driven by P fertiliser inputs (Zheng et al. 2002; Zheng Z. et al. 2004; Herlihy & McGrath 2007; McLaughlin et al. 2011; McLaren et al. 2015; Schefe et al. 2015; McLaren et al. 2016; Tian et al. 2017). Other studies have also shown that P applied in organic amendments, such as manure and composts, also accumulates in these Pi fractions (Guardini et al. 2012; Gatiboni et al. 2013; Annaheim et al. 2015; De Conti et al. 2015; Boitt et al. 2018c). And over time this applied Pi that has been bound in the NaOH I Pi fraction, or has not been utilised for plant growth from the $NaHCO_3$ fraction, becomes immobilised into organic forms, predominantly NaOH I Po (Condron & Goh 1989; McLaren et al. 2015; McLaren et al. 2016; Redel et al. 2016). This explains the source of the proportionally large amount of P that is contained in the NaOH I Po fraction in these sampled soils, and others elsewhere in New Zealand (Chen et al. 2003). In the sampled soils of this study, accumulation of Po in the NaOH I fraction may be the result of a lack of N cycling and organic matter mineralisation due to poor clover growth and biological N fixation, and this possibly relates back to the noted S deficiency in these soils, along with soil acidity and Al toxicity issues.

The application of P fertilisers to acidic soils has been reported to reduce exchangeable Al by forming insoluble Al-P precipitates (Coleman et al. 1960; Adams 1981; Bache & Crooke 1981; Sloan et al. 1995; Manoharan 1997). This renders the applied P completely plant unavailable but can be beneficial for reducing Al toxicity, while having little to no effects on soil pH. This could explain the low Al concentrations in the sampled soils relative to the low soil pH levels that were measured. Compared to Whitley et al. (2016) the critical pH_{H2O} at which Al became toxic (exceeded the 3 mg/kg general toxicity threshold) across their sampled soils was 5.8, whereas in this study it was lower at 5.1. This Al-P precipitation process could also help explain the low levels of plant available P and Olsen P in these soils. However, no correlations of soil Al concentrations and plant available P (R^2=0.19) or Olsen P (R^2=-0.12) were recorded in this study. Soil Al concentrations were shown to be moderately correlated to the NaOH II Pi fraction (R^2=-0.46), which could be the sink for Al-P precipitates.

When considering the potential for a P sparing effect due to the application of lime to occur in these soils it would be expected that any released P would most likely come from the moderately labile NaOH I Pi

and Po fractions which contained the greatest amount of P of all the fractions across all of the sampled soils. There could also be potential for P release from the NaOH I Po to occur if nutrient deficiencies, especially S, in these soils are corrected as to increase legume growth and inputs of biologically fixed N resulting in increased nutrient cycling and organic matter mineralisation in these soils. Future research should focus on the methods and effects of overcoming nutrient deficiencies and liming on these soils, and investigating how this may affect sustained P availability and potential release from moderately and non-labile P pools.

3.5 Conclusions

Concentrations of S were found to be deficient in the majority of the soils sampled in this study and it is likely that S deficiency is a major limiting factor to production in South Island hill and high country soils. These soils were predicted to be low in P, but soil Olsen P levels were generally within economically optimal concentrations. P fractionation showed large quantities of P are stored in moderately labile inorganic and especially organic forms that have the potential to become plant available in these soils. Statistical analysis showed that soil acidity and Al concentrations were not driving factors of P speciation and fertiliser P fixation in South Island high country soils as hypothesised. Al toxicity was more prominent at 7.5-15 cm below the soil surface compared to 0-7.5 cm, and therefore deep testing for Al is recommended before trying to establish deep rooting Al sensitive legumes on these soils. Overall, the productive potential of these soils will be driven by confounding factors of nutrient deficiencies and soil acidity.

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Chapter 4

The effects of lime on phosphorus availability and legume growth in exhaustively cropped acid hill and high country soils

4.1 Introduction

Phosphorus (P) availability in acid hill and high country soils has been shown to be a limitation for legume growth and persistence (Edmeades et al. 1984a; Moir 2000; Dodd & Sheath 2003; Moir & Moot 2010). But when applied to these acid soils, fertiliser P is rapidly adsorbed by Al and Fe hydroxides, rendering it unavailable to plants (Haynes 1982, 1984; Parfitt et al. 1989; McDowell & Condron 2001). Soil acidity can also limit legume production by limiting the availability of other nutrients, such as Mo, and inhibiting biological N fixation (Bolan et al. 2003).

Liming is required to reduce soil acidity, and its other effects on soil properties and processes have been well studied. However, there has been much debate on the effects of liming on the availability of P to plants, but it can potentially be increased due to desorption and mineralisation processes and is known as the P-sparing effect (Haynes 1982; Edmeades et al. 1989). Results from Chapter 3 show that acid hill and high country soils contain, and have accumulated, large quantities of P in plant unavailable fractions, especially as moderately labile inorganic and organic forms. It is uncertain though how these soils will react to liming, and whether liming will increase the availability of this currently plant unavailable P, or if improved soil pH conditions simply allow plants to greater access available soil P. Given that liming can be expensive, if a 'P-sparing effect' were to occur on these soils it is important to quantify the size of the effect and whether it will be sufficient to improve and sustain plant growth.

The aim of this experiment was to investigate the effect liming will have on the availability and uptake of soil P and applied P, and on the growth of legumes in a range of acid high country soils that have been fertilised but not limed in the past but P fertility remains low. By using a modified Stanford and DeMent (1957) technique, in which only a small quantity of soil is exhaustively cropped using plants as bio-indicators of treatment effects on nutrient availability, the effects of lime on short term P availability, and utilisation of applied P, will be quantified. For this experiment four soils collected from the field trial sites in Chapters 5 and 6 will be treated with four rates of lime; 0, 1, 2, and 4 t lime/ha equivalent, and supplied with no or additional P fertiliser (7 kg P/ha), and will then be exhaustively cropped by Russell lupins (*Lupinus polyphyllus*) and *Lotus pedunculatus* (cv. Grasslands Maku) in a controlled glasshouse environment.

These legumes were selected to be used as the bio-indicators based on their agronomic potential in acid soils due to their tolerance to soil acidity, Al toxicity and low P fertility compared to other species, such as lucerne (Brock 1973; Nordmeyer & Davis 1977; Davis 1981; Morton 1981; Scott & Mills 1981; Floate et al. 1985; Scott 1989a; White 1995; Caradus et al. 2001; Moot & Pollock 2014; Moir et al. 2016a). Both lotus and other members of the lupin family have also been shown to solubilize P forms in soil that are otherwise plant unavailable (Borie 1990; Trolove et al. 1996; Lambers et al. 2013; Dissanayaka et al. 2017) making them useful legumes for hill and high country.

The specific objective for this experiment is to quantify P availability and extraction by legumes in response to lime and P applications to acid high country soils. The null hypothesis is that applying lime to acid high country soils will not affect P availability and the utilisation of applied P, and will not affect the growth of Russell lupins and *Lotus pedunculatus*.

4.2 Methodology

4.2.1 Soil collection and preparation

Soils were collected from Omarama Station (OM), Glenmore Station (GM), Mt Grand Station (MG) in 2014 (Whitley et al. 2016) and from The Dasher (TD) in July 2016. These soils were collected in addition to the core samples collected for Chapter 3, and were selected for use in this experiment based on their low pH, Al content, their range in Olsen P and P fractions, and in correspondence to the field trial at Mt Grand Station (Chapter 5) and the locations of the deep placed lime field trials (Chapter 6). Details of sampling locations are in Table 3.1. Soils were spade sampled and trimmed to 15 cm depth with a knife from within the core sampled area.

The collected soils were then crushed, air dried at 30˚C for one week, passed through a 2 mm sieve and then thoroughly mixed. A 70 mL subsample of each soil was further dried at 65˚C for 24 hours for P fractionation analysis. The soil was stored at ambient air temperature until the experiment began.

4.2.2 Soil Chemical analysis

Prior to beginning the experiment soil chemical analysis was performed on each soil (Table 4.1) according to the methods described in section 3.2.2.

Phosphorus Fractionation

Soil P fractionation (Table 4.2) was carried out according to the method of Boitt et al. (2018a) developed from the methods of Hedley et al. (Hedley et al. 1982), Condron and Goh (1989), Condron et al. (1996), Olsen and Sommers (1982), and Condron and Newman (2011), as described in Appendix A.

Table 4.1 Initial chemical analysis of each experimental soil.

Soil Analysis	Unit	Glenmore	Omarama	Mt Grand	The Dasher
pH_{H2O}		4.6	5.2	4.8	4.5
Exch Al	mg/kg	10.7	4.6	3.7	30.9
Olsen P	µg/mL	24	7	11	5
Sulphate S	µg/g	19	1	3	11
Organic S	µg/g	4	<1	5	6
Total C	% w/w	6.12	2.14	3.10	6.05
Total N	% w/w	0.54	0.19	0.32	0.39
C:N Ratio	C/N	11.3	11.3	9.7	15.5
CEC	me/100g	15	10	13	17
Base Saturation	%	36.9	31.6	45.2	12.9
Calcium	me/100g	4.3	2.1	4.3	1.2
Magnesium	me/100g	0.73	0.52	0.96	0.51
Potassium	me/100g	0.42	0.31	0.51	0.26
Sodium	me/100g	0.09	0.08	0.04	0.13
ASC	%	42	23	25	59
Bulk Density	g/mL	0.75	1.00	0.97	0.74

Table 4.2 Initial P fractions within each experimental soil.

P Fraction (mg P/kg)	Glenmore	Omarama	Mt Grand	The Dasher
NH_4Cl Pi	0.64	0.16	0.59	0.25
$NaHCO_3$ Pi	40.3	14.5	26.5	10.3
$NaHCO_3$ Po	84.8	35.1	40.7	49.8
NaOH-I Pi	148	80.9	102	65.3
NaOH-I Po	682	197	459	393
HCl Pi	199	92.3	35.8	3.89
NaOH-II Pi	48.7	50.3	59.9	32.5
NaOH-II Po	102	64.2	117	94.3
Residual P	114	104	150	131
Total Pi	423	238	225	112
Total Po	869	296	616	537
Total P	1400	638	991	779

4.2.3 Trial Design and treatments

A modified Stanford and DeMent (1957) bioassay technique was used to exhaustively crop two legume species (Russell lupins and Lotus) in four soils (GM, OM, MG, and TD) treated with four application rates of lime (0, 1, 2, and 4 t/ha), and two rates of P fertiliser (0 and 7 kg P/ha), in a fully factorial design, totalling 64 treatment combinations. Each treatment combination was replicated four times to give a total of 256 pots. There was an additional fifth replicate of the 0 and 2 t lime/ha treated pots, bringing the total number of pots to 288. These pots were to be destructively sampled halfway through the experiment to investigate the amount of P remaining in the soil at this stage of the experiment, but this did not occur as planned and the pots were carried through to the end of the experiment. The trial was held in the Aluminex Glasshouse at Lincoln University with all the Russell lupin pots on one table and all the Lotus pots on an adjacent table. Pots were blocked by soil type in a randomised block design. For the full trial layout see appendix C.

For the experiment just 75 g of soil per pot was used as the only source of P to plants. This amount of soil was selected based on calculations of an expected 5 g of dry matter production per pot in a six month time frame with a shoot P content of 0.2%, therefore requiring 1 mg P/pot. Given the average Olsen P across the four selected soils of 12 µg/mL there would be an expected 900 µg Olsen P/pot contained in 75 g of soil. These calculations are based on the results of similar pot trials in previous studies (Moir 2000; Chirino-Valle 2013).

The four application rates of lime (lab-grade $CaCO_3$, Sigma-Aldrich) applied to each soil were; 0 (control), 0.64, 1.28, and 2.56 g/pot, equivalent to a surface application of 0, 1, 2, and 4 T lime/ha respectively. The lime was thoroughly mixed through the soil for each individual pot before the trial commenced.

Phosphorus (mono-calcium P (MCP), Sigma-Aldrich) was applied at rates of 0 (control) and 4.5 mg P/pot, equivalent to 7 kg P/ha or 78 kg/ha of superphosphate, in order to examine the effects of lime application rate on the utilisation of this applied P. This P was applied by incorporating 1 mL of 0.07 M MCP solution to the soil for each individual pot before the trial commenced.

4.2.4. Pot Design

Square plastic 500 mL pots (80 x 80 x 100 mm, slightly tapered to base, RXT90, RX Plastics, Ashburton NZ) were lined with very fine polyester gauze 200x200 µm and filled with 165 mL (255 g) of high purity silica sand (99.32 % SiO_2, J61w, Industrial Sands Ltd, Auckland, NZ). A layer of 330x330 µm polyester gauze was then laid down over the sand and up the sides of the pot before the soil with added lime and P treatments was then added to the pot. The pot size was selected to generate a 2.5 cm thick layer of soil (Figure 4.1). A second layer of 330 µm polyester gauze was laid down over the soil and another 130 mL (200 g) of sand

was added to the pot. The purpose of the sand was to provide support and growing space for plant roots given the restricted quantity of soil and to hold moisture in the pot. While the fine 200x200 µm mesh gauze lining the pot was fine enough to prevent the sand passing through it, the 330 µm gauze between the soil and sand layers was not. To prevent the sand leaking through into the soil the pots were wetted up as each layer was laid down during pot establishment to form cohesive layers, and the moisture content of the pots was then maintained throughout the experiment. The 330x330 µm gauze was not expected to limit the capacity of the plant roots to fully explore the depth of soil in each pot (Scholefield & Hall 1985).

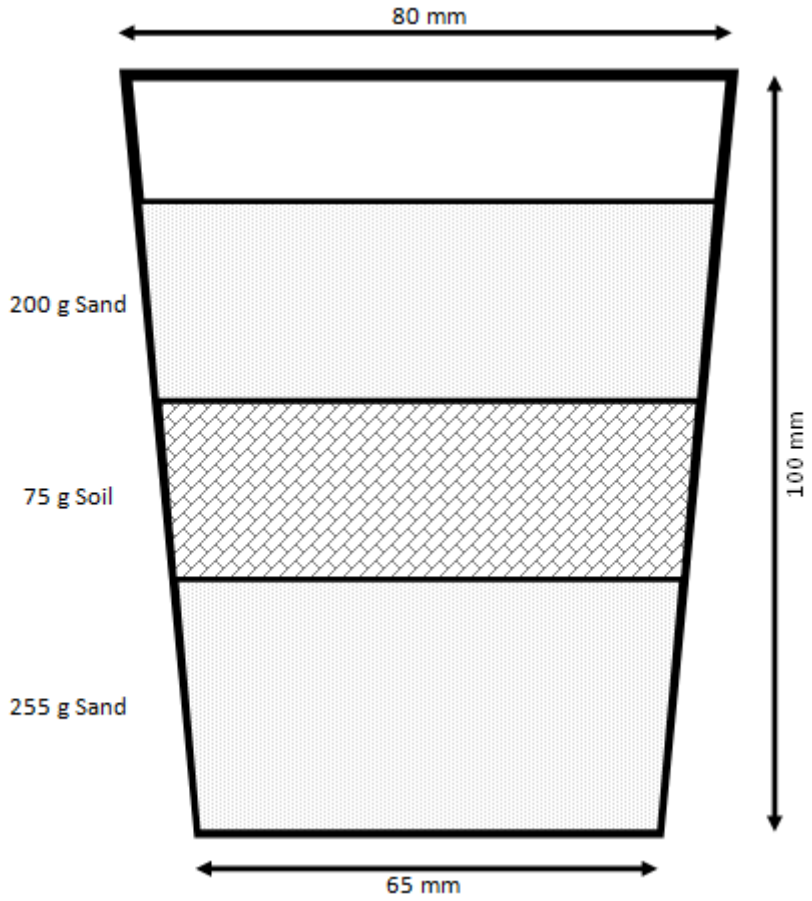


Figure 4.1 The modified Stanford and DeMent (1957) pot design used in the experiment.

4.2.4 Trial Management

Plant establishment

Prior to sowing Russell lupin seed was scarified in 97% sulphuric acid (H_2SO_4, Scharlau, 1.84 g cm^{-3}) at 2:1 acid to seed for 20 minutes, followed by 20 minutes of rinsing with tap water then deionised water (Hartmann et al. 2002; Berenji 2015), which lifted germination to 90 % after ten days.

The pots were sown on the 13th of October 2016. Two scarified lupin seeds which were inoculated with fresh, ground functional nodules collected from field grown Russell lupins, were sown per pot and then

thinned to one plant per pot as they emerged. Six lotus seeds, which were inoculated with *Bradyrhizobium japonicum* (Kirchner 1896, ICMP 5798) were sown and then thinned to four plants per pot as they emerged.

Insect and Disease Control

All plants were sprayed with the insecticides Success naturalyte® (spinosad, Dow Agrosciences) and Pliarking 200 SL® (imidacloprid, Adria Crop Protection) on days 37 and 49 post sowing, and then with Karate Zeon® (lambda-cyhalothrin, Syngenta) on days 57, 69, 90 and 131 post sowing for the control of thrips, which were mostly present on the lupin plants. Alto 100 SL® fungicide (cyproconazole, Syngenta) was also applied on day 131 post sowing.

Automated Irrigation System

Prior to the beginning of the experiment 5TM soil moisture sensors (Decagon Devices, Pullman, WA USA) were calibrated by establishing volumetric soil water content (% v/v) calibration curves for each soil. The moisture sensors were installed horizontally through the walls of the pot and sealed with silicon between the soil and upper sand layer. Incremental amounts of water (10 % of the combined soil-sand volume) was added to a representative pot for each of the four soil types, allowing the sensor reading to equilibrate between additions. The top of the pots was sealed to avoid evaporative losses and water was added up to the point of field capacity as determined by the point which water drained from the holes in the bottom of the pots.

In the experiment a total of 12 5TM moisture sensors were installed, as above, across all of the pots (4 per soil, 6 per plant species). Soil moisture data, along with soil temperature, was measured by the sensors and was recorded by a data logger (Campbell Scientific CR23X, Logan, UT USA).

The irrigation system (Plate 4.1) consisted of a series of eight 15 mm alkathene pipes, fed by a single 25 mm alkathene pipe, which supplied water to each pot through a 4 mm 2 L/hr regulated dripper (Antelco Pinch Grip PC, Murray Bridge, SA Australia) and 4 mm diameter rubber tubing. The volumetric water content of the pots was maintained at an average of 25 % v/v over the 12 sensors. The whole system was fed through a single solenoid valve (Hunter ICZ Drip Control Zone valve, San Macros, CA USA) controlled by the data logger which opened when the average volumetric water content dropped to 22 % v/v, supplying 11 mm of water to each pot per irrigation event.

Plate 4.1 The automated irrigation system tubing in the Russell lupin pots. The data logger was stored in the grey box below the table.

Basal Nutrient Solution

A basal nutrient solution was applied to each pot twice weekly up to the fourth harvest of plant material, and once per week thereafter. The nutrient solution contained all the major and trace elements required to sustain plant growth excluding N and P which plants were required to fix or extract from soil themselves. Two applications of N (Table 4.3) did occur in the first week of the experiment to aid in plant establishment, but no more was applied thereafter.

The nutrient solution applied to each pot consisted of a combination of four stock solutions (Table 4.3) which were then made up to 2.25 L with DI water in order to apply 7.5 mL of solution by pipette to each pot per application. In total 80 applications of nutrient solution were applied over the course of the experiment (Table 4.4).

Table 4.3 Basal nutrient stock solution recipes.

Stock Solution	Ingredient	Ingredient g/L in stock solution	Solution applied per application[a]
Major Element Solution A	K_2SO_4	2.22	105 mL
	$KHCO_3$	6.0	
Major Element Solution B	$MgCl_2.6H_2O$	2.02	105 mL
	$CaCO_3$	0.51	
	1M HCl	10 mL	
	Na_2SO_4	1.555	
Minor Element Solution	H_3BO_3	0.03	11 mL
	$CoCl_2.6H_2O$	0.004	
	$CuCl_2.2H_2O$	0.01	
	$MnCl_2.4H_2O$	0.20	
	$(NH_4)_6Mo_7O_2.4H_2O$	0.004	
	$ZnCl_2$	0.015	
Ferric Citrate Solution	$FeC_6H_5O_7$	0.0585	6 mL
N Solution	NH_4NO_3	12.19	500 mL*

[a] Applied across all 288 pots. * Only applied twice in the first week of the experiment.

Table 4.4 The total amount of each basal nutrient applied to each pot during the experiment.

Nutrient	Total amount of nutrient applied per pot (mg)
K	97.4
S	22.2
Ca	6.0
Mg	7.0
Na	14.7
B	0.02
Co	0.003
Cu	0.01
Mn	0.17
Mo	0.01
Zn	0.02
Fe	0.02
N*	14.8

* Only applied twice in the first week of the experiment.

4.2.5 Plant Yield Measurement

Six harvests of plant material were conducted during the course of the experiment. These were carried out 68, 128, 189, 316, 372, and lastly 407 days after sowing. Measurements of maximum plant height were recorded, as were the number of leaves produced by lupin plants, excluding the youngest leaf. The harvest procedure involved cutting leaves from lupins at the plant base, leaving the youngest leaf intact, and cutting the lotus plants to a height of 1.5 cm, level with the top of each pot, leaving some green leaves on each stem. At the final harvest on day 407 all above ground herbage material was cut to the base of the plant.

Harvested material was dried at 65˚C for 48 hours and the dry weight was recorded. Dried samples from each harvest were then bulked by pot and ground for chemical analysis.

4.2.6 Final Root and Soil Sampling

Following the final harvest of shoot material on day 407, the 24th of November 2017, the sand and soil layers from each pot were carefully separated and all the sand was discarded. The soil and root material was then placed on a 2 mm sieve and all of the soil passed through into a 70 ml container for each pot. The collected root material was then washed, placed in a paper bag and air dried at 65˚C for 48 hours and weighed to determine root dry matter yield. The soil from each pot was placed in a clean paper bowl and air dried at 25˚C for one week before being analysed for pH_{H20}, pH_{CaCl2}, Al, and Olsen P.

4.2.7 Plant Tissue Analysis

At the conclusion of the experiment the collected dried shoot material from each harvest was bulked by pot and finely ground (enclosed grinder, Glen Creston DFH 48, Stanmore, England). In order to have enough material for analysis, the dried root material was bulked to create two replicates of each treatment combination by combining the root samples from either two or three pots (depending on whether that treatment was replicated four or five times) of the same treatment together.

The ground shoot and root material was then chemically analysed for a full suite of nutrients, excluding C and N, by digesting 0.2 g of the ground material in material in 2.0 ml HNO_3 (69%) and 2.0 ml H_2O_2 (30%) in a microwave digester (CEM MARS Xpress, CEM Corporation, Matthews, NC, USA), followed by ICP-OES analysis (Varian 720, Varian Australia Pty Ltd, Melbourne, Australia) (Nolte 2003).

4.2.8 Statistical Analysis

The effects of liming and applying additional P on measured variables in each soil were statistically analysed by general linear models in Genstat 16 to account for the differences in the number of replicates for different treatments. A Fisher's LSD *post hoc* performed where significant ($P<0.05$) results were

observed. The differences between plant yields, shoot and root P concentrations, and shoot and root P uptake between the first three harvests and final three harvests were analysed by paired t-tests. The correlation coefficients between shoot yield, P concentration and P uptake were determined by simple linear and non-linear regression analysis. All plotted results are ±1 SE.

4.3 Results

4.3.1 The effects of lime and fertiliser on soil pH

Plant species (P=0.103), and the application of additional P (P=0.225), did not affect soil pH in any of the four soils. Therefore, only the mean pH_{H2O} for each soil across all plant species and P treatments for each of the four application rates of lime are presented (Figure 4.2).

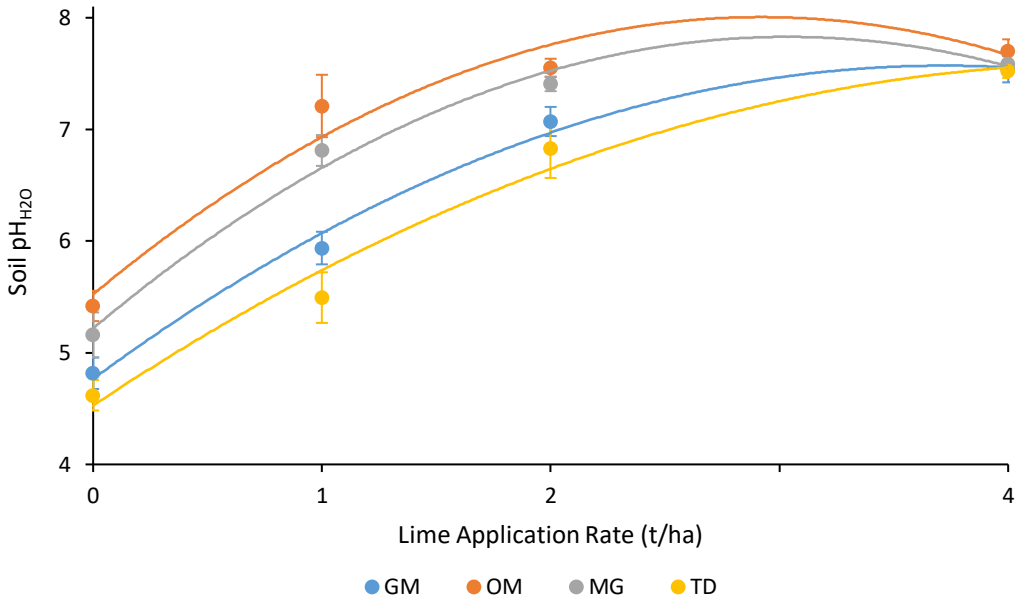


Figure 4.2 The effect of incorporated lime on soil pH_{H2O} in each soil at the conclusion of the experiment. For regression equations and correlation coefficients see Appendix D.1.

Incorporating lime into each soil caused large, non-linear increases (P<0.001) in soil pH_{H2O}. Despite the initial differences in pH_{H2O} between the different soils, incorporating lime increased pH_{H2O} in each soil up to a constant pH_{H2O} of 7.5±0.05 in the 4 t lime/ha treatment. In the GM and TD soils, the trend in pH_{H2O} increase was constant at 1.0±0.1 pH units for the application of 1 and 2 t lime/ha respectively. In the OM and MG soils there was a larger initial response (P<0.001) of 1.8±0.1 pH units for the application of 1 t lime/ha in the OM and MG soils, but the response between the 1 t lime/ha treatment and the 2 t lime/ha treatment was only to 0.6±0.1 pH units for both soils.

As for pH_{H2O}, soil pH_{CaCl2} followed the same trends in pH increase as lime application rate and rose to a final pH_{CaCl2} of 7.1±0.03 in each soil in the 4 t lime/ha treatment (data not presented).

4.3.2 Exchangeable Aluminium

Incorporating lime into each soil decreased (<0.001) soil exchangeable Al concentrations. In general 1 t lime/ha was sufficient to reduce soil Al to below 3 mg/kg in each soil. The addition of P had no effect on soil Al concentrations (P=0.249), so presented results (Figure 4.3) exclude P treatment effects.

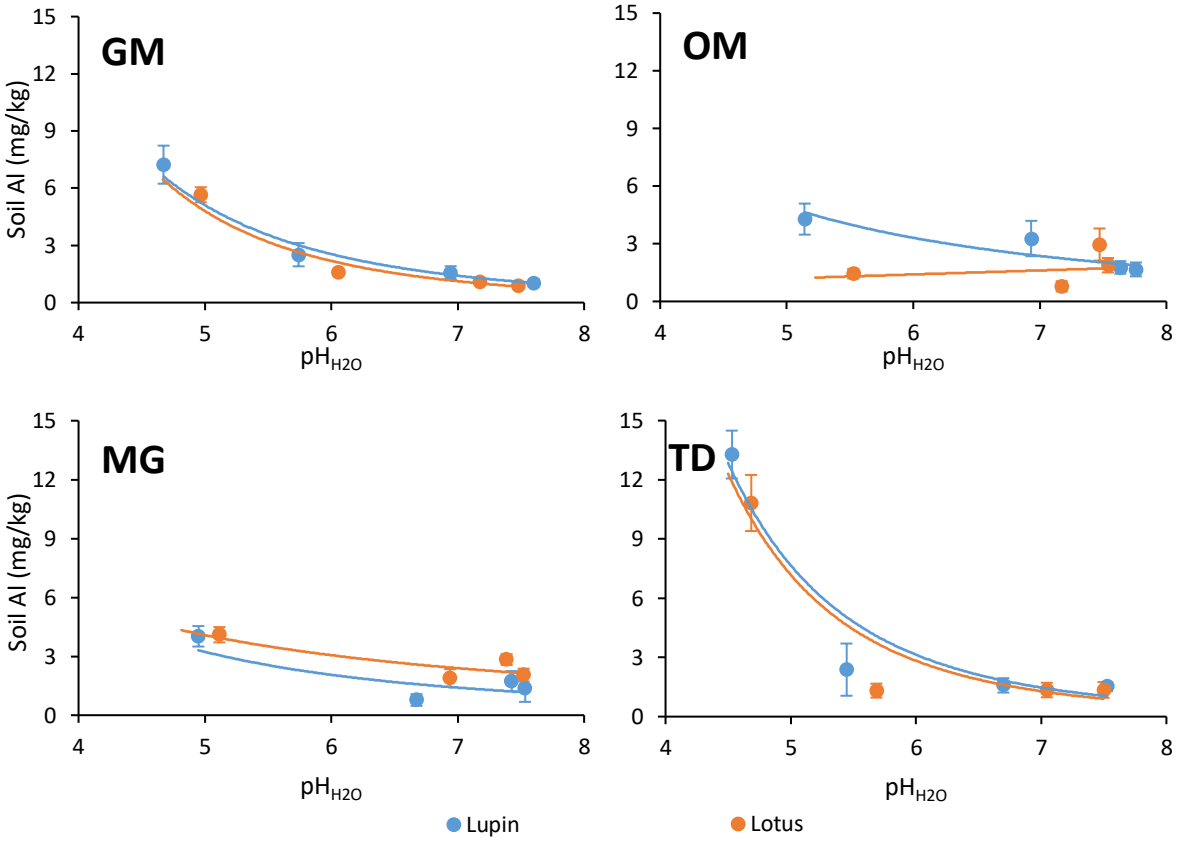


Figure 4.3 Soil Al concentration (mg Al/kg soil) in relation to soil pH$_{H2O}$ in each soil. For regression equations and correlation coefficients see Appendix D.1.

Despite plant species having no effect on soil pH$_{H2O}$, across all treatments the lotus did reduce soil Al concentration across all soils more than the Russell lupins (P<0.001). Even without the application of lime, both plant species reduced the concentration of Al in each soil during the experiment, especially in the TD soil which had the greatest initial concentration of Al before beginning the experiment (30.9 mg/kg).

This species effect on the reduction in Al concentration can mostly be explained by the accumulation of Al in plant roots, which was consistent across all lime treatments (P=0.360). Al in lotus roots was much greater than that of Russell lupin roots (P<0.001), averaging 2.58±0.41 mg Al/pot, compared to 0.97±0.33 mg Al/pot for the Russell lupins. Al in plant roots was also not correlated to root dry matter production (R^2=0.352). Compared to the roots, there was very little total Al in plant shoots (0.18±0.02 mg Al/pot and 0.21±0.04 mg Al/pot for lotus and Russell lupin pots respectively).

4.3.3 The effect of soil pH on shoot yield

Across all six harvests of shoot material, shoot yield was in general highest at the second harvest (Figure 4.4), and then declined as subsequent harvests occurred (P=0.005). Slightly larger yields were measured at the final harvest on day 407 compared to previous harvests due to the destructive harvesting of plant crowns.

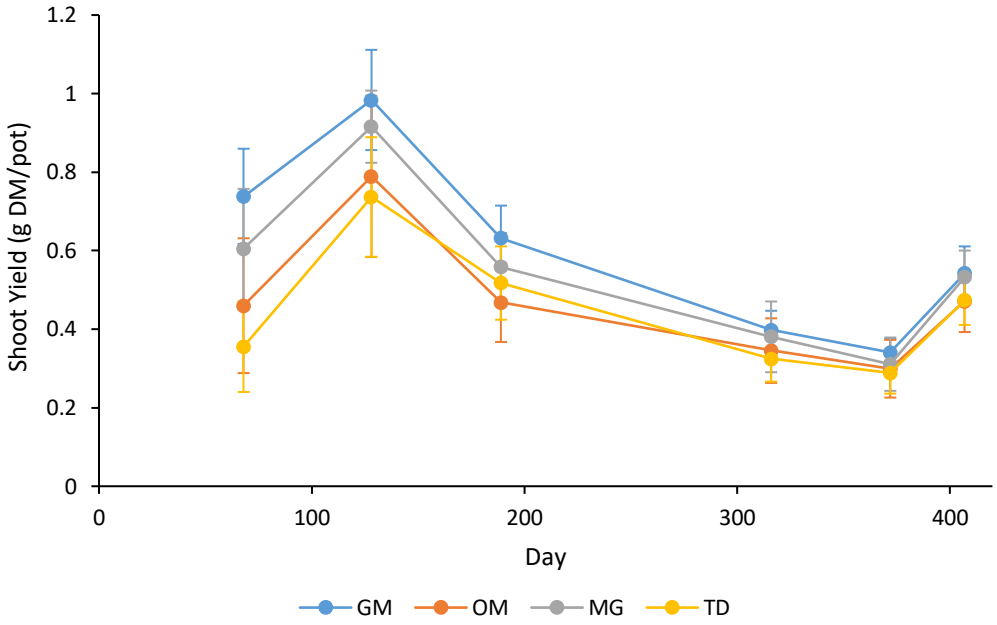


Figure 4.4 Shoot yield (g DM/pot) averaged across all species, lime and P treatments at each harvest over the course of the experiment.

Giving the exhaustive nature of the experiment some, but not all of the pots, were dead by the time of the final harvest of shoot material. The experiment was stopped prior to all of the pots becoming completely starved of P, as intended, due to time restraints and slow plant growth. However, most plants, especially the Russell lupin plants, showed signs of severe P deficiency (Plate 4.2).

Plate 4.2 A Russell lupin plant growing in TD soil treated with 2 t lime/ha with no additional P showing severe P deficiency on the 7th of July 2017, day 267 of the 407 day experiment.

Total shoot yield of the lotus and Russell lupins responded differently to increasing soil pH_{H2O} and the application of additional P in each of the four soils (Figure 4.5). The shoot yield of both generally followed a parabolic growth curve, increasing as pH rose to an optimal level beyond which it decreased. This optimum was approximately pH_{H2O} 6.0 for Russell lupins, but was slightly higher in the fertilised GM and OM soils, and pH_{H2O} 6.2-6.8 for the lotus. The shoot yield of the lotus was generally greater ($P=0.006$) than that of Russell lupin, except for at low pH (>6.0) in the OM soil. Across all soils, species and lime application rates the application of the additional P treatment lead to an average increase ($P<0.001$) of 0.65±0.08 g DM/pot of shoot yield.

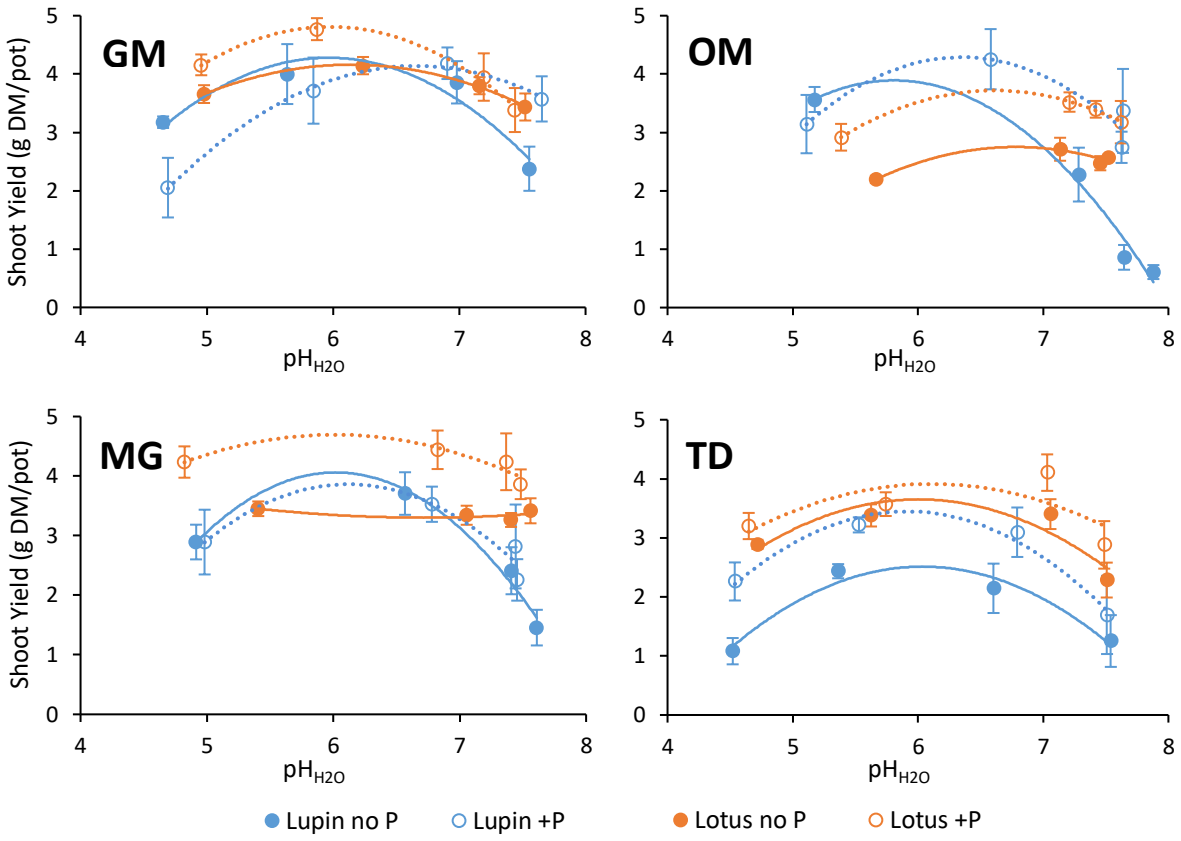


Figure 4.5 The relationship between Russell lupin and lotus shoot yield (g DM/pot) and soil pH in each soil. For regression equations and correlation coefficients see Appendix D.1.

In the GM soil without additional P the shoot yield of Russell lupins was increased (P=0.002) by the application of 1 t lime/ha but declined at higher lime application rates when pH rose above the optimum. However, with the addition of P Russell lupin shoot yield was maintained at the higher rates of lime (P=0.048), and did not decline as much as it did without the additional P. Adding P alone did not affect Russell lupin shoot yield in this soil (P=0.950). As with the Russell lupins, the application of 1 t lime/ha increased (P=0.005) the shoot yield of the lotus but at rates above this the yield again declined. The decline in Lotus shoot yield above their optimal pH created by the 2 and 4 t lime/ha treatments in each of the four soils was not as great as that in the Russell lupins. The application of P had no overall effect on lotus shoot yield in the GM soil (P=0.093), and did not create an interaction effect with the applied lime (P=0.541).

The application of lime to the OM soil caused large increases in soil pH with even the 1 t lime/ha application rate raising pH to beyond the optimal pH for Russell lupin growth. This caused a reduction in Russell lupin shoot yield (P<0.001), which was further reduced by the higher application rates of lime. However, as with the GM soil, Russell lupin shoot yield was sustained, but was no greater than in the untreated soil, at these higher pH's when additional P was applied (P=0.003), and was only increased when additional P was applied with 1 t lime/ha. Unlike the Russell lupins, the shoot yield of the lotus was

unaffected by the large pH increases due to liming the OM soil (P=0.411). And in this soil the application of additional P caused a constant increase in lotus shoot yield (P<0.001) across the measured pH range.

As with the GM soil, the application of 1 t lime/ha increased the shoot yield of the Russell lupins in the MG soil (P=0.009). However, at application rates higher than 1 t lime/ha their shoot yield declined. The application of additional P had no effect on Russell lupin shoot yield (P=0.439) in the MG soil, but did increase the shoot yield of the lotus (P<0.001). And as with the OM soil, lotus shoot yield was unaffected by liming in the MG soil (P=0.821).

The largest Russell lupin shoot yield response to added P at low pH occurred in the TD soil, and this response was consistent across the pH range caused by liming (P=0.003). As with the GM soil, the application of the 1 and 2 t lime/ha treatments increased Russell lupin shoot yield (P=0.002) in the TD soil, but the highest, 4 t lime/ha, raised soil pH too far above the optimum pH and shoot yield again declined and was no different to the unlimed soil. The shoot yield of the lotus was greater than that of the Russell lupins in the TD soil (P=0.006), but followed the same trajectory in relation to increasing pH, with the 1 and 2 t lime/ha treatments increasing shoot yield (P<0.001) compared to the control but the yield in the 4 t lime/ha treatment was no different to the unlimed control. And the application of additional P increased shoot yield (P=0.013). As with the Russell lupins this P effect was consistent across the pH range and no interaction effect between applied lime and P occurred (P=0.764).

Overall, the shoot yield of the lotus was less affected by liming than that of the Russell lupins, and the greatest yield of both occurred in the GM and MG soils. Without liming the shoot yield of the Russell lupins was greatest in the OM soil but rapidly declined when lime was applied. And the shoot yield of both the Russell lupins and lotus was the least in the TD soil.

4.3.4 The effect of soil pH on root yield

The effects of liming and application of additional P were much less prominent in the root yields of the Russell lupins and lotus (Figure 4.6) compared to that of the shoots (Figure 4.5). Again, the lotus generally yielded greater in each soil than the Russell lupin roots did (P=0.025). However, the mean root yields between the different soils was more consistent than the shoot yields were.

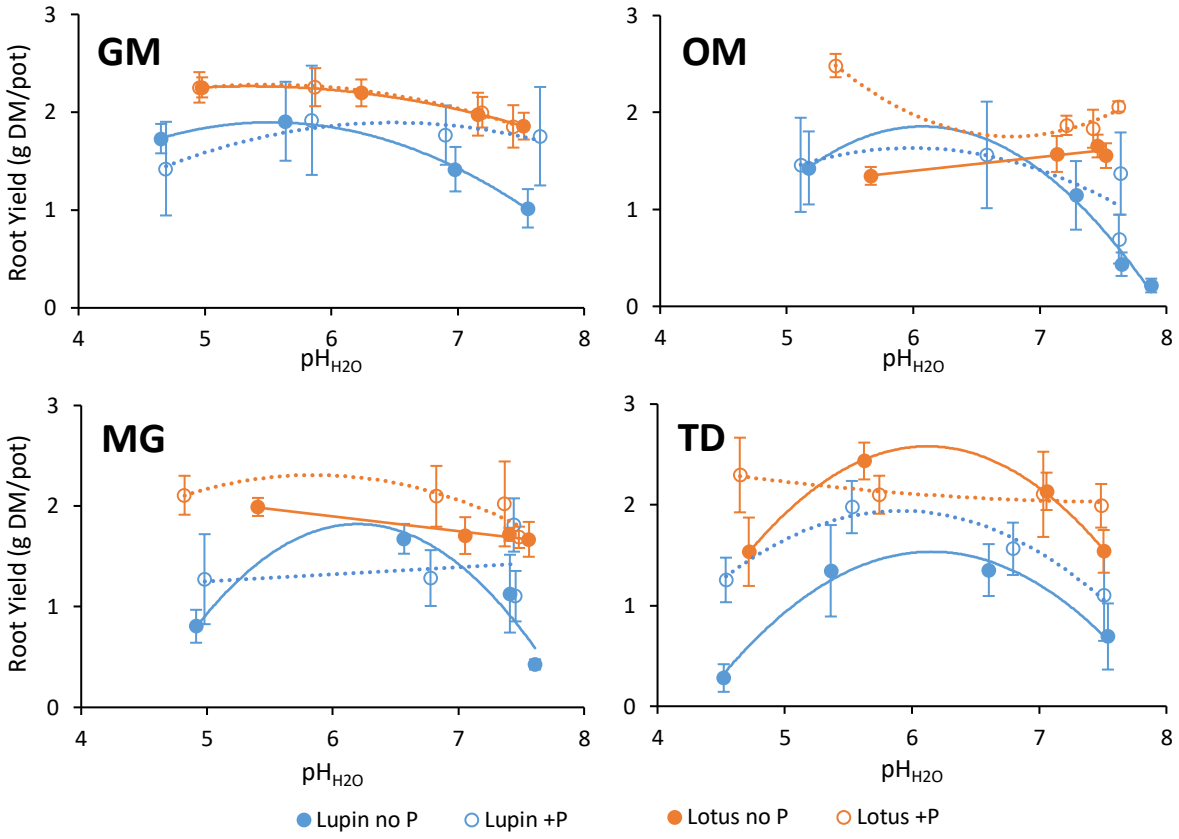


Figure 4.6 The relationship between Russell lupin and lotus root yield (g DM/pot) and soil pH in each soil. For regression equations and correlation coefficients see Appendix D.1.

Compared to shoot yield, and despite showing similar trends, the application of both lime (P=0.069) and P (P=0.134) had no effect on Russell lupin root yield in the GM soil. The application of additional P also had no effect on lotus root yield (P=0.639). The application of the 4 t lime/ha treatment reduced lotus root yield (P=0.032) compared to the control and 1 t lime/ha treatments.

As with shoot yield, liming the OM soil reduced the root yield of the Russell lupins in that soil (P=0.040). However, unlike shoot yield, root yield was reduced by applying additional P (P=0.492), and overall the additional P had no effect on Russell lupin root yield (P=0.087) in the OM soil. And as with shoot yield, the addition of P to the OM soil increased the root yield of the lotus (P=0.005), especially at low pH in the unlimed soil (P=0.041), whereas liming alone had no effect (P=0.562).

Despite following a similar trend as shoot yield, Russell lupin root yield, without the application of additional P, in the MG soil was not affected by increasing soil pH by liming (P=0.061). The addition of P also had no overall effect (P=0.075). Lotus root yield was also not affected by liming (P=0.466) or adding P (P=0.209) to the MG soil.

As with shoot yield, the root yield of Russell lupins in the TD soil was initially increased by the application of the 1 and 2 t lime/ha treatments (P=0.001), but then declined again in the 4 t lime/ha treatment. The

application of additional P also consistently increased Russell lupin root yield (P<0.001) across the whole pH range. The root yield of the lotus in the TD soil was however not affected by liming (P=0.102) or applying additional P (P=0.259).

4.3.5 Lime and P effects on shoot P concentration

As with shoot yield, increasing soil pH by applying lime, and adding additional P to each soil also affected the amount of P plants took up from each soil. The concentration of P in the bulked shoot material collected from the first three harvests of shoot material (987±38.8 mg P/kg) was no greater than that in the bulked shoot material from the final three harvests of shoot material (953±26.2 mg P/kg) across all of the lime and P treatments in either species in the GM soil (P=0.457). However, in the OM (P=0.024), MG (P=0.003) and TD (P<0.001) soils the concentration of P in shoot material was less in the final three harvests than the first three harvests across all treatments. The mean shoot concentration of P in the OM soil across all treatments was 806±22.2 mg P/kg in the first three harvests and reduced to 742±17.1 mg P/kg in the final three harvests. In the MG soil shoot P concentration was 936±30.3 mg P/kg in the first three harvests and reduced to 816±24.4 mg P/kg in the final three harvests. And in the TD soil shoot P concentration reduced from 893±18.3 mg P/kg in the first three harvests to just 618±14.9 mg P/kg in the final three harvests across all treatments.

Having corrected for shoot yield, the average concentration of P contained in the shoots of each plant species over the six harvests of shoot material (Figure 4.7) differed between soils (P<0.001), and was greatest in the GM soil and lowest in the OM and TD soils. Although lotus shoot yield was greater, shoot P concentration was generally greater in the Russell lupins than in the lotus shoots (P=0.002).

Applying additional P increased the concentration of P in Russell lupin shoots in the GM soil (P=0.014), but without the application of additional P Russell lupin shoot P concentration decreased at the high pH caused by the 4 t lime/ha treatment (P=0.015). Without applying additional P liming increased the concentration of P in lotus shoots in the GM soil (P<0.001) by an average of 100 mg P/kg from 827 mg P/kg in the unlimed control to an average of 927 mg P/kg across the three application rates of lime. Applying additional P increased lotus P concentration at low pH (P<0.001), but at higher pH in the 2 and 4 t lime/ha treatments the concentration was no different to the unlimed soil.

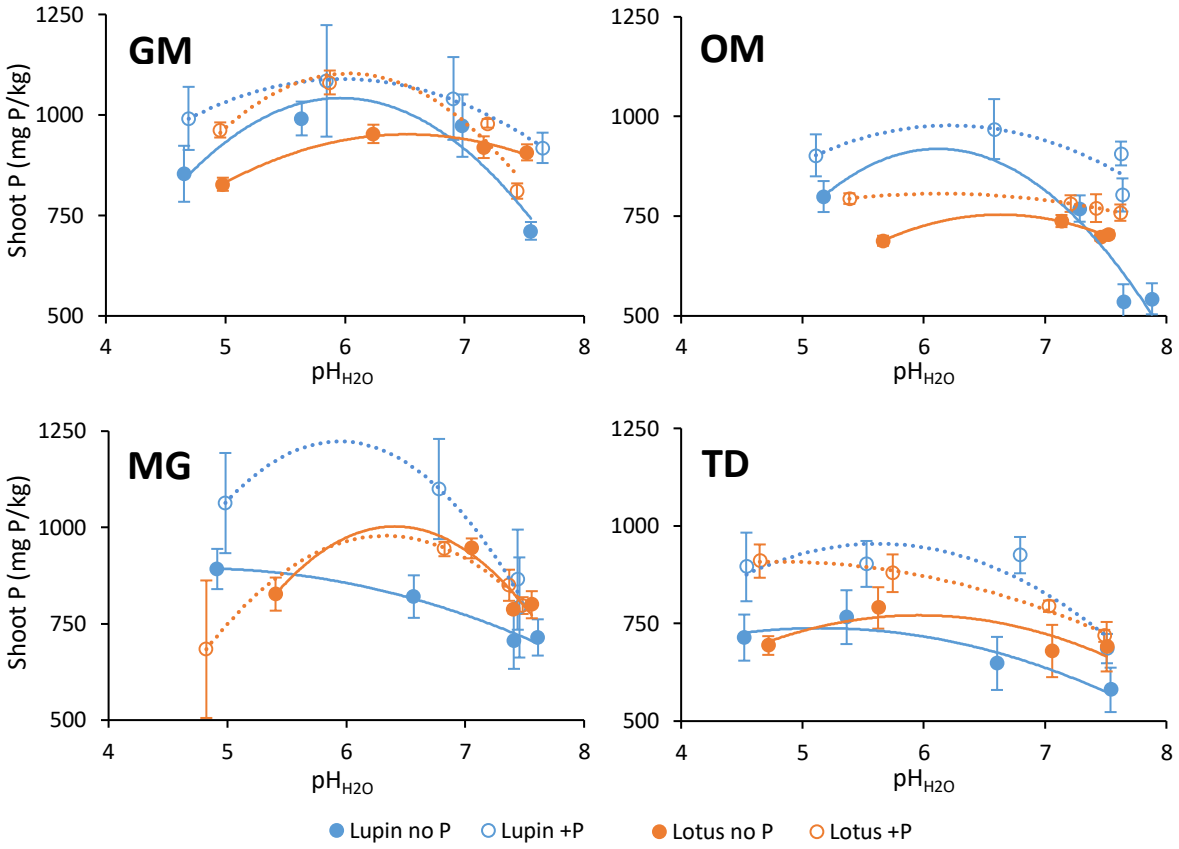


Figure 4.7 The relationship between Russell lupin and lotus shoot P concentration (mg P/kg DM), averaged over the six harvests of shoot material, and soil pH in each soil. For regression equations and correlation coefficients see Appendix D.1.

In the OM soil the application of additional P increased the concentration of P in the Russell lupin shoots ($P<0.001$) but unlike the GM soil this effect was consistent across the pH range caused by liming. However, in both the fertilised and unfertilised Russell lupin plants shoot P concentration decreased at high pH in the 2 and 4 t lime/ha treatments. This decrease did not occur in the lotus plants and pH did not affect shoot P concentration ($P=0.405$). But as with the Russell lupins applying additional P increased lotus shoot P concentration across the pH range ($P<0.001$).

As with the lotus in the GM soil, in the 0 and 1 t lime/ha treatments the concentration of P in the shoots of the Russell lupins in the MG soil was increased by the application of additional P ($P<0.001$), but was no different to the unfertilised shoots at the higher pH caused by the 2 and 4 t lime/ha treatments. This higher pH also lead to a decrease in shoot P concentration of the Russell lupins ($P<0.001$) compared to the 0 and 1 t lime/ha treatments. In the MG soil the concentration of P in the lotus shoots was not affected by either pH and lime application ($P=0.110$), or the application of additional P ($P=0.724$).

In the TD soil the application of additional P increased the concentration of P in the Russell lupin shoots ($P<0.001$). However, P concentration decreased gradually as soil pH increased ($P=0.006$), with the P concentration in the shoots of the Russell lupins treatment with 4 t lime/ha being lower than the

concentrations in the other lime treatments. In the lotus shoots P concentration was not affected by liming when no additional P was applied, but with the application of additional P, which increased P concentration in the unlimed control soil ($P<0.001$), the concentration decreased as pH increased ($P=0.024$) and shoot P concentration was no different to the unfertilised shoots.

As well as having these effects on shoot P concentration, as soil pH increased in each of these soils the concentration of magnesium (Mg, $P<0.001$), manganese (Mn, $P<0.001$), sodium (Na, $P<0.001$), and zinc (Zn $P<0.001$) all decreased in both the Russell lupin and lotus shoots in each soil. Shoot zinc concentration was especially reduced as pH increased in the OM soil ($P<0.001$). Conversely the shoot concentration of molybdenum (Mo) was increased ($P<0.001$) three-fold as pH increased in both species. The concentrations of boron (B, $P=0.347$) and sulphur (S, $P=0.315$) were unaffected by pH increases.

4.3.6 Lime and P effects on root P concentration

Compared to shoot P concentration, liming had no effect on root P concentrations in the four soils ($P=0.129$). And unlike shoot P concentrations there was no difference in root P concentrations between the Russell lupins and the lotus ($P=0.108$). Root P concentrations were consistently 70 % of shoot P concentration in each soil, averaging 713±27.2 mg P/kg in the GM soil, 536±27.4 mg P/kg in the OM soil, 618±30.7 mg P/kg in the MG soil, and 555±15.8 mg P/kg in the TD soil. Applying additional P to the OM soil increased the P concentration of the root of both the Russell lupins ($P=0.018$), increasing it from an average of 503±47.3 mg P/kg to 669±58.5 mg P/kg, and the lotus ($P=0.003$), increasing it from 457±19.8 mg P/kg up to 515±22.0 mg P/kg. Applying additional P also increased the average P concentration in Russell lupin roots in the MG soil ($P=0.015$) from 503±48.0 mg P/kg up to 669±68.9 mg P/kg, but it did not affect the lotus roots ($P=0.184$).

As with shoot nutrient concentrations the concentration of B ($P=0.895$) and S ($P=0.942$) in plant roots were unaffected by increases in soil pH. And conversely to liming effects that occurred in plant shoots, the concentrations of Mg ($P=0.593$), Mo ($P=0.259$), Na ($P=0.588$), and Zn ($P=0.317$) in plant roots were unaffected by pH increases.

4.3.7 Lime and P treatment effects on plant P uptake

By factoring in the yield of shoot material at each harvest and the concentration of P in shoot material, as measured on bulked shoot material from harvests 1-3 and 4-6, the amount of P taken up in plant shoots can be measured. Shoot P uptake in the first three harvests of shoot material combined was 88%, 65%, 66%, and 70% greater than in the combined final three harvests across both species for the GM, OM, MG, and TD soils respectively.

Across all six harvests, shoot P uptake was greatest in the GM soil (P<0.001), followed by the MG soil, and was lowest in the OM and TD soils (Figure 4.8). And as with shoot yield was greater in the lotus than the Russell lupins (P<0.001), and in the added P treatment compared to the unfertilised treatment (P<0.001), with an additional 0.90±0.08 mg P/pot taken up by plant shoots where the additional P treatment was applied. Across all of the soils the application of 1 t lime/ha generally increased soil P uptake, but as pH increased in the 2 and 4 t lime/ha treatments shoot P uptake was reduced. Shoot P uptake was closely correlated to both shoot P concentration (R^2=0.54, P<0.001) and shoot growth (R^2=0.74, P<0.001).

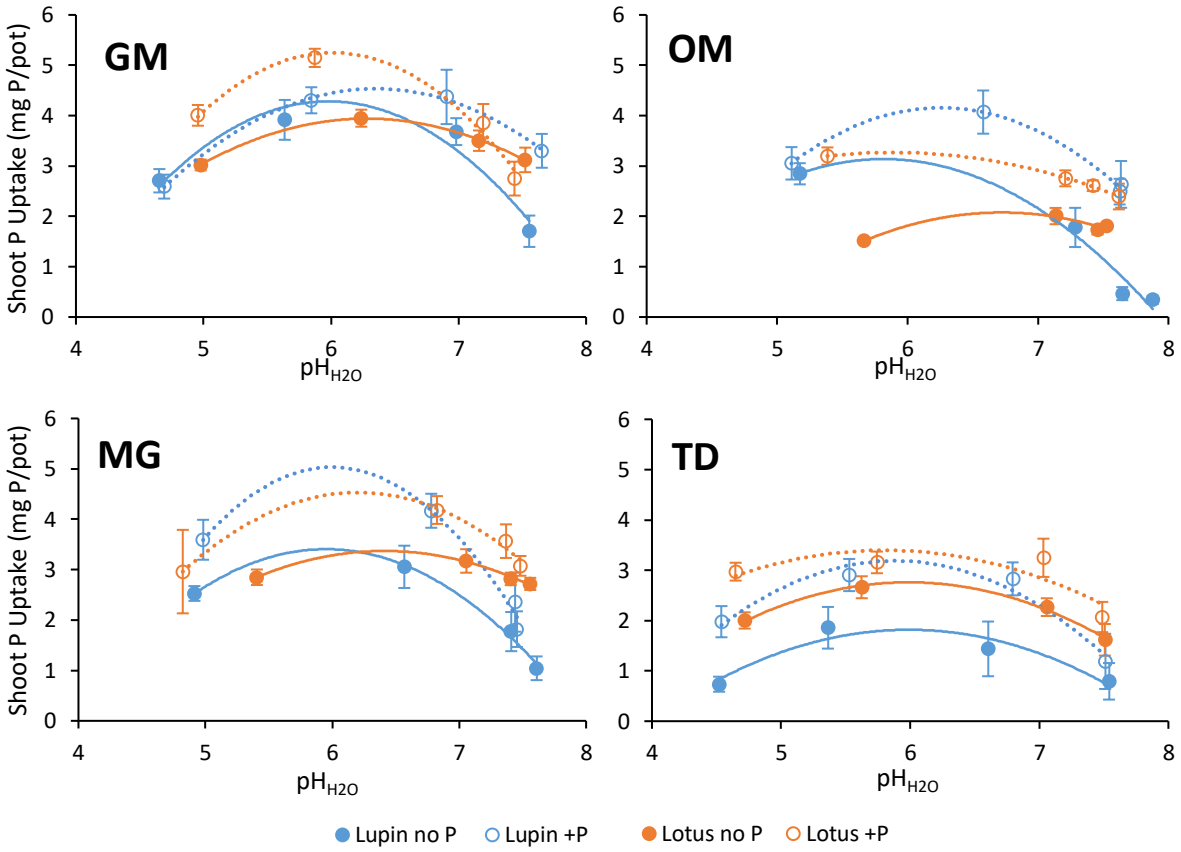


Figure 4.8 The relationship between Russell lupin and lotus total shoot P uptake (mg P/pot) and soil pH in each soil. For regression equations and correlation coefficients see Appendix D.1.

As with the shoot yield and shoot P concentration in the GM soil, the uptake of P by the Russell lupins (P<0.001) and lotus (P<0.001) initially increased as pH increased, but then again declined as pH rose above optimal pH levels for each species. For the Russell lupins this was pH 5.5-6.5, and for the lotus pH 6.2-6.8 for the lotus. Up to pH 6.0 there was no difference between shoot P uptake of the unfertilised Russell lupins and those with added P, and as pH rose beyond pH 6.0 shoot P uptake diverged (P=0.127) and was greater in the Russell lupin pots with added P (P=0.020). In contrast, the added P treatment increased shoot P uptake over the unfertilised treatment (P=0.003) in the lotus plants at below the optimal pH, and above pH 6.0 P uptake in the unfertilised and added P treatments converged. When measuring shoot yield and shoot P concentration both lime and the added P treatment had separate effects on the lotus in the

GM soil, but when combined to determine shoot P uptake the treatment effects combined to cause shoot P uptake to be increased (P=0.020) greater in the 1 t lime/ha with added P treatment combination pots compared to the other treatments.

As with the GM soil, shoot P uptake by unfertilised Russell lupin plants and those with added P diverged as pH increased in the OM soil, while shoot P uptake by unfertilised and fertilised lotus pots converged as pH increased. Russell lupin shoot P uptake was not affected by the application of additional P in the unlimed soil, but when applied with 1 t lime/ha greatly increased P uptake (P=0.003). Increasing the pH of the OM soil by liming caused P uptake by Russell lupin plants to be greatly reduced (P<0.001), but P uptake was sustained (P<0.001) and no different to the unlimed control treatment by the addition of the added P treatment at the high pH caused by the 2 and 4 t lime/ha treatments. As will both shoot yield and shoot P concentration, the uptake of P by lotus in the OM soil was not affected by increases in soil pH due to liming (P=0.135), but was consistently increased by the application of additional P (P<0.001).

As with the GM soil, the shoot uptake of P by the Russell lupins in the MG soil initially increased (P<0.001) as pH was increased by liming up to pH 6.0, but as pH increased above 6.0 Russell lupin shoot P uptake rapidly declined. And unlike the GM soil this decline in Russell lupin shoot P uptake was not sustained by the application of additional P, which had increased P uptake (P=0.004) in the unlimed and 1 t lime/ha treated pots. As with shoot yield and shoot P concentration in the MG soil the shoot uptake of P by the lotus was unaffected by increases in pH due to liming (P=0.194). However, when coupled with the application of additional P, lotus shoot P uptake was increased (P=0.031) in the 1 t lime/ha treatment compared to all other treatment combinations. This response to liming and the application of additional P occurred when pH was within the optimal range for the lotus of pH 6.2-6.8.

In the TD soil the shoot P uptake was greatest across both legume species and P treatments at pH 6.0. For the Russell lupins increasing pH initially increased shoot P uptake (P<0.001) and was greatest in the 1 and 2 t lime/ha treatments, but was again decreased in the 4 t lime/ha treatment. The application of additional P caused a consistent increase (P<0.001) in Russell lupin shoot P uptake, apart from at high pH caused by the application of the 4 t lime/ha treatment where it was no different to the unfertilised treatment. As with the Russell lupins, shoot P uptake by the lotus in the unfertilised pots was increased (P<0.001) compared to the unlimed soil by the 1 t lime/ha treatment only, and was greatest at pH 6.0. Above pH 6.0 shoot P uptake declined due to the high pH caused by the application of the 2 and 4 t lime/ha treatments. Where additional P was applied, which also increased (P<0.001) shoot P uptake, increasing soil pH by liming did not affect shoot P uptake by the lotus until pH rose above 6.8, beyond which P uptake decreased. In neither the Russell lupins (P=0.368) or the lotus (P=0.493) did increasing soil pH increase the effect of applying the additional P treatment.

For plant roots the uptake of P by plant roots generally followed similar trends to liming and the application of additional P as root yield did, given that these treatments had little effect on the concentrations of P in root material. Root P uptake was generally very low (<2.0 mg P/pot) and was again greatest in the GM soil (P<0.001), and greater in the lotus roots than Russell lupin roots (P<0.001). By adding the amount of P taken up by plant roots to respective shoot P uptake results the total amount of P taken up by plants in each treatment was determined (Figure 4.9). As with shoot P uptake, total P uptake by plant shoots and roots combined was greatest in the GM soil (4.81±0.17 mg P/pot averaged across all treatments, P<0.001), followed by the MG soil (3.76±0.17 mg P/pot) and was equally lowest in the OM (2.97±0.17 mg P pot) and TD (2.99±0.16 mg P/pot) soils, and generally followed a parabolic curve as pH was increased. Total P uptake was also greater (P<0.001) in the lotus plants (4.05±0.14 mg P/pot) than in the Russell lupins (3.21±0.10 mg P/pot). Across all of the treatments, the application of additional P to the pots caused P uptake to be increased (P=0.008) by an average of 1.16±0.11 mg P/pot.

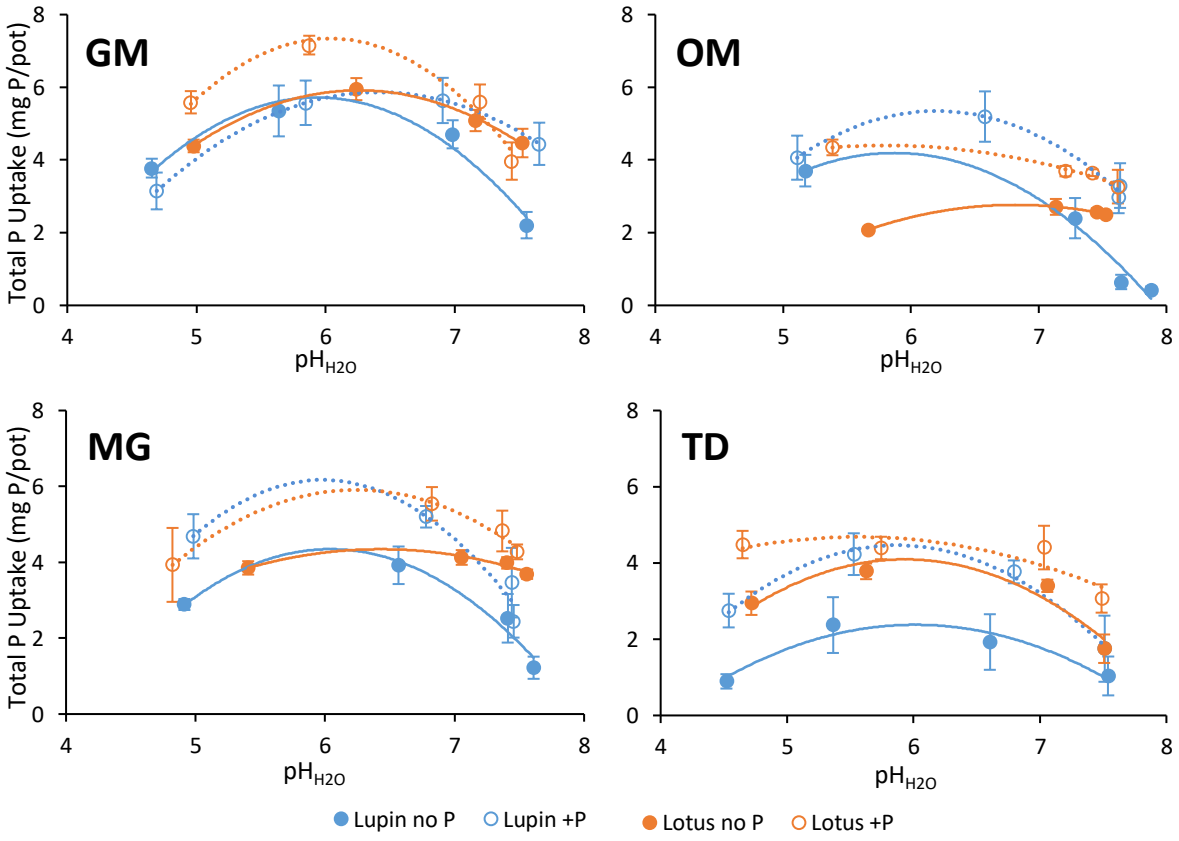


Figure 4.9 The relationship between Russell lupin and lotus total P uptake (mg P/pot) and soil pH in each soil. For regression equations and correlation coefficients see Appendix D.1.

By multiplying the initial concentration of soil plant available P (P contained in the NH_4Cl, $NaHCO_3$ Pi and $NaHCO_3$ Po combined, Figure 4.2) in each soil by the amount of soil in each pot (75 g) the initial amount of plant available P in each pot can be calculated. For the GM soil this was 9.43 mg P/pot, for OM it was 3.73 mg P/pot, for MG it was 5.08 mg P/pot, and for TD it was 4.53 mg P/pot. The application of the additional P treatment added a further 4.5 mg P/pot to treated pots. Using P uptake results the effects of

liming and increases in soil pH on the proportion of initial plant available P, with or without the additional applied P, taken up by plants during the timeframe of this experiment were able to be calculated (Figure 4.10).

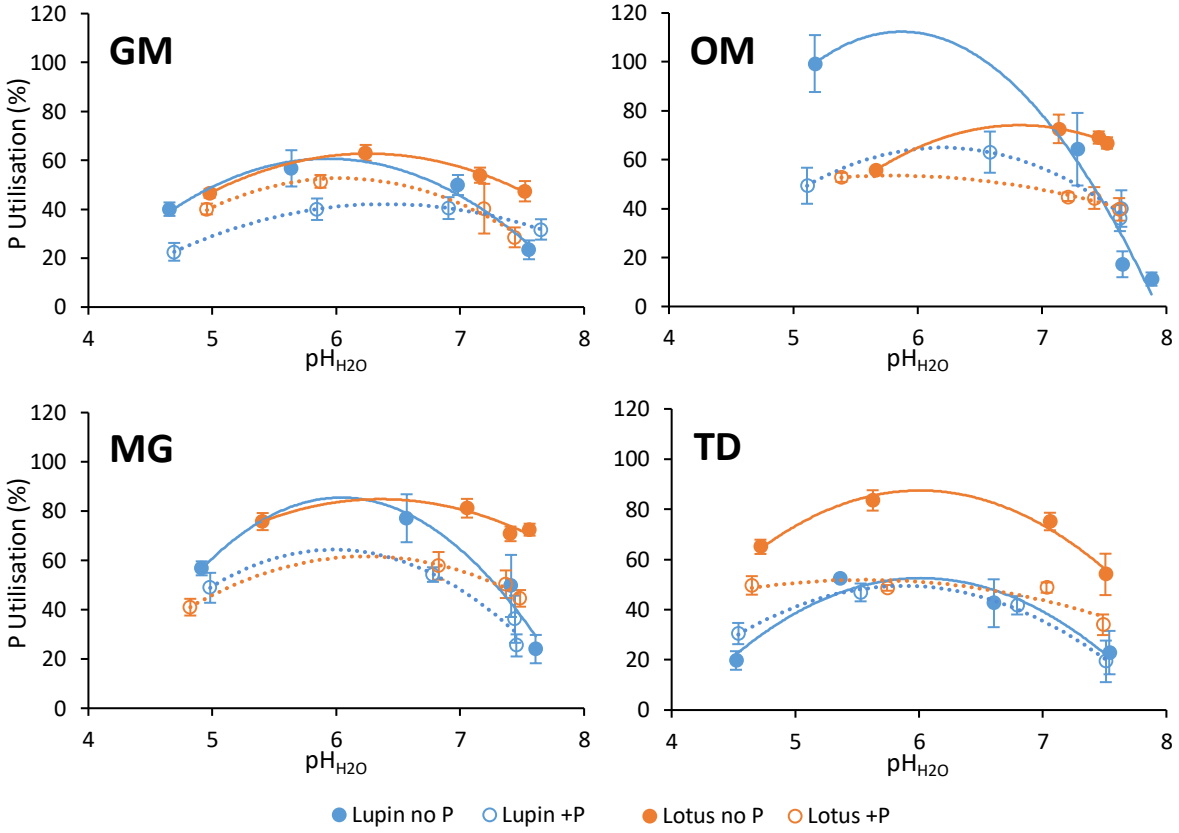


Figure 4.10 The relationship between soil pH and the utilisation (%) of initial plant available P by Russell lupin and lotus in each soil. For regression equations and correlation coefficients see Appendix D.1.

Although plant growth and P uptake was greatest in the GM soil, when considering the uptake of P by plants in terms of the proportion of plant available that was in the GM soil at the beginning of the experiment, P utilisation was the lowest (on average 42.2±1.6% across all treatments) in this soil ($P<0.001$). In the MG and OM soils P utilisation across all treatments during the experiment was roughly half the amount of initially available P in each soil (54.1±2.5% and 51.8±2.9%). In TD P utilisation across all treatments averaged 46.0±2.4%. Lotus was able to utilise more ($P<0.001$) of the initial plant available P (55.2±3.6%) compared to the Russell lupins (41.8±6.1). The application of 4.5 mg P/pot in the added P treatment only resulted in an average increase in P uptake of 1.16±0.11 mg P/pot (Figure 4.9), and due to this the overall utilisation of plant available P in pots with this added P was less (42.0±4.7%, $P<0.001$) compared to unfertilised pots (55.1±5.1%).

When calculating the amount of Olsen P in each pot at the beginning of the experiment, initial Olsen P contents in each soil were much lower than the corresponding amounts of plant available P measured by P fractionation. In the GM soil the initial amount of P available to plants as Olsen P was 2.4 mg P/pot

(25.4% of plant available P measured by fractionation), in the OM soil it was 0.53 mg P/pot (14.1% of plant available P measured by fractionation), in the MG soil it was 0.85 mg P/pot (16.7% of plant available P measured by fractionation), and in the TD soil it was only 0.51 mg P/pot (11.2% of plant available P measured by fractionation). Due to this, when measuring plant P uptake in terms of the utilisation of the initial soil Olsen P, P utilisation is actually much greater than the initial amount of Olsen P available to plants. In the GM soil, the average utilisation of initial soil Olsen P across all species, lime and added P treatments was 130±11.0%, in the OM soil it was 241±24.6%, in the MG soil it was 230±21.4%, and in the TD soil it was 268±27.3%. However, the utilisation of initial P quantity results do not account for any potential liming effects on the quantity of P available for plant uptake. At the conclusion of the experiment P fractionation was unable to be analysed on the treated soils. However, soil Olsen P concentrations were shown to be affected by liming (Figure 4.11).

4.3.8 Treatment and plant growth effects on soil Olsen P concentrations

Despite having been exhaustively cropped, at the end of the experiment soil Olsen P, apart from in the GM soil, was generally unchanged from the initial concentration in each soil below pH 6.0 (Figure 4.11), and above pH 6.0 Olsen P was increased as pH increased in all soils ($P<0.001$).

In the GM soil, which had an initial Olsen P of 24 µg/ml, the growth of Russell lupins in the unlimed soil without additional P reduced Olsen P ($P<0.001$) by 10±1.6 µg/ml, and with additional P Olsen P was increased ($P<0.001$) by 10±4.0 µg/ml. The application of 1 t lime/ha lead to a further reduction ($P<0.001$) of soil Olsen P in the unfertilised soil, and also caused a reduction ($P<0.001$) in the fertilised soil. However, beyond the 1 t lime/ha treatment the applications of 2 and 4 t lime/ha caused Olsen P to increase as pH increased in both unfertilised and fertilised Russell lupin pots. With increasing pH the difference in Olsen P between the unfertilised and fertilised soil decreased and the Olsen P content of both converged ($P=0.086$) due to greater rates of Olsen P increase in the unfertilised soil. In comparison, the increase in Olsen P as the result of applying additional P ($P<0.001$) was constant across the pH range in the lotus pots. Without lime Olsen P in the lotus pots was reduced more than it was in the Russell lupin pots ($P<0.001$), and it was also reduced compared to the initial soil Olsen P concentration in the soil with added P. Compared to the Russell lupin pots, soil Olsen P increased ($P<0.001$) immediately as pH began to rise due to liming, but the final Olsen P at the highest pH in the 4 t lime/ha treatment was not as great as it was in the Russell lupin pots.

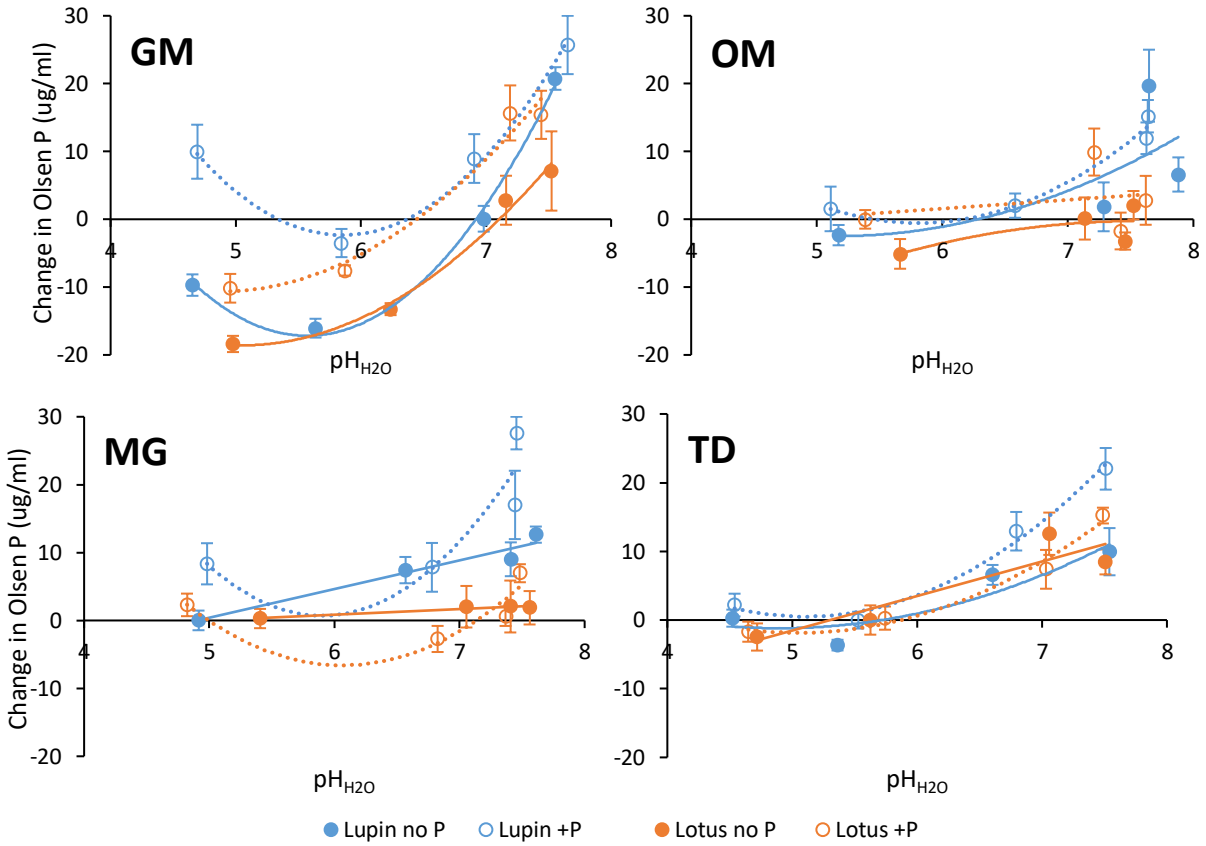


Figure 4.11 The relationship between change in soil Olsen P in Russell lupin and lotus pots and soil pH at the conclusion of the experiment in each soil. For regression equations and correlation coefficients see Appendix D.1.

Despite the Russell lupin growth in the OM soil, which had an initial Olsen P concentration of 7 µg/ml, Olsen P was unchanged in the unlimed and 1 t lime/ha treatments but was increased (P<0.001) as pH rose above 6.5 in the 2 and 4 t lime/ha treatments. In this soil the application of the additional P treatment had no effect on final Olsen P concentration in the Russell lupin pots across the pH range (P=0.702). Compared to the Russell lupins the growth of the lotus had a greater effect (P<0.001) on reducing Olsen P in the OM soil, and the rate at which Olsen P was increased as pH increased was less (P=0.048). The application of additional P also increased Olsen P in the lotus pots (P=0.021) but not as greatly as it did in the GM soil.

In the MG soil despite plant growth Olsen P in the Russell lupin pots increased (P<0.001) as pH was increased by liming from an initial 11 µg/ml up to 23.6±1.2 µg/ml in unlimed soil and 36.6±1.1 µg/ml in fertilised soil in the 4 t lime/ha treatment. Compared to unfertilised soil, Olsen P was only increased (P=0.001) by the application of additional P in the 4 t lime/ha treatment. The growth of the lotus in the MG soil was the only combination in which liming (P=0.077) and additional P (P=0.302) treatments had no effect on final soil Olsen P concentration.

Similar to the OM soil, Olsen P was increased as pH increased due to the application of the 2 and 4 t lime/ha treatments in the TD soil for both the Russell lupins (P<0.001) and the lotus (P<0.001), which had

the lowest initial Olsen P of 5 µg/ml. In the unlimed and 1 t lime/ha treatments the final Olsen P was no different to the initial Olsen P despite the growth of the Russell lupins and lotus in the respective treatments. The application of additional P had no effect on Olsen P in the lotus pots ($P=0.242$) as pH increased, but increased Olsen P in the 2 and 4 t lime/ha treated Russell lupin pots ($P<0.001$).

Despite the liming effects on soil Olsen P, in no soil did pH increase alter the size of the effect that additional P had on the Olsen P of the soils at the end of the experiment.

4.4 Discussion

The results from this experiment indicate that no sizeable 'P-sparing effect' occurred due to liming these acid soils as was originally hypothesised. Instead plant growth and P uptake increases were due to plant physiological responses and increased ability of plants to access existing plant available P in soil up to an optimal pH for each species. For Russell lupins the optimal pH was found to be 5.5-6.5, and for lotus it was within a range of 6.2-6.8. Above these pH levels plant growth and shoot P concentration declined. This result was determined by using shoot P uptake as an indicator of soil P availability, and due to there being differences in optimal pH levels between these species. Had an increase in soil P availability occurred, shoot P concentration would have been expected to remain elevated at high pH, despite reductions in shoot yield due to luxury P uptake by plants.

In general, the lotus was more tolerant of extreme pH conditions and physically showed less signs of P deficiency during the experiment than the Russell lupins. Compared to the study by Moir et al. (2016a) the optimal pH range for lotus found to be a broad 5.4-6.5. And in the study by Berenji et al. (2018) the growth of both Russell lupins and lotus was unaffected by liming over a pH range of 5.2-5.7. Overall, plant growth and P uptake was greatest in the GM soil and this, and the amount of plant growth in the other soils, was reflective past fertiliser history and the initial concentrations of soil Olsen P, and also to a lesser extent total N, in each soil before beginning the experiment (Table 4.2).

In the experiment only relatively small amounts, compared to standard field application rates, of lime were required to cause large pH increases in each soil. In the field at GM, Berenji et al. (2018) recorded an increase of 0.5 pH units, increasing pH from 5.2 to 5.7, when 4 t lime/ha was surface applied. Moir and Moot (2014) also recorded pH increases of 0.5-0.8 pH units when 4-5 t lime/ha was applied to three high country sites. This is most likely due to the application of lime on a per area basis of the pots but there only being a shallow depth of soil in each pot. Pure calcium carbonate was also used in the experiment whereas common agricultural lime generally only contains 80-90% calcium carbonate and its reactivity is dependent on how finely it is ground (Elphick 1955; Craighead 2005). Therefore, the application rates of

lime in this experiment are irrelevant to field lime application rates. However, in a field situation it would be expected that the same results would be observed in relation to the magnitude of pH increase.

Soil Al concentrations reduced as pH increased in each soil, as expected. Soil Al concentrations were also reduced in unlimed soil following the exhaustive cropping, indicating that both the Russell lupins and lotus plants were able reduce soil Al concentrations. At the conclusion of the experiment large amounts of Al were contained in plant roots, especially in the lotus roots. Root exudates by plants, including carboxylic acids, could also have reduced Al concentrations by complexation reactions (Foy et al. 1978). Using these Al tolerant species in this experiment ensured that P, and not Al, was the main limiting nutrient to plant growth and the effects of applied treatments on P dynamics could be fully measured. Had an Al sensitive species, such as lucerne, been grown in the experiment, a greater response to reduced soil Al concentrations would have been expected (Moir et al. 2016a; Berenji et al. 2017).

As with shoot yield, shoot P concentrations and uptake in both the Russell lupins and lotus increased as soil pH increased up to the optimal pH for each species. Root P concentrations were less affected by changes in soil pH. These increases may relate to a variety of interacting factors, including increased biological N fixation resulting from improved pH conditions for rhizobia function, decreased Al concentrations and possibly increased soil P availability. Shoot N concentrations in relation to soil pH were not measured in this experiment, and unfortunately soil P fractionation was unable to be carried out on the soils after the exhaustive cropping. Having these measurements may have provided clearer explanation of the recorded results, and it is recommended that further analysis is perform to measure these.

As pH rose above the optimal levels for each species, plant yield and P uptake greatly reduced. Despite the application of the nutrient solution to the pots, the observed reductions in plant yield could be attributed to Zn deficiency. However, a Zn deficiency would not be expected to cause the observed reductions in shoot P concentrations, which instead indicate the main driver of these yield and P uptake reductions was the reduction in soil P availability. This reduction it likely to be due to the formation of non-plant available Ca-P minerals in soil at high pH (Pierzynki et al. 2005).

Interestingly, the shoot P concentrations for both species were much lower (generally >1000 mg P/kg) compared to other studies. Across all lime and applied P treatments in the study by Whitley (2013) the average shoot P concentration in lotus plants was 2002±130 mg P/kg DM, and in lupin species it was 2278±516 mg P/kg DM and in unlimed soil it was as high as 3770 mg P/kg DM. In the study by Moir et al. (2016a) mean shoot P concentration across all added P treatments in lotus plants was 2400 mg P/kg DM, and at the biological optimum yield (97% of relative maximum yield) lotus shoot P concentration was 3100 mg P/kg DM. This indicates that despite the positive lime and added P effects on shoot P concentration

and uptake, P availability was likely to still be a limitation to plant growth at this optimum pH in the experiment. However, in this experiment, and others (Caradus & Snaydon 1987; Whitley 2013; Moir et al. 2016a), concentrations of P contained in plant material are shown to be good measures of P availability in soil.

Soil Olsen P concentrations were shown to increase at high soil pH (>6.0) in each soil. In the GM soil, which had the greatest initial Olsen P concentration of 24 µg/ml before beginning the experiment, Olsen P decreased as plant P uptake increased up to pH 6.0 as would be expected. However, in the OM, MG, and TD soils which had low initial Olsen P concentrations (>11 µg/ml), soil Olsen P at low pH was not affected by the removal of P from soil by plant uptake. This may be due to the ability of both the Russell lupins and lotus plants to access soil P in sparingly soluble forms that would otherwise be plant unavailable (Borie 1990; Trolove et al. 1996; Lambers et al. 2013; Dissanayaka et al. 2017; Imai et al. 2019).

In each soil, substantial increases in soil Olsen P concentrations compared to initial concentrations were recorded at the conclusion on the experiment, especially at high pH (>6.0). This Olsen P increase was also observed in the field at GM by Berenji et al. (2018). However, the Olsen P increases were not reflected in herbage P concentrations as would be expected, and instead shoot P concentrations were actually decreased at high pH, as was shoot yield. Other studies (Whitley 2013; Moir et al. 2016a) have shown that when soil Olsen P is increased by the addition of fertiliser P, rather than liming, that shoot P concentration increases, regardless of whether increases in yield occurred or not. That literature strongly demonstrates that soil P availability is reflected by shoot P uptake, as a function of shoot P concentration and yield.

From these results it can be concluded that the Olsen P test results at the conclusion of the experiment did not accurately reflect the availability of P in these soils at pH >6.0 following the large applications of lime. The high Olsen P concentrations measured at these higher pH levels may partially be the result of mineralisation of soil microbial P that would otherwise be plant unavailable when the soils are air dried before analysis (Turner & Haygarth 2001). In contrast, Sorn-Srivichai et al. (1984) and Lambert and Grant (1980) has shown Olsen P concentrations to be reduced by liming even though plant growth and P uptake was unaffected, this was possibly due to the precipitation of P by Ca in the Olsen extract. Conversely, in this experiment much greater amounts of P were extracted from unlimed pots than was originally in them as measured by the Olsen P test. Bowman et al. (1978) also showed that Olsen P test results did not well reflect soil P availability in neutral to alkaline soils, as only 52.0±0.1% of the total amount of P that was taken up by plants was extracted by the Olsen P test in an exhaustive cropping trial. Roberts et al. (1994) also showed Olsen P to underestimate soil P availability. For this experiment, further analysis of soil P by fractionation is required to fully understand the effects that liming and exhaustively cropping these soils had on P speciation.

The application of additional P treatment to the pots supplied an additional 4.5 mg P/pot, which was roughly half the amount of plant available P in the GM soil pots and equal to the amount of plant available P already in the OM, MG, and TD soil pots. However, utilisation of this additional P by plants was low, and across all lime treatments for both species shoot yield and P uptake were generally only increased by 0.65±0.08 g DM/pot and 0.90±0.08 mg P/pot respectively by the application of this P. This low utilisation of applied P is likely to be due to the rapid absorption of applied P by soil microbes, and adsorption to Al and Fe oxides in unlimed soil at low pH (<6.0), and conversely to Ca at high pH (>6.0) in limed soils. Applied P may also have been precipitated in hydroxy-Al polymers as soil pH was increased and concentrations of Al in exchangeable form were reduced, given that the additional P and lime treatments were applied conjointly (Haynes 1982). It was expected that as soil pH was increased up to optimal levels for each species that the response to added P would have been increased due to improved soil pH conditions for plants growth and P uptake. However, this was not observed in this experiment which indicates that other soil, and possibly plant, processes were controlling the availability and uptake of this applied P to plants.

The purpose of exhaustively cropping these soils was to examine the amount of P that could be extracted from them to the point that no more P was plant available. After the relatively short time period of the experiment; 407 days, some plants had died but in most pots plant growth had slowed right down and were showing symptoms of severe P deficiency, especially in the Russell lupins. Plant growth may have continued at this slow rate for much longer but allowing plants to continue to grow at this rate would have been impractical. Across the experiment, plant growth and P uptake was shown to be relative to the initial amount of plant available P in each soil, with the greatest amount of growth and P uptake occurring in the GM soil. However, in terms of the utilisation of plant available P it was in the OM, MG, and TD soils where greater utilisation of plant available P occurred. It would therefore be expected that the GM soil would have been able to sustain plant growth for longer but, as with the other soils, plant growth in the GM soil had become very slow by the end of the experiment. Although plant growth and P uptake was increased by liming, up to optimal pH levels, plant P uptake did not exceed the quantity of plant available P that was initially in each soil at the beginning of this experiment, and this would suggest that P-sparing did not occur.

The biggest limitation to plant growth and P uptake in the experiment was liming to alkaline pH levels, and without performing detailed P fractionation analysis to determine the extent P is made plant unavailable by being bound to Ca at these pH levels, it is difficult to determine the extent that reduced P availability is having on these results. However, it is most unlikely that liming to these alkaline pH levels would occur in a field situation so this result is of low relevance in a practical sense. Performing P fractionation analysis on the exhaustively cropped soils may improve the understanding of the results obtained in this experiment, and whether increases in P uptake were related to increases in soil P

availability or were predominantly due to improved ability for plants to access existing soil plant available P.

During the experiment plant N content and the amount of N being fixed by plant rhizobia was not monitored. Although plant growth and P uptake were shown to be closely correlated to soil P availability, changes in N fixation due to liming may have affected plant growth. It is recommended that this is a factor that needs greater consideration in future experiments.

4.5 Conclusions

Overall, this exhaustive cropping trial showed that Russell lupins and lotus respond positively to liming up to their respective optimal pH levels, and to the application of additional P in these four acid high country soils. For Russell lupins the optimal pH was 5.5-6.5 and for lotus it was 6.2-6.8. The concentration of P contained in plant material was shown to be a good indicator of soil P availability, however plant P uptake did not exceed the quantity of plant available P that was initial in each soil at the beginning of this experiment. This would suggest that little or no plant unavailable P was made available due to liming and P-sparing did not occur, and instead the plant response to liming was due to their increased capacity to extract P from soil. The utilisation of applied P was also shown to be low, and was not increased by liming as expected. This suggests this applied P was made plant unavailable by soil processes before it could be utilised by plants. Further P fractionation analysis on these exhaustively cropped soils may improve the understanding of these results, and better quantify the changes in P uptake that were related to changes in soil P availability as measurements of soil Olsen P were shown to be unreliable following large applications of lime.

Chapter 5

Soil fertility, legume growth and pasture composition responses to applied lime, fertiliser and herbicide at Mt Grand Station

5.1 Introduction

Clovers are relied upon by hill and high country farmers for both biological nitrogen (N) fixation and for the production of high quality stock feed. N is usually the most limiting nutrient to pasture production on most hill and high country farms and biologically fixed N is the primary source of N on these farms (Haynes & Williams 1993b; White 1995; Lambert et al. 2003; Stevens et al. 2014). For optimal growth clovers require sufficient soil phosphorus (P), sulphur (S), and molybdenum (Mo) fertility (Caradus 1980; Haynes & Ludecke 1981b; Moir 2000; Kaiser et al. 2005). To maintain adequate soil fertility and clover abundance farmers have traditionally aerially applied superphosphate based fertilisers. However, with rising costs, the removal of farm subsidies, lower commodity prices (Ledgard et al. 1991; Brown & Green 2003), and reductions in pasture response to applied fertiliser, potentially due to ongoing soil acidification (Lambert et al. 1998), farmer focus has shifted away from hill and high country to development and intensification of lower, cultivatable land (Gillingham et al. 2003; Moot 2012). Therefore, over time fertiliser inputs to hill and high country have continued to reduce. The escalation of soil acidity, and associated aluminium (Al) toxicity also restricts clover and other legume production, and has resulted in the proliferation of low quality grass (especially browntop) and weed species (especially heracium). These species outcompete clovers for available nutrients, space and soil water, resulting in reductions of pasture yield and quality (Harris 1974; Jackman & Mouat 1974; Harris & Fan 1996; Steer & Norton 2013).

Large areas of low to mid altitude hill country in the South Island high country have good histories of regular superphosphate fertiliser applications. Despite this, Olsen P levels remain low (Lambert et al. 1998). This is the situation at Mt Grand Station at Lake Hawea, Central Otago. Due to the acid nature of these soils, applied P rapidly becomes bound to Al and Fe hydroxides and made plant unavailable (Parfitt 1978; Haynes 1982). Applied P can also precipitate with exchangeable forms of Al, which reduces Al concentration but also renders the applied fertiliser P plant unavailable (Coleman et al. 1960; Adams 1981; Sloan et al. 1995).

There is potential for development of this country to increase farm productivity and profitability where slope and climate allow. However, despite a history of regular superphosphate fertiliser applications, very little of this country has been sufficiently limed (Moir & Moot 2010). It is uncertain how high country soils will react to lime and to what extent lime will affect legume growth and abundance in these areas. A

limitation of lime application which has prevented it past use is the high cost involved in transporting large quantities and having it aerially applied. Increasing soil pH is known to increase the availability of many essential nutrients for plants (Wheeler & O'Connor 1998; Moir et al. 2016a), but how it affects P availability and uptake in high country soils is unknown. In Chapter 4, liming acid high country soils was not shown to increase the concentration of Olsen P in soil. However, reduced soil acidity and Al concentrations increased legume growth and uptake of P up to the biological optimal pH levels for studied species.

Furthermore, to increase pasture legume abundance and yield in hill and high country the resistant grass dominant vegetation needs to be suppressed. This could be done with the use of selective herbicides to reduce interspecies competition. Using a selective grass herbicide, such as Gallant® (haloxyfop-P, 520g/L), has been shown to reduce grass competition and increase legume yield and abundance in hill country (Rolston et al. 1985; Casey et al. 2000; Hepp et al. 2003). So there is potential for the use of selective herbicides as tools to increase legume production in South Island hill and high country.

Given the acid nature of high country soils, and their low P availability, the objective of this experiment was to investigate the effects of lime, fertiliser and herbicide application on pasture yield, specifically legume yield, pasture quality, and soil fertility at Mt Grand Station over a three year period. The experiment also aims to identify specific combinations of lime, fertiliser and herbicide which can be realistically and cost effectively broadly applied to develop hill and high country pastures.

5.2 Methods

5.2.1 Site Description

Mt Grand Station is a 2131 ha sheep and beef high country farm located southeast of Lake Hawea in the Central Otago district. The experimental site was located on a directly north facing, moderate to steep (varying from 20˚ to 30˚) slope that is one-third of the way up the farm at 680 to 700 m altitude (44˚38’01.95” S; 169˚19’42.30”E), within a 70 ha paddock known as the ‘Broadspur Block’. The soil at the site is an Arrow steepland Pallic soil with schist and loess parent material (Duncan et al. 1997). Exposure to the sun and northerly winds cause the site to dry out in summer, and the steepness at the top of the site are factors that may limit pasture growth and development.

The experimental site consisted of 16 5x5 m plots (four blocks of four plots each, 12 fully fenced and 4 open to continuous grazing). It was originally established in October 2008 and was actively managed through to May 2010 (Maxwell 2013; Maxwell et al. 2014, 2016). In this work a factorial split plot design was used to implement four grazing management treatments (main plots) and two fertiliser treatments

(split plots, 2.5 x 5 m), each replicated four times. The four grazing management treatments were continuous spring grazing by Merino sheep with and without plot fences, early spring plot closure to exclude stock, and late spring closure. The two fertiliser treatments were low (75 kg/ha) and high (200 kg/ha) application rates of 30% sulphur superphosphate fertiliser (7% P, 9% sulphate-S, 21% elemental-S, 'SS30'). The fertiliser treatment was applied once in November 2008 and the grazing management treatments were implemented in both the spring of 2008 and 2009. Since the conclusion of that experiment fenced plots remained closed off and ungrazed until the beginning of this experiment in March 2015.

Rainfall (Figure 5.1 and 5.2), air temperature and soil temperature at 10 cm depth (Figure 5.3) was recorded using Onset® weather recording equipment at another north facing site below the experimental site, at 620 m altitude. The 60 year average rainfall at the site is 703 mm (Power et al. 2006; Maxwell et al. 2016). During the experimental period annual rainfall was below average in each of the three years at 432 mm in 2015/16, 579 mm in 2016/17, and 688 mm in 2017/18 (Figure 5.1).

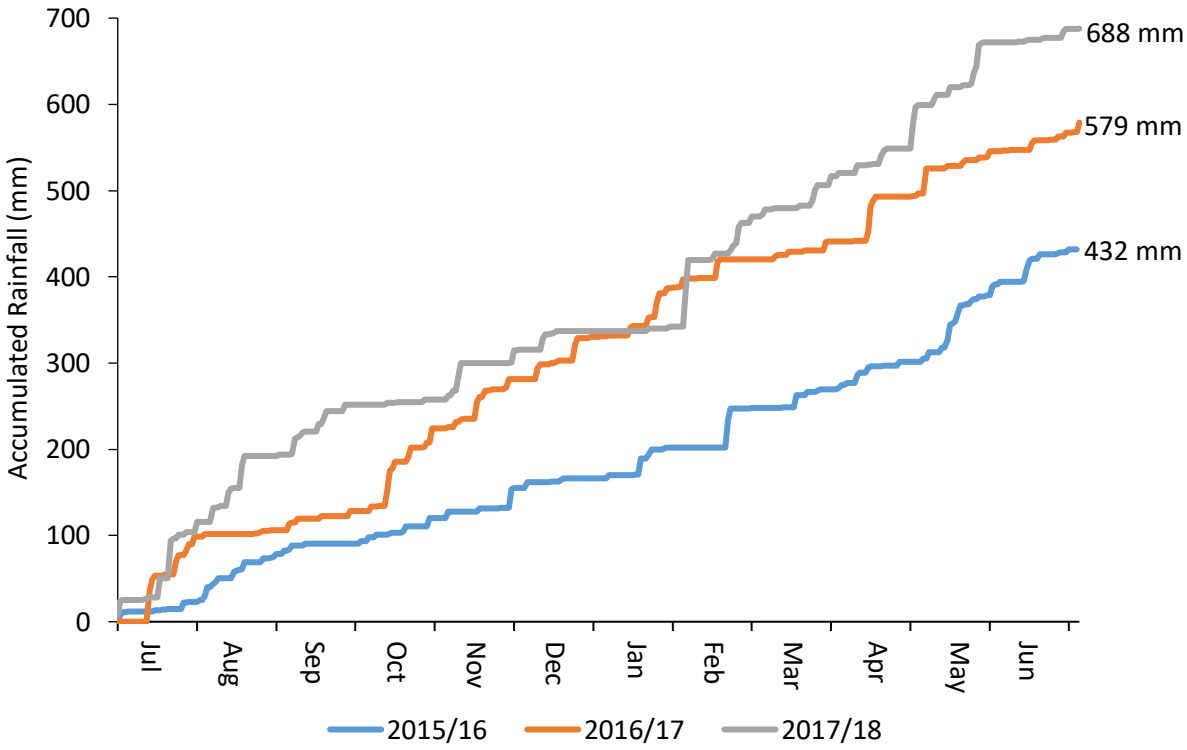


Figure 5.1 Accumulated annual rainfall at Mt Grand in each year of the experiment on a 1st of July to 30th of June year basis.

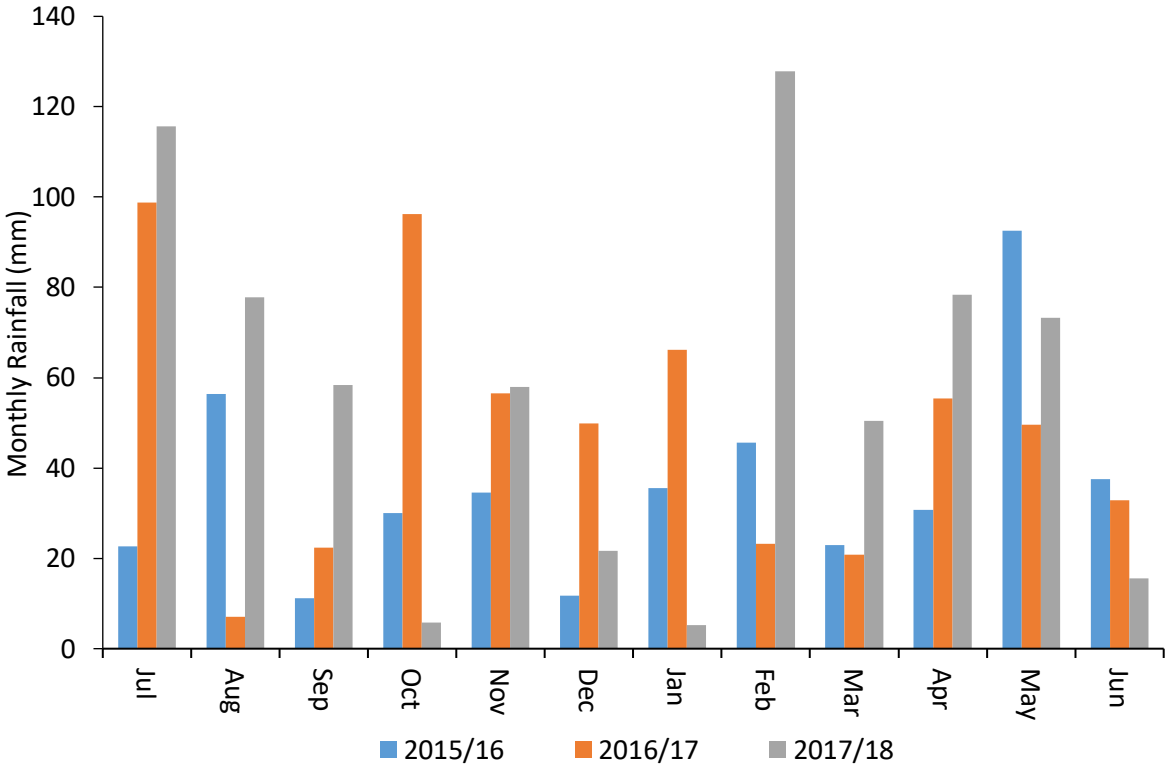


Figure 5.2 Monthly rainfall at Mt Grand between 1st of July 2015 and 30th of June 2018.

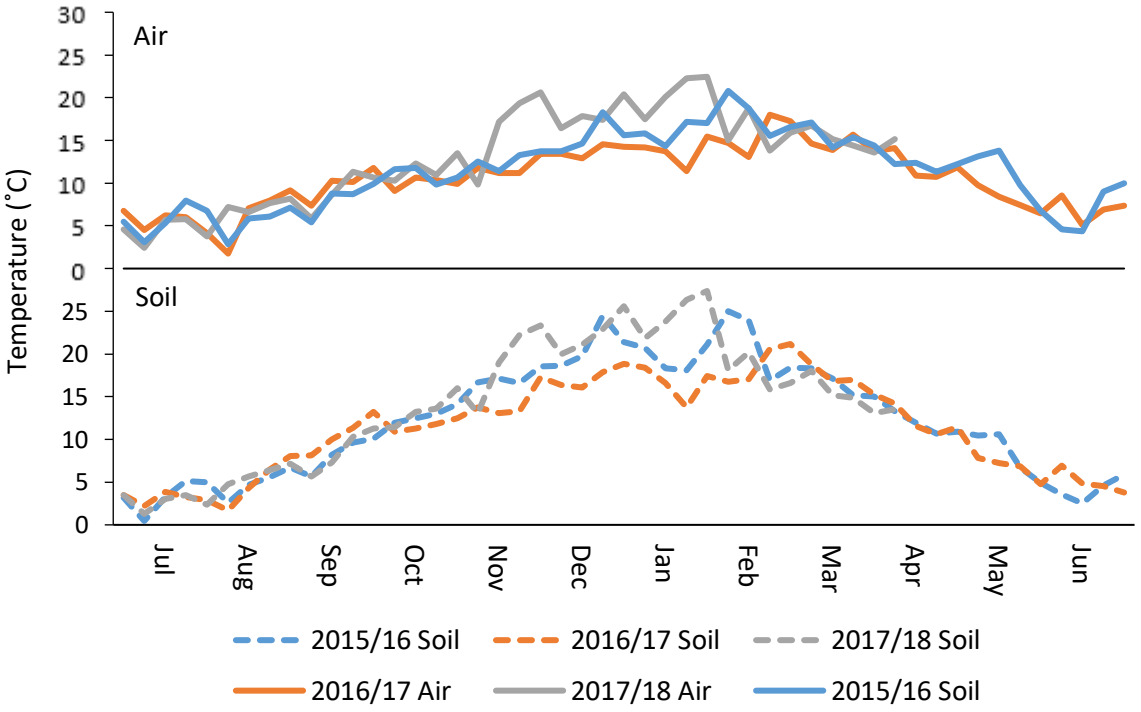


Figure 5.3 Weekly air and soil (10 cm depth) temperature at Mt Grand between 1st of July 2015 and 30th of June 2018.

Prior to beginning this experiment, five 7.5 cm deep, 2.5 cm diameter soil cores were collected from each of the original high and low fertiliser plots and bulked by block (Table 5.1). The collected soil was air dried

at 20˚C for seven days and passed through a 2 mm sieve. Soil chemical analysis was performed according to Section 3.2.2.

Table 5.1 Soil chemical analysis of the original low (75 kg/ha) and high (200 kg/ha) fertiliser split plots in March 2015, before beginning this experiment.

Soil Analysis	Unit	Original Fertiliser Treatments		Method
		75 kg/ha	200 kg/ha	
pH		5.5	5.5	Blakemore *et al.* (1987)
Exch Al	mg/kg	1.8	1.9	Edmeades *et al.* (1983) ($CaCl_2$)
Olsen P	µg/mL	10.5	10.8	Olsen *et al.* (1954)
Sulphate S	µg/g	3.3	3.0	Watkinson & Kear (1994)
Organic S	µg/g	3.0	2.5	Watkinson & Kear (1996)
Total C	% w/w	5.36	5.38	Horneck & Miller (1998)
Total N	% w/w	0.46	0.47	Horneck & Miller (1998)
C:N Ratio	C/N	11.7	11.4	-
CEC	me/100g	15.3	15.8	Hesse (1971)
Base Saturation	%	54.1	54.9	Hesse (1971)
Calcium	me/100g	6.78	6.95	Schollenberger & Simon (1945)
Magnesium	me/100g	0.85	1.06	Schollenberger & Simon (1945)
Potassium	me/100g	0.51	0.51	Schollenberger & Simon (1945)
Sodium	me/100g	0.09	0.19	Schollenberger & Simon (1945)

The results of the analysed soil parameters showed that there was no longer any measurable differences between the high and low fertiliser treatments used in the initial experiment 7 years after they were applied.

5.2.2 Experimental Design

The experiment was established in March 2015 as a split-split plot design. Each 5x5 m plot was split in half both vertically and horizontally to create four 2.5x2.5 m subplots in each main plot (Plate 5.1). The 30% sulphur superphosphate (7% P, 9% sulphate-S, 21% elemental-S) fertiliser treatment was applied to each vertical split plot at both 0 and 200 kg/ha (14 kg P/ha) in each of the 16 main plots. For the horizontal split plots, lime (crushed Ag-lime, 84 % neutralizing value) was applied at rates of 0 or 1 t/ha to each half of two main plots per block, and was applied at rates of 0 or 3 t/ha to the other two main plots per block respectively. In doing so each main plot contained subplots of a control, fertiliser only, lime only at 1 t/ha or 3 t/ha, and a combination of fertiliser and lime. These fertiliser and lime treatments were applied annually in March 2015, April 2016 and April 2017.

At the establishment of the experiment, two subterranean (sub) clover (*Trifolium subterrareum*) cultivars; mid-spring flowering 'Woogenellup' and late-spring flowering 'Denmark' (Nichols et al. 2013), and a single balansa clover (*Trifolium michelianum*) cultivar; early to mid-spring flowering 'Bolta' (Monks et al. 2010), were sown on the main plots at rates of 15, 15, and 10 kg/ha respectively. The germination rates of the sown clovers were measured as 98, 82 and 52% after 10 days at 20˚C respectively, which then corresponds to effective sowing rates of 14.7, 12.3 and 5.2 kg/ha of viable seed. This seed mixture was sown again in April 2016 at rates of 15, 15, and 10 kg/ha of viable seed (corrected for germination rate) respectively. This clover mix was not sown in the third year of the experiment.

Additionally, agricultural salt (NaCl) was spread on all of the plots in an effort to improve grazing intensity, pasture utilisation, and clover establishment by increased treading by Merino sheep due to the soil of the experimental site being deficient in sodium (Aspinall et al. 2004; Gillespie et al. 2006). The salt was initially applied at 30 kg/ha when the experiment was established in March 2015 and then again at a higher rate of 150 kg/ha in April 2016 when the fertiliser, lime and seed were applied.

In early September 2016 a selective grass herbicide treatment was added to the experiment to examine the effect of grass suppression on clover growth along with the respective fertiliser and lime treatments (Rolston et al. 1985; Hepp et al. 2003; Nori et al. 2015). Gallant (haloxyfop-P, 520 g/l, 12 ml in 15 l water) was applied with Uptake spray oil (582 g/L paraffinic oil and 240 g/L alkoxylated alcohol non-ionic surfactants) using a knapsack sprayer with two nozzles to half of the plots (Plate 5.1).

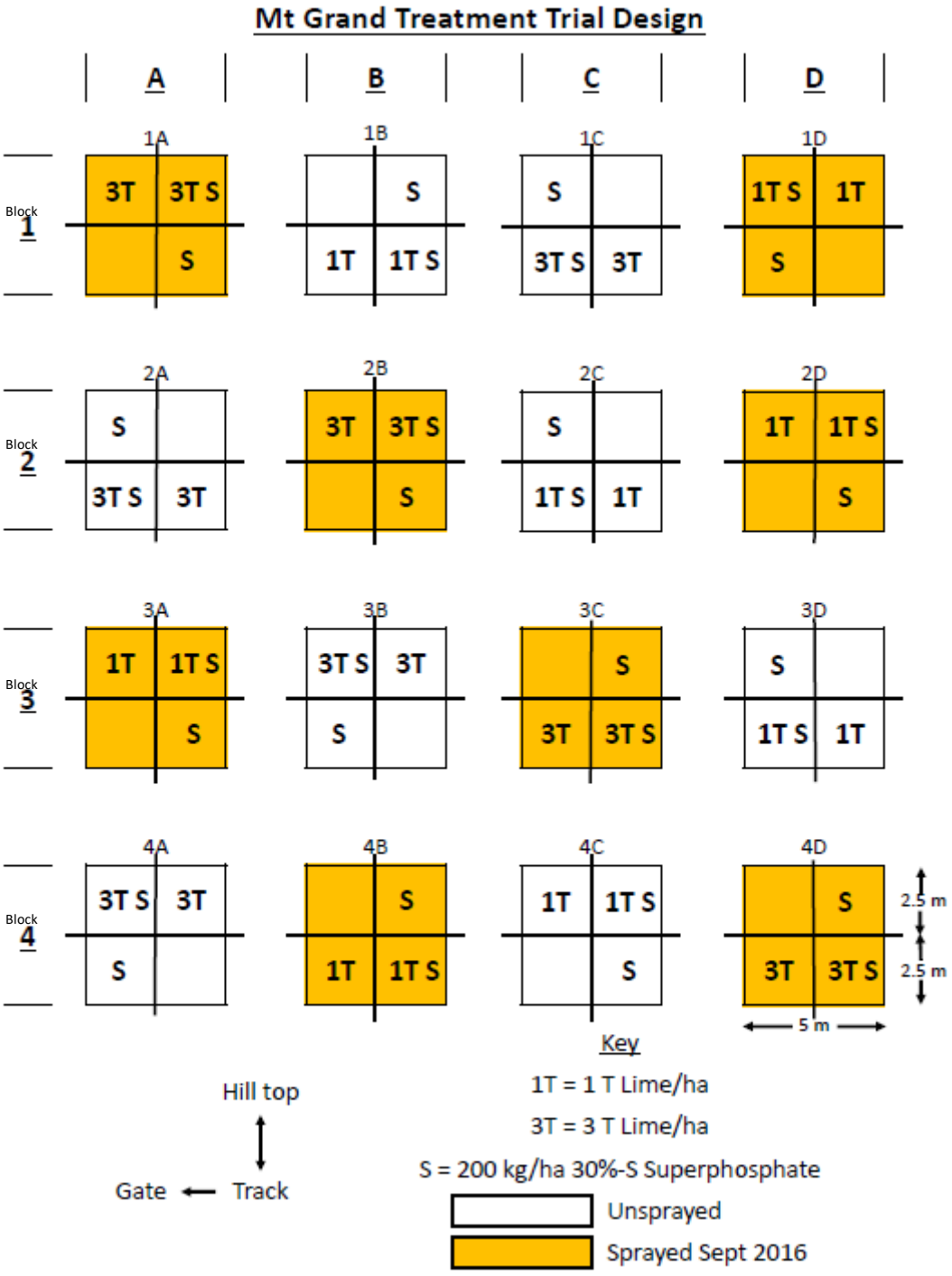


Plate 5.1 Mt Grand Station lime, fertiliser and herbicide treatment experimental plan.

5.2.3 Herbage Sampling

Measurements of herbage yield and botanical composition occurred at the site in the spring and autumn of each of the three measurement years. The plots were grazed by set-stocked Merino sheep after each sampling for 8-10 weeks, and sheep were then excluded from the plots by netting fences. To collect pasture samples electric shears were used to cut the pasture to a height of 1-2 cm within a randomly placed quadrat of known area (Plate 5.2), and the cut sample material was collected. Quadrat size was dependant on the amount of pasture in the plots at the time of harvest. Herbage sampling occurred on

the 9th-11th November 2015 using one 0.2 m^2 quadrat in each plot, on the 6th-7th April 2016 (0.4 m^2 quadrat), the 28th-29th November 2016 (0.5 m^2 quadrat), the 26th-27th April 2017 (0.2 m^2 quadrat), the 6th-7th December 2017 (0.5 m^2 quadrat), and finally on the 18th-19th June 2018 (0.5 m^2 quadrat).

Plate 5.2 Pasture sampling with electric shears, Mt Grand Station, 6th December 2017.

Following each spring sampling and the final autumn sampling pasture samples were weighed fresh before being thoroughly mixed and subsampled. A 20-30 g subsample was used to assess botanical composition and a larger sample (150-350 g) was air dried at 65˚C for 48 hours to determine dry matter content. This was then factored up to determine the total dry matter content of the whole sample. The dried material was then finely ground and analysed for a wide spectrum of nutrients, excluding C and N, by digesting 0.2 g of ground sample material in 2.0 ml HNO_3 (69%) and 2.0 ml H_2O_2 (30%) in a microwave digester (CEM MARS Xpress, CEM Corporation, Matthews, NC, USA), followed by ICP-OES analysis (Varian 720, Varian Australia Pty Ltd, Melbourne, Australia) (Nolte 2003).

For the autumn collected pasture samples from April 2016 and 2017, pasture yield was very low (<1500 kg DM/ha) so the whole collected sample was air dried at 65˚C for 48 hours to determine dry matter content. These samples were not ground or sorted for botanical composition. Instead, the yield of each was added to the corresponding spring yield in equal proportion to the botanical composition from the spring sampling to determine the overall annual pasture yield.

5.2.4 Soil Sampling

Following the April 2016, November 2016 and December 2017 herbage sampling events, six 7.5 cm deep soil core samples were taken from each subplot where the herbage sampling had occurred. These soil samples were bulked by lime-fertiliser treatment per block. Any effects of the spray treatment on soil fertility were not considered. The bulked samples were then air dried at 30˚C for seven days and passed through a 2 mm sieve. Each was then chemically analysed for pH, Olsen P, Exch Al, Sulphate-S, Organic-S, CEC and QT cations according to the methods in Section 3.2.2. P fractionation was carried out on the soil collected at the November 2016 sampling, according to the method in Appendix A.

5.2.5 Statistical Analysis

The yields of all the pasture components and uptake of nutrients was analysed by a split-split plot design ANOVA in Genstat 2016 for each individual year of the experiment. In the ANOVA the spray treatment was assigned to the whole plot treatment (all plots designated as non-sprayed in year 1), and lime and fertiliser as subplot treatments. The significance level was set at P=0.05.

For the soil chemistry results (excluding the P fractionation results), repeated measures two-way ANOVA's were used to analyse the effects of lime and fertiliser on soil chemistry. Soil P fractionation results were analysed by split plot design two-way ANOVA in Genstat 16.

5.3 Results

5.3.1 Treatment Effects on Pasture Yield and Composition

In the first year of the experiment, 2015/16, the application of neither lime nor fertiliser, and any combination of the two, had no effect on annual pasture yield. No treatment effects were measured for the sown clover, naturalised annual clover (NAC), grass, weed, or dead components of the pasture within the total pasture yield (Figure 5.4).

Within the sown clover yield in 2015/16 there were no treatment effects on the yield of each of the three sown species. The yield of the sown clovers was dominated by 'Denmark' sub clover which comprised 57.2±9.7% of the sown clover yield, followed by 'Bolta' balansa clover, 22.5±5.5%, and then 'Woogenellup' sub clover, 20.3±7.6%. Of the NAC's lime and fertiliser treatment had no effect on the yield of the striated, cluster or suckling clovers. Fertiliser treatment had no effect but lime significantly reduced the yield of the haresfoot clover (P=0.045) within the total NAC yield. However, the haresfoot clover was the lowest yielding NAC species and on average only made up 1.6±0.7% of total NAC yield. The striated clover was the most dominant of the NAC's, making up 55.5±6.6% of total NAC yield, followed by suckling clover (36.2±6.7%), and cluster clover (6.6±2.5%). The yield of the individual grass and weed species within the

grass and weed components was not assessed. Nor was it within the dead component of the sward but this mostly consisted of old, senesced grass leaves and stems.

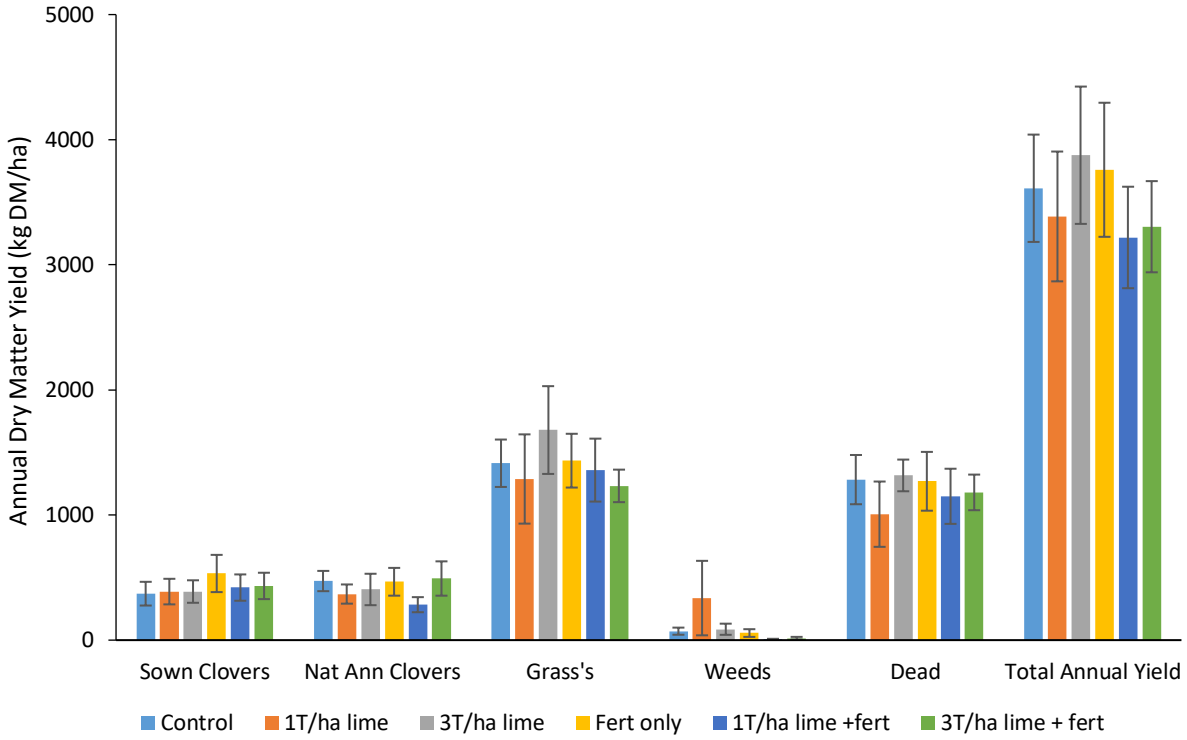


Figure 5.4 Annual dry matter yield and composition at Mt Grand in the first year of the experiment, 2015/16.

The herbicide treatment was applied at the beginning of the second year of the experiment, 2016/17. As expected, it reduced (P<0.001) grass yield in the sprayed plots (Figure 5.5). Conversely, this led to an increase (P<0.001) in pasture weed content. In terms of overall total pasture yield, when coupled with the high fertiliser and lime treatments, the spray treatment increased (P=0.014) total pasture yield. In all other treatments the reduction in grass yield resulted in a loss of total pasture production (Fig 5.7). The warm, wet spring of 2016 provided excellent growing conditions which resulted in exceptional NAC growth, and very little development of low quality dead material. Applying lime alone had no effect on the growth of sown clovers (P=0.070), NAC's (P=0.548), grass (P=0.352), weeds (P=0.469), dead material (P=0.778) or total pasture yield (P=0.450). The application of fertiliser alone increased the yield of the sown clovers (P=0.019), the NAC's (P<0.001), and the total pasture yield (P<0.001). When applied with either 1 t/ha or 3 t/ha of lime, the fertiliser further increased the yield of the NAC's (P=0.032), and inversely reduced the yield of the grass (P<0.01).

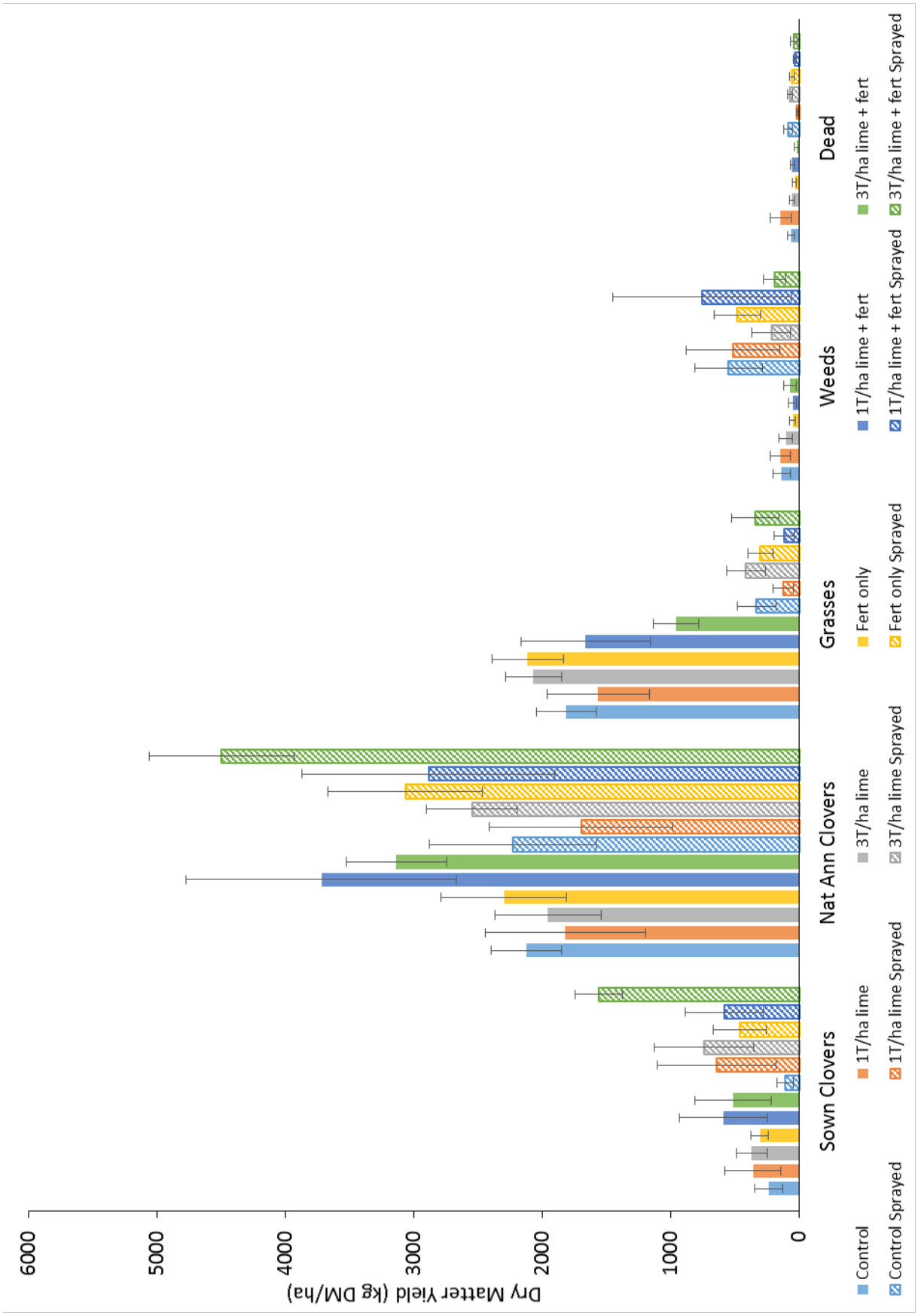


Figure 5.5 Annual dry matter yield and composition at Mt Grand in the second year of the experiment, 2016/17.

The increase in sown clover yield in response to fertiliser addition in the second year resulted from an increase in yield of both the 'Woogenellup' sub clover (P=0.003) and the 'Denmark' sub clover (P=0.009). They contributed an additional 60.0±17.3 kg DM/ha and 107±37.0 kg DM/ha of each species respectively (Plate 5.3). This fertiliser effect was further enhanced by the application of the herbicide for both species (P=0.019 and P=0.034, respectively).

Plate 5.3 Sub clover production (dark green) in Plot 2A (no herbicide applied) on the 28th of November, 2016. The four subplots divided by the light blue lines are clockwise from top left; fertiliser only, control, 3 t/ha lime only, fertiliser and 3 t/ha lime.

The lime treatment increased the production of the 'Bolta' balansa clover (P=0.019), increasing yield from 87.0 kg DM/ha (control) to 335 kg DM/ha (1 t lime/ha), and to 569 kg DM/ha (3 t lime/ha). This lime effect was evident in the plots by a visual increase in the number of white Bolta balansa flowers at the time of the spring harvest (Plate 5.4).

Plate 5.4 The effect of lime on clover growth in Plot 2C (no herbicide applied) at the 28th of November, 2016. Photo taken from the right hand side of the plot. The white flowers on the left hand side (limed side) of the picture are 'Bolta' balansa clover, while the red flowers are sorrel. The subplots are clockwise from top left; fertilised with 1 t/ha lime, fertiliser only, control, 1 t/ha lime only.

Of the NAC's striated clover was again the highest yielding, averaging 1510±172 kg DM/ha/yr, followed by the cluster (551±95.6 kg DM/ha/yr), suckling (519±61.7 kg DM/ha/yr), and with little yield the haresfoot clover (30.8±9.08 kg DM/ha/yr). Traces of resident 'Mt Barker' sub clover were also found in several plots. Despite the large increase in NAC yield with the application of lime and fertiliser, there was a high degree of variability among plots, with large yields occurring in several plots and very little in others. Of the NAC's, cluster clover was the only species to consistently respond to any of the applied treatments, and it especially responded to fertiliser treatment (P=0.002) which resulted in an additional 576±160 kg DM/ha in this 2016/17 year. .

When considering the combined total clover yield (sown and NAC combined), the fertiliser treatment increased total clover yield (P<0.001) by an additional 1250 kg DM/ha. There was also a strong lime by fertiliser interaction (P=0.034), which doubled total clover yield from 2420±510 kg DM/ha in the control treatment to 4860±328 kg DM/ha when 3 t lime/ha with fertiliser was applied.

In 2016/17, the individual species composition of the grass, weed, and dead material components of the pasture were not assessed.

In the third year (2017/18) of the experiment the fertiliser (P=0.005) and fertiliser by spray (P=0.002) treatments again had beneficial effects on overall total pasture yield, which averaged 5790 kg DM/ha across all treatments (Figure 5.6). This was the greatest annual yield of the three measurement years. This high yield resulted from much larger autumn yields, especially by the sown clovers, than those measured in the previous two years. The high fertiliser treatment increased sown clover yield from 1420±263 kg DM/ha to 1950±425 kg DM/ha (P=0.011). There was positive interaction of spray by fertiliser (P=0.044), spray by lime (P=0.038), and a three way interaction between the spray, lime and fertiliser treatments (P=0.018) on sown clover yield. NAC yield was much lower in 2017/18 (911±81.8 kg DM/ha) compared with 2016/17 (2610±186 kg DM/ha), but it too responded to the high fertiliser treatment (P=0.050). The grass was responsive to the fertiliser treatment (P=0.049) but with the return of annual grasses the effect of the spray treatment on suppressing grass yield was shown to have worn off in 2017/18. No lime or fertiliser treatment effects were observed on the yield of the weeds or dead material in the sward.

The large sown clover yield in 2017/18 was due to large sub clover growth in the autumn of 2018, particularly the 'Denmark' sub clover. 'Woogenellup' sub clover yield was greater than the previous two years, but was not affected by any of the applied treatments. The yield of the 'Denmark' sub clover averaged 1131±90.7 kg DM/ha in the control treatment as was increased by 316±125 kg DM/ha by the high fertiliser treatment (P=0.021). Within the sprayed plots it was increased by both the lime (P=0.033), fertiliser (P=0.026), and the combined lime plus fertiliser (P=0.009) treatments. The yield of the 'Bolta' balansa clover was no different to the previous season but was increased by both the lime (P=0.007), fertiliser (P=0.002), and the combined lime plus fertiliser (P=0.007) treatments. 'Bolta' yield increased from 76.0±44.5 kg DM/ha in the control treatment up to 652±154 kg DM/ha in the 3 t lime/ha with high fertiliser treatment. Of the NAC's, the striated clover was again the highest yielding (638±75.9 kg DM/ha) and was not affected by any of the applied treatments. In contrast, the cluster clover responded to the high fertiliser treatment (P=0.011), producing an additional 115±40.9 kg DM/ha. This year it also responded to the spray treatment (P=0.040), producing an additional 108±30.9 kg DM/ha. There was also an interaction between the spray and fertiliser treatments (P=0.050). The suckling clover yield was lower than in previous years, but it did respond to the high fertiliser treatment (P=0.007), producing an additional 55.3±18.0 kg DM/ha, and to the combined lime by fertiliser treatments (P=0.019). The spray treatment reduced (P=0.043) suckling clover yield by 28.7±8.53 kg DM/ha. The haresfoot was again the lowest yielding of the NAC's and was not affected by any of the applied treatments.

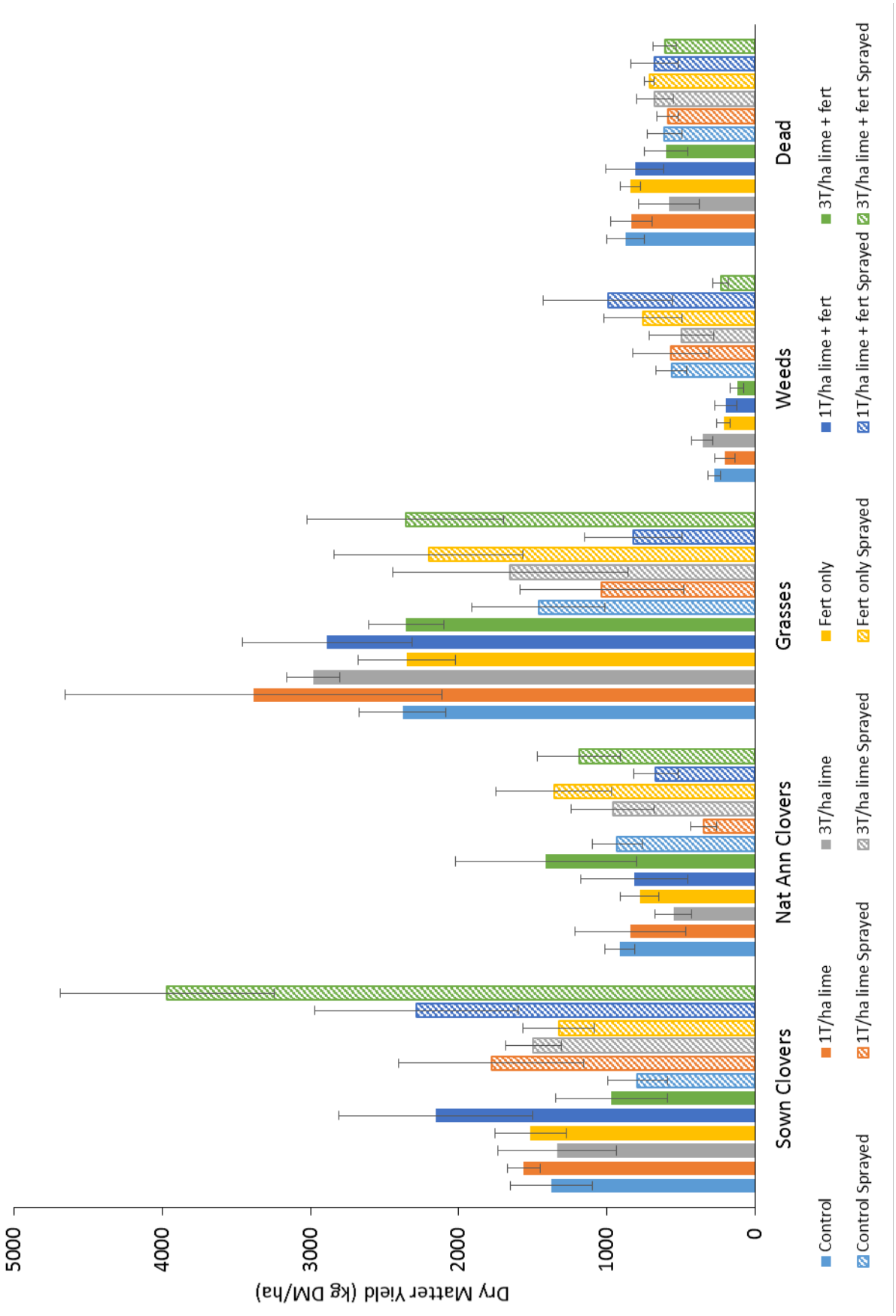


Figure 5.6 Annual dry matter yield and composition at Mt Grand in the third year of the experiment, 2017/18.

The species composition of the grass and dead material components of the sward were not assessed in 2017/18 but the composition of the weed component was sorted into heracium (*Pilosella officinarum*), sorrel (*Rumex acetosella*), and remaining other weeds. The sorrel was the most dominant weed and contributed on average 246±48.6 kg DM/ha of the total 424±55.8 kg DM/ha of weed yield. No treatment effects on any of the weeds were recorded.

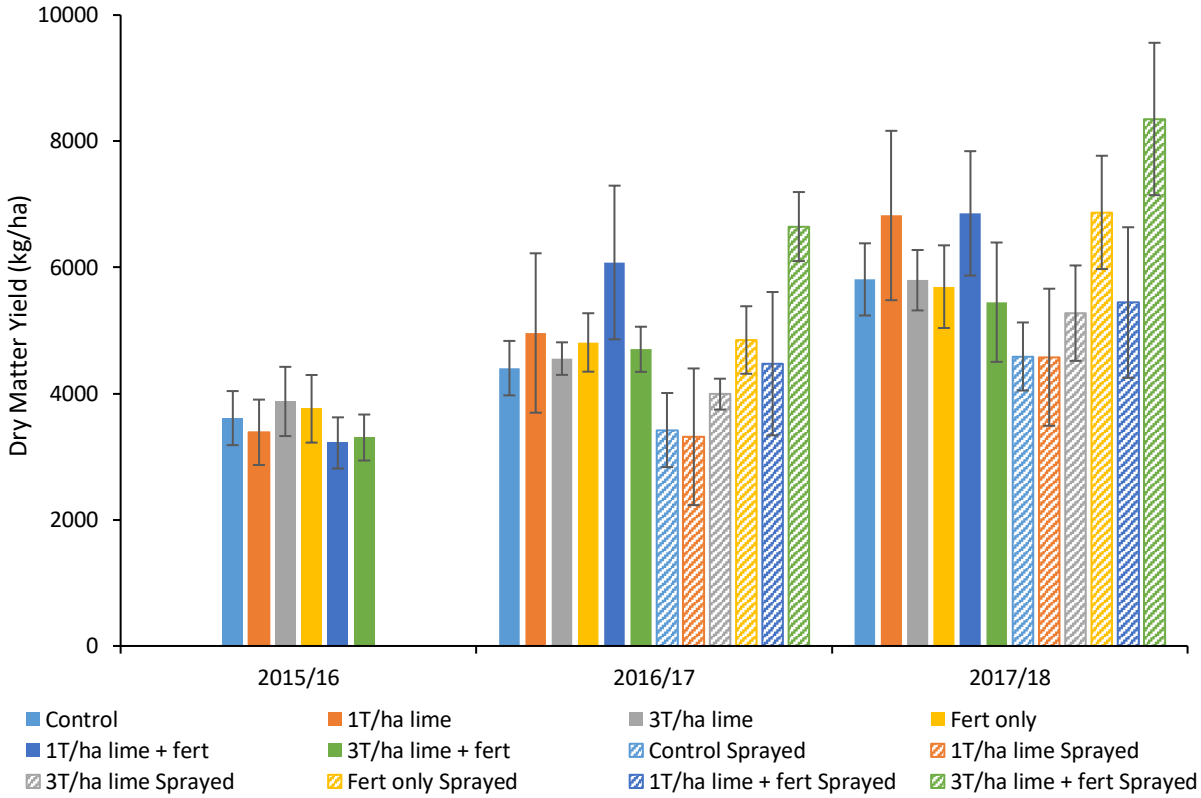


Figure 5.7 Total annual dry matter yield at Mt Grand Station during each of the three years of the experiment.

In the dry year (below average rainfall) of 2015/16 (Fig 5.1), there were no effects of the applied lime or fertiliser treatments on the total amount of pasture grown at the site (Figure 5.7), or the total percentage of clover in the pasture (Figure 5.8). The herbicide treatment had no effect (P=0.484) on total pasture yield when applied at the beginning of 2016/17. However, it did increase (P=0.049) the percentage of the total pasture yield that was clover (Figure 5.8). In 2016/17, the fertiliser treatment alone increased both the overall total pasture yield (P<0.001) and the percentage of clover in the pasture (P=0.010). Applying lime alone had no effect (P=0.583) on total pasture yield in 2016/17 or on the percentage of clover in the pasture (P=0.187).

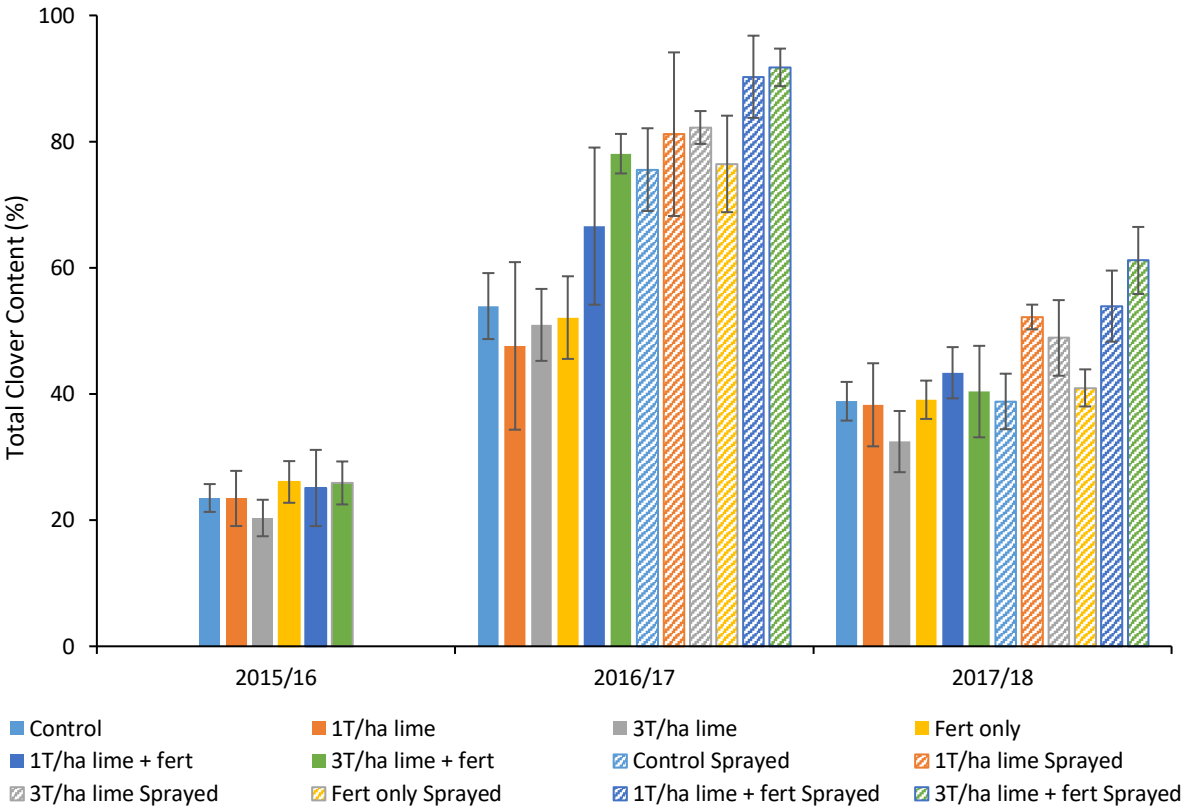


Figure 5.8 Overall sward clover content of the annual pasture yield (% DM) at Mt Grand during each of the three years of the experiment.

By 2017/18, one year following the herbicide application, its positive effect on the percentage of clover in the pasture had begun to wear off (P=0.081). However, overall it still increased the total annual dry matter production of the clovers (P=0.027) from 2370±441 kg DM/ha to 2850±459 kg DM/ha, and again had no effect on overall total pasture yield (P=0.797). The effects of the fertiliser treatment on clover percentage in the pasture were also not as profound in 2017/18 (P=0.096), as was the effect of the fertiliser when combined with the lime treatments (P=0.067). However, the application of fertiliser still increased the total clover yield in the pasture (P=0.001), and overall total pasture production (P=0.004), especially in the sprayed plots (P=0.001).

5.3.2 Treatment effects on nutrient uptake

The application of lime or herbicide did not increase the concentration (mg/kg DM) or uptake (kg/ha) of either P or S in herbage material in each spring of the experiment (Table 5.2). Only the application of the 'sulphur super 30' fertiliser increased both P and S in each spring of the experiment. P concentration within the pasture (mg/kg DM) was increased on average 6.7%, 20.2%, and 16.1% by the annual application of SS30 in each year of the experiment, respectively. S concentration was also increased by 45.9%, 95.8%, and 59.3% by the annual application of SS30 in each year of the experiment, respectively.

Table 5.2 The effects of applied treatments on pasture P and S uptake at Mt Grand Station.

Year				P uptake		S uptake	
				mg/kg DM	kg/ha	mg/kg DM	kg/ha
2015/16	Nutrient Uptake	Control		2010	9.51	1390	6.43
		1 T lime/ha/yr		2080	8.59	1400	5.81
		3 T lime/ha/yr		2160	10.4	1390	6.44
		SS30 only		2260	10.9	2040	9.82
		1 T lime/ha/yr + SS30		2120	7.45	2030	7.10
		3 T lime/ha/yr + SS30		2290	8.49	2040	7.51
	Main Treatment Effects	Lime	SED	79.9	1.55	82.7	1.22
			LSD (5%)	163	3.18	169	2.50
			P Value	ns	ns	ns	ns
		Fert	SED	64.3	1.25	66.5	0.98
			LSD (5%)	131	2.56	136	2.01
			P Value	**	ns	***	*
	Interaction Effects	LimexFert	SED	114	2.21	118	1.73
			LSD (5%)	232	4.51	240	3.55
			P Value	ns	ns	ns	ns
Spray Treatment applied Sept 2016				Untreated Sprayed	Untreated Sprayed	Untreated Sprayed	Untreated Sprayed
2016/17	Nutrient Uptake	Control		2320 2130	9.88 7.32	1380 1210	5.77 3.94
		1 T lime/ha/yr		2250 2400	10.9 7.00	1370 1550	6.39 4.40
		3 T lime/ha/yr		2620 2440	12.1 9.80	1870 1390	8.71 5.87
		SS30 only		2780 3100	13.5 13.7	2670 3260	13.0 12.7
		1 T lime/ha/yr + SS30		2580 3040	15.8 12.7	2620 2740	16.4 11.0
		3 T lime/ha/yr + SS30		2890 2630	13.5 17.0	3030 2850	14.2 18.5
		Spray	Untreated	2570	12.6	2120	10.7
			Sprayed	2620	11.3	2190	9.40
	Main Treatment Effects	Lime	SED	138	1.31	176	1.17
			LSD (5%)	302	2.64	383	2.34
			P value	ns	ns	ns	0.084
		Fert	SED	93.1	1.08	107	0.96
			LSD (5%)	197	2.16	226	1.92
			P value	***	***	***	***
		Spray	SED	71.7	1.07	108	0.95
			LSD (5%)	228	2.15	343	1.91
			P value	ns	ns	ns	ns
	Interaction Effects	LimexFert	P value	ns	ns	ns	ns
		LimexSpray	P value	ns	ns	ns	ns
		FertxSpray	P value	ns	*	0.057	ns
		LimexFertxSpray	P value	ns	ns	ns	*
2017/18	Nutrient Uptake	Control		2380 2430	13.8 10.4	1520 1610	8.71 6.75
		1 T lime/ha/yr		2150 2770	14.6 11.8	1520 1930	9.70 8.11
		3 T lime/ha/yr		2250 2500	12.8 13.2	1520 1610	8.68 8.38
		SS30 only		2800 2860	16.1 17.6	2600 2550	14.9 15.6
		1 T lime/ha/yr + SS30		2790 3040	18.6 16.4	2730 2640	17.9 14.2
		3 T lime/ha/yr + SS30		2680 2640	14.4 20.6	2530 2410	13.8 18.8
		Spray	Untreated	2530	15.0	2070	12.2
			Sprayed	2610	14.1	2050	11.4
	Main Treatment Effects	Lime	SED	184	1.73	143	1.29
			LSD (5%)	400	3.76	311	2.81
			P value	ns	ns	ns	ns
		Fert	SED	114	1.10	105	0.94
			LSD (5%)	240	2.32	220	1.98
			P value	*	**	***	***
		Spray	SED	76.6	2.09	61.6	1.77
			LSD (5%)	244	6.66	196	5.62
			P value	ns	ns	ns	ns
	Interaction Effects	LimexFert	P value	ns	ns	ns	ns
		LimexSpray	P value	ns	ns	ns	ns
		FertxSpray	P value	ns	ns	0.086	ns
		LimexFertxSpray	P value	ns	ns	ns	ns

Significance levels: ns not significant, * P<0.05, ** P<0.01, *** P<0.001

No relationship was found between lime treatment and the concentration of P and S in herbage material (mg/kg DM), or total P and S uptake (kg/ha) in each year of the experiment. Total P and S uptake was only a factor of total pasture yield for each year of the experiment. The maximum increase in both P and S concentration and uptake was in the second year of the experiment following two applications of SS30 fertiliser. The third application at the beginning of 2017/18 had no additional effect on pasture P and S concentration or uptake.

Of the other nutrients measured, the application of the lime treatments reduced the concentration of manganese (Mn) taken up by the pasture, although concentrations did not exceed toxicity thresholds (Smith et al. 1983), in both the second ($P=0.025$) and third ($P<0.001$) years of the experiment. However, the application of the fertiliser increased the total uptake (kg/ha) of Mn in both these years ($P=0.008$ and $P=0.001$ respectively) as a function of increased total pasture yield.

In the second year of the experiment, 2016/17, the application of the fertiliser treatment resulted in an increase in both the concentration (mg/kg DM) and uptake (kg/ha) of calcium ($P=0.002$ and $P<0.001$ respectively), potassium (K) ($P<0.001$), and magnesium (Mg) ($P<0.001$), and again this was not due to only an increase in pasture clover content. In contrast the concentration ($P=0.004$) and uptake ($P=0.049$) of molybdenum (Mo) was reduced by the application of the fertiliser in 2016/17. The application of the herbicide treatment increased the concentration of boron (B) in the herbage material ($P=0.018$), and along with fertiliser treatment, increased total B uptake ($P=0.043$) in response to increases in pasture clover content (%) ($P<0.001$). The herbicide treatment also increased the overall uptake of iron (Fe) by the pasture ($P=0.023$) but not in relation to increased clover content (%).

The fertiliser treatment did not increase the concentration of Ca, K and Mg in the herbage material in the third year of the experiment, 2017/18, as it did in 2016/17. But overall the total uptake of each (kg/ha) was still increased by the fertiliser treatment as a function of increased total pasture yield ($P=0.011$, $P<0.001$, and $P<0.001$, respectively). The concentration of Mo in herbage material (mg/kg DM) was again decreased by the fertiliser treatment in 2017/18 ($P=0.001$). In contrast, the application of the lime treatments increased Mo concentration ($P=0.040$), and overall neither the lime nor fertiliser treatments affected the total amount of Mo that was taken up in the herbage material (kg/ha).

5.3.3 Treatment effects on soil chemistry

Applying 3 t/ha of lime was sufficient to raise the soil pH at the Mt Grand experimental site from the initial pH of 5.5 up to the recommended pH range of 5.8-6.0 (Edmeades et al. 1984a; Edmeades et al. 1991a). There was no difference in pH increase by applying this 3 t/ha of lime in one single application or three split applications over successive years (Figure 5.9).

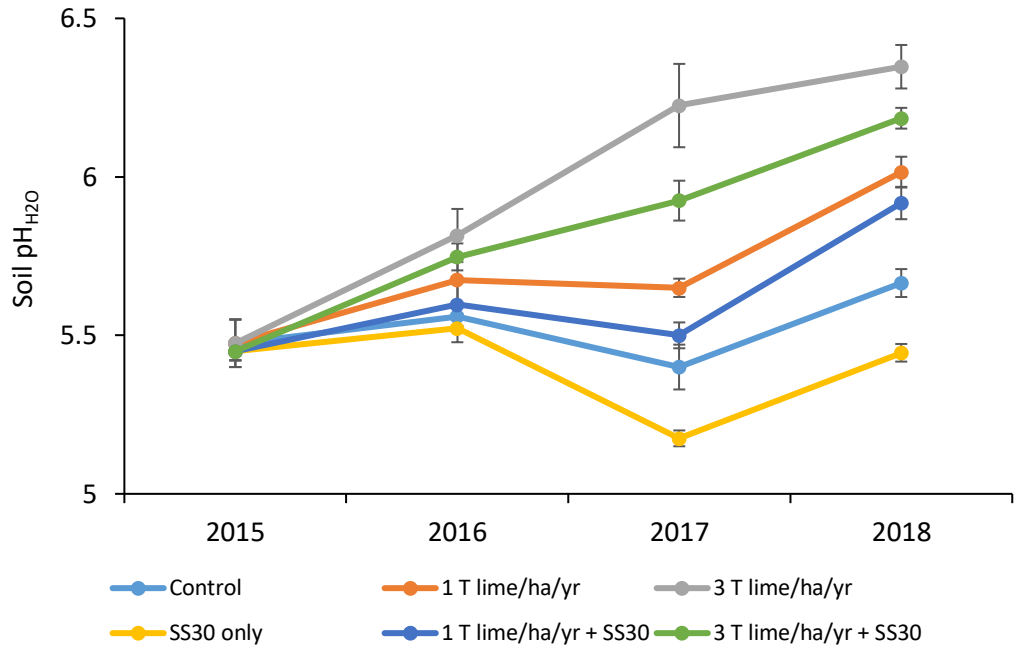


Figure 5.9 Soil pH responses to annual lime and fertiliser applications at Mt Grand Station.

Beyond the second year of application, applying 200 kg/ha/yr of 'sulphur super 30' fertiliser without lime resulted in the acidification ($P<0.001$) of the soil compared with the control. Applying 3 t lime/ha annually increased the soil pH ($P<0.001$) by 0.7 pH units over three years, and applying 1 t lime/ha over three years increased pH by 0.4 units ($P<0.001$) over that of the control. When applied with fertiliser, the pH in the limed plots was not increased as much as when lime was applied alone ($P<0.001$) and this effect became greater as the experiment went on ($P=0.010$).

As expected soil Al concentrations responded inversely to the applied lime and fertiliser treatments as the pH did (Figure 5.10). The application of the SS30 alone increased Exch Al compared to the control plots in the second and third year of the experiment ($P<0.001$). The application of lime reduced soil Al at the site ($P<0.001$). Despite the initial Al concentration being below the 3 mg/kg toxicity threshold, after three years of the experiment both lime treatments had reduced Al concentration to <0.5 mg/kg. In this instance applying 3 t lime/ha as split annual 1 t lime/ha application was more effective at reducing soil Al at the site than applying a single application of 3 t lime/ha. As with the soil acidity, when the SS30 fertiliser was applied with the lime the soil Al was not reduced as much was when the lime was applied by itself ($P<0.014$).

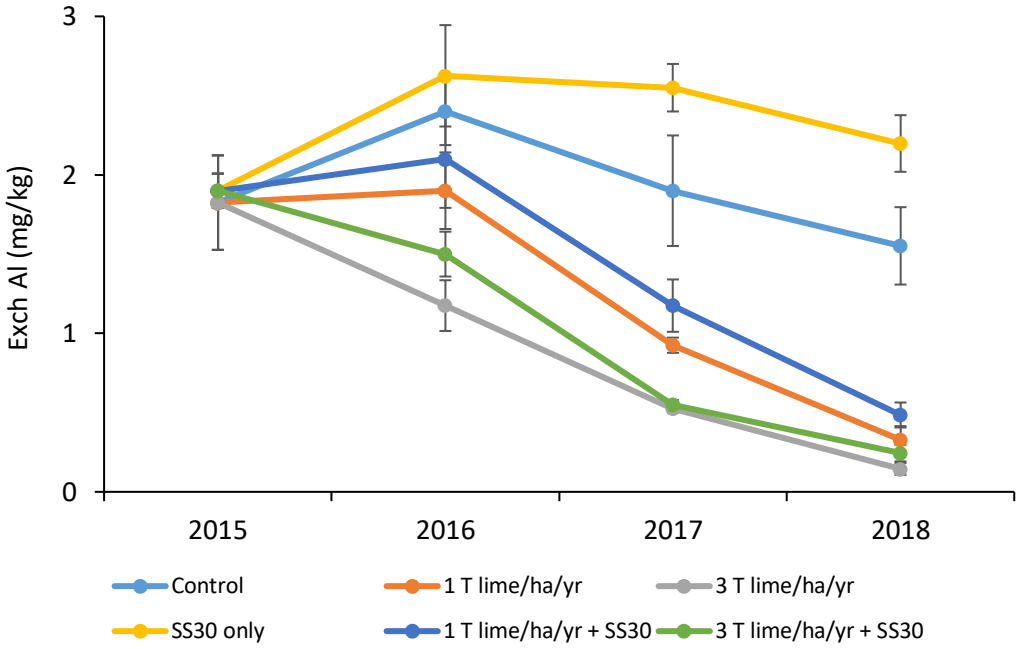


Figure 5.10 Soil exchangeable aluminium responses to annual lime and fertiliser applications at Mt Grand Station.

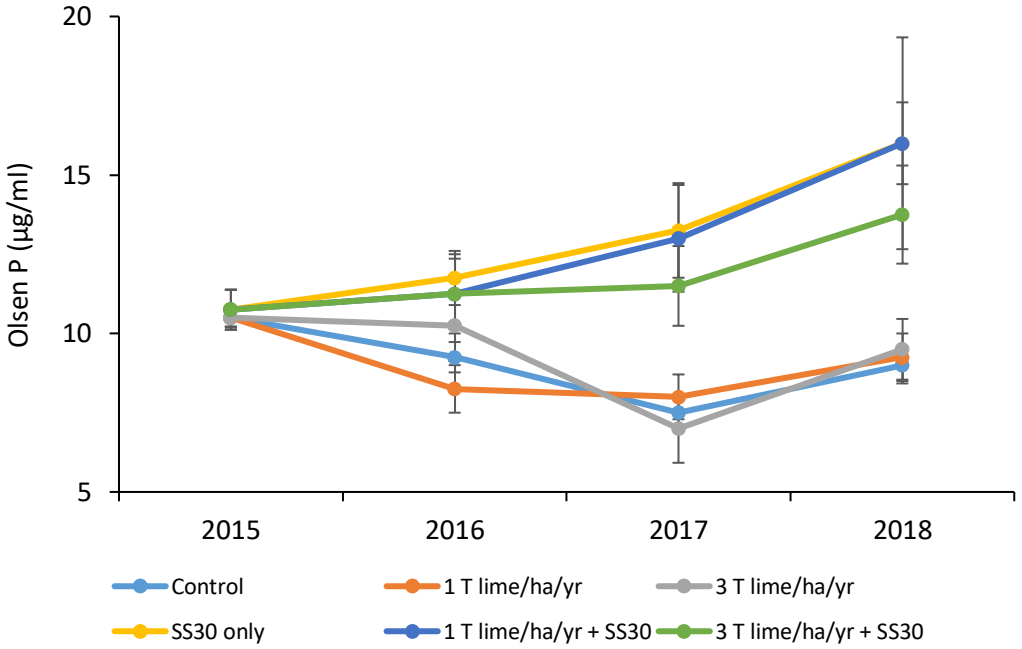


Figure 5.11 Soil Olsen P responses to annual lime and fertiliser applications at Mt Grand Station.

The application of lime alone had no effect on soil Olsen P at Mt Grand (P=0.775, Figure 5.11). The annual application of the SS30 fertiliser, irrespective of lime application, increased soil Olsen P (P<0.001) in each year of the experiment, and the rate of increase rose every year as the experiment went on (P<0.001).

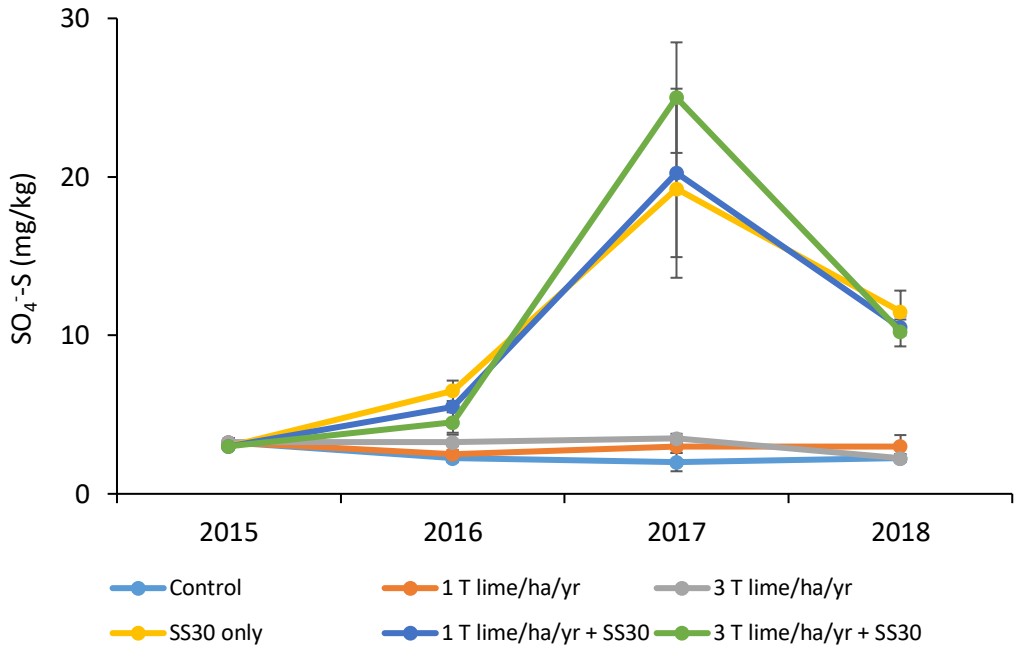


Figure 5.12 Soil sulphate sulphur responses to annual lime and fertiliser applications at Mt Grand Station.

The application of lime alone had no effect (P=0.815) on soil sulphate sulphur (SO_4^--S) concentrations at the site (Figure 5.12). Only the application of the fertiliser increased (P<0.001) SO_4^--S, and there were no effects (P=0.671) of different application rates of lime along with the fertiliser.

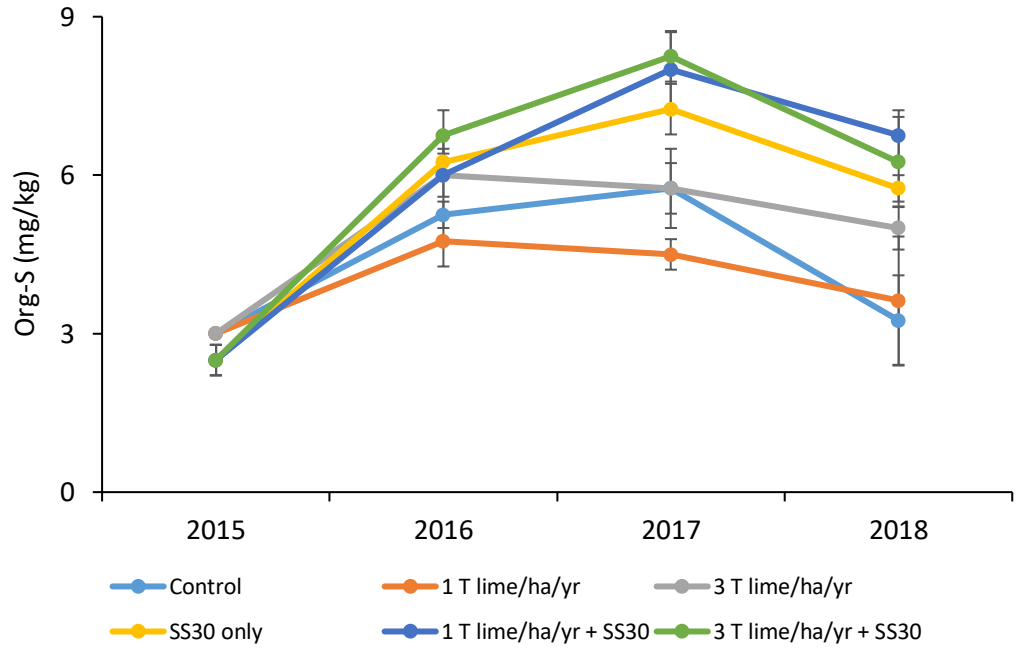


Figure 5.13 Soil organic sulphur responses to annual lime and fertiliser applications at Mt Grand Station.

The application of lime alone had no effect on soil organic sulphur (Org-S) at Mt Grand during the experiment (Figure 5.13). Only the application of the SS30 fertiliser, with or without lime, increased Org-S (P<0.001) to a 6.8±0.2 mg/kg from 2016 to 2018.

5.3.4 Treatment effects on soil phosphorus fractions in December 2016

Following two years of lime and fertiliser treatment applications, in December 2016, neither treatment had affected the total amount of organic P (P_O) in the top 7.5 cm of soil at the site. The total amount of inorganic P (P_i) was increased by the two applications of the SS30 fertiliser (P=0.001), and this lead to an overall increase in the total amount of P in the soil (P=0.018).

Of the total P pool at the experiment site on average only 8.1±0.4% was plant available (Figure 5.14). After two years of lime and fertiliser applications, neither treatment affected the total amount of plant available P at the site, with only the fertiliser treatment causing a small increase (P=0.058). However, within the components of the plant available P pool (NH_4Cl, $NaHCO_3$ P_i, and $NaHCO_3$ P_o), the two applications of fertiliser, up until December 2016, increased the amount of $NaHCO_3$ P_i in the soil (P=0.023) from 24.3±1.84 mg/kg to 40.0±8.27 mg/kg. The addition of lime had no effect on $NaHCO_3$ P_i, but reduced the amount of P_O in this $NaHCO_3$ extractable fraction (P=0.020). In the unlimed soil $NaHCO_3$ extractable P_O was 45.2±5.29 mg/kg, by applying 1 t lime/ha/yr it was reduced to 41.0±4.38 mg/kg, and by applying 3 t lime/ha/yr it was reduced to just 30.5±3.96 mg/kg at December 2016. The ammonium chloride (NH_4Cl) extractable P accounted for a small amount of P within the plant available P pool (<2 mg/kg), and was unaffected by lime or fertiliser application.

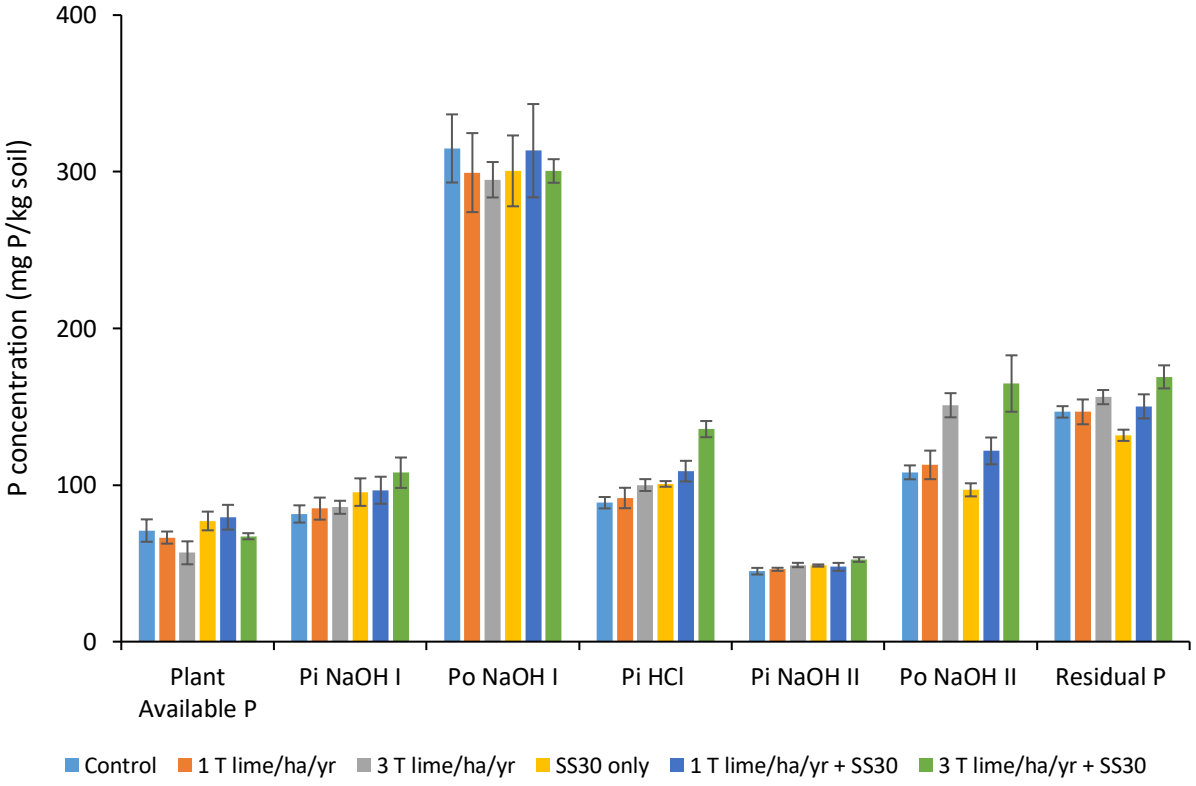


Figure 5.14 Concentrations of phosphorus contained within increasing insoluble P fractions at Mt Grand in December 2016.

Of the moderately labile P contained within the first sodium hydroxide extractable fraction (NaOH I) the amount of P_i was increased (P=0.004) by the application of the fertiliser treatment by 15.9±4.21 mg/kg, and was unaffected by lime. The P_O in this NaOH I fraction was by far the largest of all the P pools and not affected by the application of either fertiliser or lime. The P_i in the hydrochloric acid (HCl) extractable fraction is that which is bound to calcium. It was increased by the application of both lime (P=0.023) (added calcium for P to bind to), and fertiliser (P<0.001) (added P to bind to calcium), and lime and fertiliser combined (P=0.029). The more stable P_i in the second sodium hydroxide extractable fraction (NaOH II) was again only affected by the application of the fertiliser treatment (P=0.046), increasing it from 46.7±1.50 mg/kg (unfertilised) to 49.6±1.57 mg/kg (fertilised). In contrast, the P_o in this stable NaOH II fraction was not affected by the fertiliser treatment, but was increased by lime application (P=0.020), increasing from 102.5±4.31 mg/kg in the unlimed plots to 117.3±8.83 mg/kg (1 t lime/ha/yr) to 157.9±12.9 mg/kg (3 t lime/ha/yr). In the final fraction the application of either lime (P=0.106) or fertiliser (P=0.878) alone had no effect on residual P concentration. However, when applied together residual P concentration was increased (P=0.016), specifically in the 3 t lime/ha/yr + 'SuphurSuper30' fertiliser treatment.

5.3.5 Regression analyses between measured soil chemistry, plant yield and nutrient uptake parameters

The only parameter that soil pH significantly correlated to at the site was soil Al, which decreased exponentially as pH increased (Figure 5.15). The measured soil pH in each plot at the experimental site was not correlated to increases in total herbage and clover only yield in either the unsprayed or herbicide treated plots throughout the three year experiment. This included expressing yields as whole annual yields, and standardizing yield between years to account for variations in climate and annual rainfall between years. Despite a strong interaction between lime and fertiliser treatments on pasture clover content (%) in 2016/17, soil pH was also not correlated with clover content of the pasture (%) in any year of the experiment. Soil pH was also not correlated with the uptake of P and S, measured Olsen P, or soil S levels.

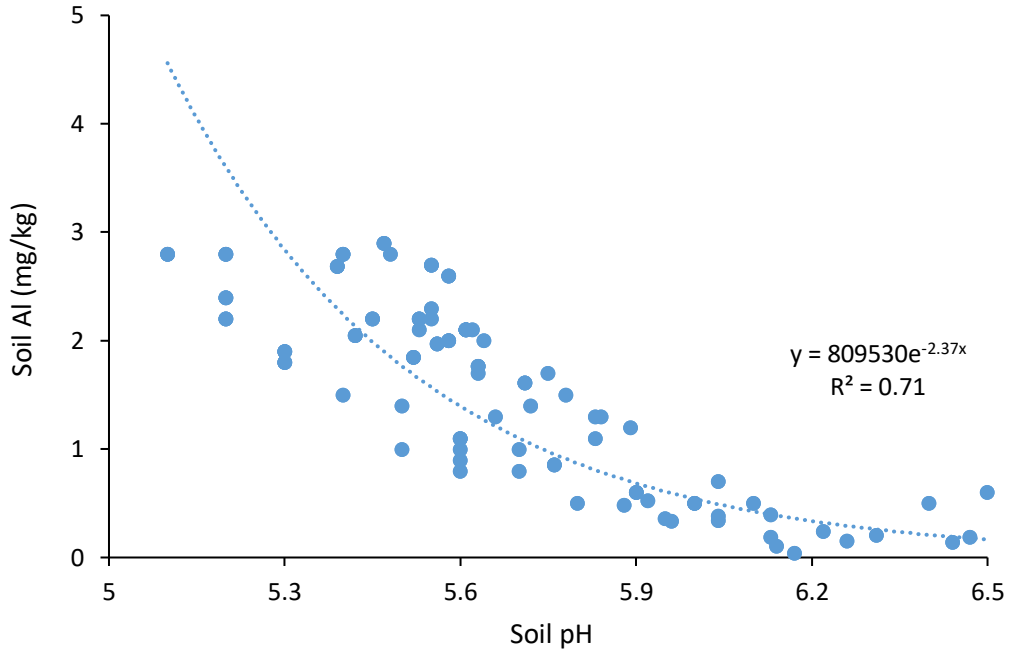


Figure 5.15 The relationship between soil pH and soil Al at Mt Grand Station.

As with soil pH, reducing Al by liming did not correlate to any effects on pasture yield, total clover content, or P and S uptake.

The rise in soil Olsen P with the annual application of the SS30 fertiliser caused an increase in pasture P uptake in both the unsprayed (P<0.001) and herbicide treated plots (P<0.001). However, the increases in Olsen P did not increase total pasture yield (P=0.144), clover only yield (P=0.138), or pasture clover content (P=0.977), irrespective of herbicide treatment. In contrast, sulphate sulphur (SO_4^--S) was associated with the total pasture yield (P=0.003), total clover yield (P<0.001), the clover content in the sward (P<0.001), and shoot S concentration (P<0.001, Figure 5.16), irrespective of the herbicide treatment.

P uptake was also positively correlated with pasture clover content (P<0.001) and clover yield (P<0.001) in the unsprayed plots, but not in the herbicide treated plots.

Despite Olsen P not being correlated to soil pH, the amount of plant available P, as measured by P fractionation at December 2016, was negatively correlated with soil pH (P=0.038). This was due to a reduction in $NaHCO_3$ extractable organic P (P=0.003). $NaHCO_3$ P_O was halved from 49.9 mg/kg to 25.0 mg/kg by the 0.6 unit increase in soil pH from pH 5.4 to 6.0. In contrast, the organic P in the NaOH-II extractable fraction increased with increasing soil pH (P=0.029), increasing by 87.7 mg/kg per 1.0 unit increase in soil pH. These were the only P fractions affected by changes in soil pH.

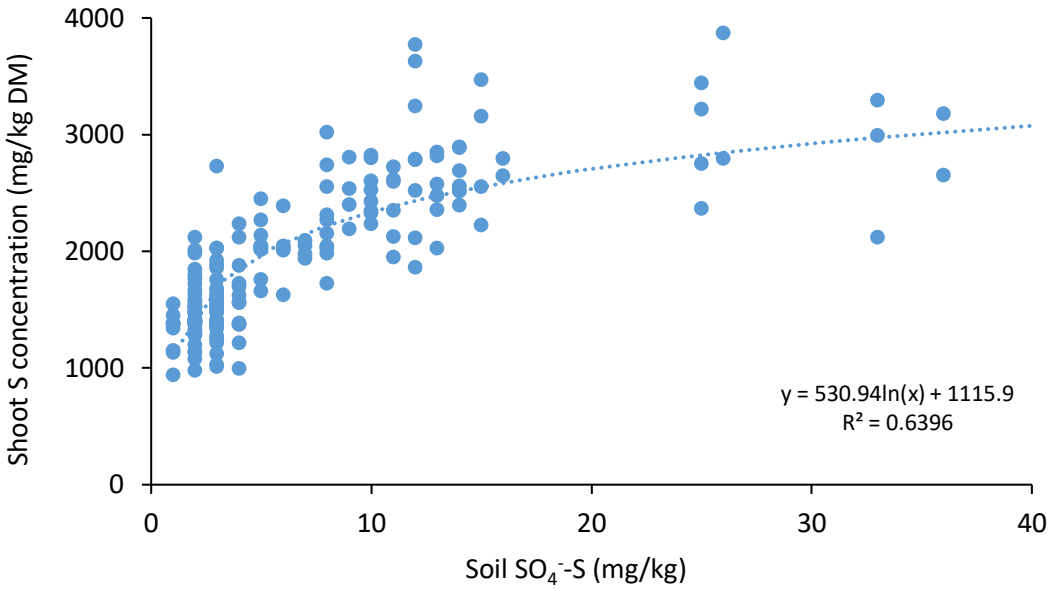


Figure 5.16 The relationship between SO_4^--S and S uptake by the pasture (mg/kg DM) at Mt Grand Station over three years.

5.4 Discussion

5.4.1 Treatment effects on soil fertility and pasture production

Sulphur was found to be the most limiting nutrient to clover and total pasture production, and pasture quality (as a measure of pasture clover content) at this site. Soil SO_4^--S was the only nutrient which correlated with increases in clover and total pasture yield, pasture clover content, and sulphur uptake. It was therefore the most important nutrient required to enable clover growth and consequently increase N fixation. The low initial soil Olsen P levels (10 µg/g) were expected to be a limitation to pasture production at the site (Roberts & White 2016). However, there were no relationships between increasing Olsen P and overall pasture or clover production. This was likely due to other nutrients being more limiting, especially S

Previous studies have focused on the importance of applying P over S to hill and high country (Percival et al. 1984; Edmeades et al. 1990; Gillingham et al. 1998; Lambert et al. 1998; Mackay & Lambert 2011), and conversely the impact on withholding P fertiliser (Gillingham et al. 1990; Lambert et al. 1990; Dodd & Ledgard 1999), as the main driver and hill and high country production. However, S deficiency has been found to be the greatest limitation to clover N fixation and pasture production in majority of the South Island hill and high country (Craighead et al. 1990; Scott 1998a; Craighead & Metherell 2006). This is consistent with the expected loss of S which is not well stored and is easily leached from soil, and therefore needs constant and frequent replenishing (Boswell 1994).

The results show there was considerably more plant available P stored in the soil than S. If we assume the field bulk density of the soil to be 1 g/cm^3 then there is approximately 50 kg/ha of plant available P and

only 20-30 kg/ha of plant available S in this soil. So when considering the amount of each that is taken up by the pasture in each year (approximately 12-15 kg P/ha and 10-12 kg S/ha), along with any S leaching, the soil S pool can be depleted much faster than the soil P pool. S will therefore require replenishment by fertiliser additions more often, preferably annually. Given the quantity of the P in the soil, but the requirement for S, there is potential for expensive P inputs to be reduced or temporarily suspended, as long as inputs of S continue. This could be coupled with the application of lime, such as by using a lime-sulphur fertiliser, and the budget that would have been used for the P component of the fertiliser used for lime instead.

The acidifying effects of the SS30 fertiliser are likely to be driven by the oxidation of the elemental sulphur component of the fertiliser (Bolan 1995; Manoharan 1997; Scott 1998a). It can be expected that by continuing to only apply superphosphate based fertilisers, especially those fortified with elemental sulphur, to hill and high country, without the addition of any lime, will continue to acidify soils. This, in turn, will detrimentally and exponentially drive up the availability of Al (Moir & Moot 2010, 2014).

At the site, 3 T lime/ha was required to increase the soil pH (0-7.5 cm) to the optimal pH range of 5.8-6.0 (Edmeades et al. 1984a; Edmeades et al. 1985; O'Connor et al. 2007). However, without the addition of fertiliser, the application of lime alone had no effect on clover growth and total pasture production. Given that soil Al in the top 7.5 cm at the site did not exceed the 3 mg/kg toxicity threshold for sensitive legumes (Moir et al. 2016a), it was not the most limiting factor to pasture and clover production at the site. Had soil Al been >3 mg/kg at the beginning of the experiment, greater responses to lime would have been expected. Despite the lack of response to lime application alone, in several of the 16 plots at the site there were obvious large, consistent responses to applied lime in each year of the experiment. This highlights the high degree of land slope and soil variability across the landscape, particularly top soil depth, which is important to consider when extrapolating the main results over a broad scale. Thus, broad treatment effects are discussed in relation to hill and high country farms with large scale subdivision. Identifying such areas that will respond to liming and the use of variable rate spreading technology has the potential to greatly increase the value in applying both lime and fertiliser to hill and high country (Gillingham et al. 2003; Murray & Yule 2007; Roberts & White 2016; White et al. 2017).

When applied in addition to lime, the clover growth response to applied fertiliser was much greater, but again not in all plots across the experimental site. Trends in lime application rates and associated responses to applied fertiliser were inconsistent across the different pasture components and herbicide treatments. These effects, especially for the NAC yield in the wetter spring of 2016, and the sown clover yield in the wetter autumn of 2018, were not directly correlated to lime effects of reducing soil acidity or Al. Instead they are most likely to be secondary effects of increasing soil pH closer to or within the optimum soil pH range, such as improved micronutrient availability, increased microbial activity,

increased organic matter mineralisation, and increased N cycling (Edmeades et al. 1986; De Vries & Breeuwsma 1987; Edmeades et al. 1990; Wheeler & O'Connor 1998). Given Al was unlikely to be a limiting factor at the site, these secondary effects can be proposed as the main drivers of the observed responses to increased soil pH.

Applying lime did not result in an increase in soil Olsen P, total plant available P (as measured by P fractionation), or pasture P uptake. This means there was no mineralisation and release of P from non-plant available P fractions, or in other terms, no 'P sparing effect' occurred. In fact, the application of lime resulted in the accumulation of P in two plant unavailable pools; stable organic P and residual P. This accumulation in the residual P pool, especially in fertilised plots, could be due to the formation of strong Al-P hydroxy bonds driven by the liming effects on the transformation of Al speciation (Haynes & Ludecke 1981b; Haynes 1982; Carran 1992), which then represents a loss of applied fertiliser P. Liming greatly reduced the amount of plant available organic P (P_O $NaHCO_3$). This could be due to an increase in organic matter cycling, which reduced the amount of readily mineralisable organic matter in the soil. Alternatively, it could be due to the fixation of this organic P into the highly stable organic P fraction (P_O NaOH-II). In fertilised plots the application of lime also caused an increase in calcium bound P (P_i HCl).

The haloxyfop-P herbicide treatment that was applied in September 2016 did not increase pasture yield overall, but greatly supressed grass yield in 2016/17. When this was coupled with the fertiliser treatment it lead to unrestricted NAC and sown clover growth (Plate 5.5). The residual effect of this herbicide treatment carried over into the 2017/18 year, contributing to the increase in sown clover yield in limed and fertilised plots, despite a later recovery in grass yield and increased weed yield. The growth of both the NAC's and sown clovers in each year was very season dependant (Maxwell et al. 2010; Nori et al. 2015). It was fortuitous that such a strong growing season for each application of the herbicide treatment. Had the herbicide been applied in a drought year it is possible total pasture yield would have been drastically reduced. Providing it is applied in a good growing season, the use of this grass herbicide treatment could be a useful tool for establishing clover populations and producing large quantities of high quality clover feed (Rolston et al. 1985; Hepp et al. 2003; Craw & Craw 2016). In addition, the reprieve from grass competition may also allow the annual clover seedbank in the soil to be increased if clovers are given a chance to set seed. Casey *et al*. (2000) also found using low rates of glyphosate to have similar benefits for increasing pasture legume content and quality. The application of the haloxyfop-P herbicide allowed the weed yield at the site to also increase. Therefore, it will be important not to over apply this herbicide in order to allow grasses to eventually recover without the sprayed area becoming weed infested. Annual clovers in hill and high country environments require careful grazing management to ensure that the seed bank is sustainably maintained (Maxwell et al. 2016). Use of this herbicide treatment could be an effective tool to help develop annual clover populations and the size of the seedbank.

However, if the initial population is low it could lead to the development of considerable open space within the pasture suitable for weed infestation. It was fortunate that the initial NAC population was already high at the experimental site at the beginning of the experiment, with NAC yield making up 14.0% of total annual yield in the untreated control plots in 2015/16.

Plate 5.5 Annual clovers growing in a 3 t lime/ha/yr subplot that was treated herbicide at Mt Grand Station, 28th November 2016. The large white flowers are 'Bolta' balansa clover, the small yellow flowers are suckling clover, and the pink-brown flowers are mostly striated clover and some cluster clover.

The growth of the NAC's was dependant on spring rainfall and they did especially well in the wet spring of 2016. The advantage of NAC's in these dryland environments is that they will quickly become reproductive and produce seed in a dry year, all they can provide high quality stock feed and keep growing into a wet spring (Maxwell et al. 2010). Of the NAC's at the site, striated clover was the most abundant and greatest yielding followed by cluster>suckling>haresfoot. In an earlier study on north facing slopes at Mt Grand, Power *et al.* (2006) also found striated clover to be the most prominent NAC species at 750 m asl. However, at lower altitudes of 420 m asl and 620 m asl cluster clover was most prominent. While on a south facing slope at 630 m asl there were virtually no NAC's and instead perennial white clover was most prominent. During this experiment there was very little haresfoot at the site (<100 kg DM/ha/yr). This was beneficial to overall pasture quality, given that haresfoot is less desirable and less palatable than

the other NAC's (Boswell et al. 2003b). There is considerable potential for developing hill and high country with these NAC's where current populations are low or non-existent, but seed for these species is not currently commercially available in New Zealand (Monk et al. 2016).

Of the sown clovers, 'Bolta' balansa was the most prominent in the second year of the experiment and the most consistent yielding of the three sown clovers during the experiment. Of all the clovers at the site it was also the most responsive to the application of lime and fertiliser. Being a top-flowering annual the 'Bolta' balansa successfully flowered and produced seed at the experimental site in each year of the experiment, highlighting its qualities for use in hill and high country environments (Monks et al. 2010; Nori et al. 2015; Monk et al. 2016). Of the two sown sub clovers at the site, only the 'Denmark' grew well, but it took until the last autumn of the experiment for it to yield >1000 kg/ha. This highlights the sensitivity of sub clover to favourable conditions for germination. This growth could have resulted from the spray treatment reducing grass competition allowing the 'Denmark' to become better established, given the response of the sown clovers to lime and fertiliser in the sprayed plots. Despite a large capital outlay in applying sub clover seed it may take considerable time to generate returns from it. A study at Omarama Station by Olykan *et al*. (2018) measured 'Denmark' and 'Woogenellup' sub clovers to yield equally there, however at Mt Grand the 'Woogenellup' sub clover yielded very little throughout the experiment (<350 kg DM/ha/yr), and therefore it would not be recommended to apply in a seed mix. This shows the importance of choosing cultivars which best match the given environments on each farm. Overall, the greater balansa clover establishment, over that of the two sub clover cultivars, is most likely related to seed size and the degree of hardseededness of each, and will have been aided by the salt application to the plots (Gillespie et al. 2006).

5.4.2 Treatment effects on pasture quality

Pasture quality in hill and high country is driven by sward legume content (Power et al. 2006; Moot 2012), and increasing legume content was an objective of this study. Thus, the single application of herbicide in 2016 had the single largest effect on clover content and yield, and hence pasture quality. Applying lime alone had no effect on pasture clover content but applying the fertiliser alone did, mainly in response to added S which supports it being the most limiting nutrient at the site. When applied together the lime and fertiliser further increased pasture clover content, and hence pasture quality. This was likely due to an increase in availability of soil micronutrients, such as Mo, that may have been limiting to clover production, along with overcoming the S limitation.

The nutritive value of NAC's have not been measured (Maxwell 2013), and were not measured as part of this experiment. However, by assuming nutritive values of the individual pasture components then theoretically the effects on the applied treatments on pasture quality can be calculated. For this purpose

the metabolisable energy (ME) content, in mega joules (MJ), of the clovers growing at the site is assumed to be approximately 12 MJ ME/kg DM and the clovers are 90% utilisable, for the grass; 8 MJ ME/kg DM and 80% utilisable, and for the weeds and dead material 7 MJ ME/kg DM and only 40% utilisable (Trafford & Trafford 2011). To sustain one relative stock unit (1 RSU) for a year 6000 MJ ME is required (Trafford & Trafford 2011). To determine the average carrying capacity (RSU/ha) for each treatment during the three year course of the experiment the average yield for each categorised pasture component (sown clover, NAC, grass, weeds, dead material) over the three years (2016/17 and 2017/18 only for sprayed plots) was multiplied by the assumed nutritive value and grazing utilisation, and divided by the annual energy requirement by 1 RSU, according to equation 5.1;

Equation 5.1

$$\begin{aligned}\text{Carrying capicity (RSU/ha)} &= ((\text{Sown clover (kg/ha)} + \text{NAC (kg/ha)x12 MJME/kgDMx 90 \%}) \\ &+ (\text{Grass (kg/ha) x 8 MJ ME/kgDM x 80 \%}) \\ &+ \frac{(\text{Weed (kg/ha)} + \text{Dead (kg/ha) x 7 MJ ME/kgDM x 40 \%}))}{6000 \text{ MJ ME/RSU}}\end{aligned}$$

From this equation the average carrying capacity for each treatment over the three years of the experiment was calculated (Table 5.3).

Table 5.3 The estimated effects of applied treatments on carrying capacity (RSU/ha) averaged over the three years of the experiment at Mt Grand Station.

Herbicide	Control	1 t lime/ha	3 t lime/ha	200 kg/ha SS30	1 t lime/ha + 200 kg/ha SS30	3 t lime/ha + 200 kg/ha SS30
Untreated	6.65±1.83	6.16±2.08	5.78±1.11	7.09±2.05	7.24±2.10	6.07±1.42
Sprayed	5.03±1.42	5.03±2.20	6.61±1.71	7.39±1.80	6.86±2.43	11.8±2.08

From these calculations it can be seen that by applying lime alone with its corresponding effects on pasture yield and composition, that the theoretical carrying capacity of the plots treated with lime only was reduced compared with the control plots. The control plots also benefited from the additional 'Bolta' balansa and sub clover seed. The application of the herbicide treatment by itself also reduced the carrying capacity by 1.52 RSU/ha. The application of the fertiliser treatment, with or without lime, generally increased the carrying capacity of the plots. The much higher carrying capacity in the sprayed 3 T lime/ha + 200 kg/ha SS30 plots can be attributed to the large yields of the NAC's in the spring of 2016/17 and sown clovers in the autumn of 2017/18.

5.4.3 Economic implications of treatment applications

Having discussed the effects of each of the treatments and treatment combinations on pasture growth, composition and quality, it is important to consider the application cost and potential economic return from each in order to select the most appropriate treatment combination to develop a profitable farm system. To increase soil pH to the biological optimum of 5.8-6.0 at the experimental site 3 t lime/ha was required, whether applied in one application or split applications. The largest cost of applying lime is the application cost using a fixed wing aircraft. In reality, the economic optimum pH for this site will be lower than the biological optimum, especially given that Al toxicity is not an issue. Edmeades et al. (2016) concludes that the economic optimum pH for most hill and high country farms is pH 5.5-5.6, and that liming these soils is only economic when the initial pH is <5.5 (Edmeades et al. 1985). Despite large initial costs of lime application to South Island hill and high country the response to the applied lime will be much longer lasting compared with the fertiliser response, where S may be quickly leached. Having identified S as the most limiting nutrient to pasture production at the site, it is therefore most important to maintain regular applications of S to maintain pasture production and clover percentage. Overall climate is a big factor in both the immediate and long term response to applied fertiliser and lime.

If 3 t lime/ha was to be applied to the 70 ha Broadspur block at Mt Grand it could either be applied as one single large application, or split into smaller applications such as 1 t lime/ha for three years. The advantage of the split application is that application costs can be spread out over numerous years to spread the cost out. The disadvantage is additional transport and application costs.

If we compare the cost of blanket applying each of the treatment combinations to the 70 ha Broadspur block, given that each plot had 'Bolta' balansa and sub clover applied, to the expected return from the predicted carrying capacity, then the economic viability of each treatment combination can be calculated (Table 5.4). As of January 2019 the current price of Ravensdown® sulphur super 30 (SS30) fertiliser was $372/T excluding GST (Ravensdown 2019). The nearest Ravensdown® store to Mt Grand Station is in Cromwell where the price of lime is $60.50/T, storage and handling fees are $35/T, and product mixing is $8/T (Ravensdown 2019). Assuming transport costs to the farm are $18/T for the 35 km distance, and application costs are $75/T (Askin & Askin 2012), then the cost of applying each can be calculated on an annual scale (2019 price). For the herbicide treatment it is assumed the cost of the chemical would be $156.70/ha to apply at 500 ml/ha, and aerial application with a helicopter of $70/ha (Askin & Askin 2012). 'Bolta' balansa seed is $10.20/kg and 'Denmark' sub clover is $9.35/kg (Askin & Askin 2012). Given that the 'Woogenellup' sub clover did not perform very well at the site it has not been considered in these costings. All prices are GST exclusive which adds an additional 15 % to the total cost.

Table 5.4 Cost to apply each treatment combination used in this experiment over the whole 70 ha Broadspur block at Mt Grand Station.

Herbicide	Control	1 t lime/ha	3 t lime/ha	200 kg/ha SS30	1 t lime/ha + 200 kg/ha SS30	3 t lime/ha + 200 kg/ha SS30
Untreated	$19650	$34660	$64690	$27700	$43480	$74800
Sprayed	$37900	$52910	$82940	$45950	$61730	$93050

The capital cost of applying the sown clover seed over the 70 ha at the rates applied to the plots is considerable ($19650), and in reality the seed is unlikely to be applied at such high rates (Table 5.5).

Table 5.5 Cost to apply each treatment combination to the 70 ha Broadspur block at Mt Grand Station, excluding the capital cost of sown clover seed.

Herbicide	Control	1 t lime/ha	3 t lime/ha	200 kg/ha SS30	1 t lime/ha + 200 kg/ha SS30	3 t lime/ha + 200 kg/ha SS30
Untreated	0	$15010	$45040	$8050	$23830	$55150
Sprayed	$18250	$33260	$63290	$26300	$42080	$73400

To determine the annual return from applying each treatment combination the average annual carry capacities (Table 5.3), have been factored up to the total carrying capacity of the 70 ha Broadspur block, assuming complete consistency across the whole block. Assuming the Broadspur block is predominantly used for sheep grazing then according to a farm statistical survey performed by Alexanders® (2017) the average sheep RSU on a South Island high country farm currently returns $99.82/yr.

Table 5.6 Annual return from each treatment combination to the 70 ha Broadspur block at Mt Grand.

Herbicide	Control	1 t lime/ha	3 t lime/ha	200 kg/ha SS30	1 t lime/ha + 200 kg/ha SS30	3 t lime/ha + 200 kg/ha SS30
Untreated	$46450	$43010	$40400	$49570	$50600	$42420
Sprayed	$35170	$35180	$46190	$51630	$47910	$82320

Given that 3 t lime/ha was required at the experimental site to increase soil pH to the biological optimum of 5.8-6.0 it can inferred that the large application cost of applying 3 t lime/ha in a single application will

only have to occur once, whereas three applications of the 1 t lime/ha application would be required. Table 5.7, below, shows the financial position of the returns from the Broadspur block over three years assuming constant pasture growth, stocking rate and pasture utilisation. This financial position was calculated by adding the generated returns to offset the initial and ongoing costs of development and treatment combinations. It is assumed that after one application of 3 t lime/ha no more lime would be applied and instead only the fertiliser would be applied for that particular treatment combination. Also, the herbicide treatment would only be applied once at the beginning of each scenario, so the application costs are only accrued in the first year. The effects of the herbicide treatment are likely to wear off over time but have been left constant over the three years for the purpose of these calculations.

Table 5.7 Annual financial position following the application of up to 3 t lime/ha, and annual applications of 200 kg/ha where applicable, to the 70 ha Broadspur block at Mt Grand.

Year	Herbicide	Control	1 t lime/ha	3 t lime/ha	200 kg/ha SS30	1 t lime/ha + 200 kg/ha SS30	3 t lime/ha + 200 kg/ha SS30
1	Untreated	$26800	$8350	-$24290	$21870	$7120	-$32380
	Sprayed	-$2730	-$17730	-$36750	$5680	-$13820	-$10730
2	Untreated	$73250	$36350	$16110	$63390	$33890	$34370
	Sprayed	$32440	$2440	$9440	$49260	$10290	$63540
3	Untreated	$119700	$64350	$56510	$104910	$60660	$36360
	Sprayed	$67610	$22610	$55630	$92840	$34340	$137810

Table 5.4 shows that lime is expensive to apply to hill country by fixed-wing aircraft, and Table 5.7 shows that, in general, it is not economic to blanket apply lime to broad areas, especially in large single applications. This is especially so when lime is applied without the application of the SS30 fertiliser. It is obvious that the economic optimal soil pH for the block is much lower than that of the biological optimum. Also, despite the positive effects of the herbicide application on clover growth and pasture quality, with the given expense of broad-scale application of the herbicide in the Broadspur block, its broad-scale use is not economic.

Overall, apart from the control, the most profitable scenario is the application of fertiliser alone, which reflects the requirement for added S at the site. Despite the liming not being as profitable as applying fertiliser alone, it is still recommended that at least some lime, say 1 t lime/ha, is applied to the block given that soil pH is low and will be further reduced by fertiliser application, and Al concentrations are approaching 3 mg/kg. In the combined 3 t lime/ha with SS30 fertiliser and herbicide applied treatment combination a large profit was shown in year 3. However, the combined effects of this treatment on

pasture production over the whole block is unlikely to be this consistent as it was in the experimental plots, and it is expected the effects of the herbicide treatment will have begun to wear off by this time.

5.4.4 Future Considerations

These results highlight the requirement for use and further development of variable rate application technology to improve fertiliser and lime use efficiency. This may be able to reduce application costs and increasing returns, based on identifying and applying each where it will be most efficiently utilised (Gillingham et al. 1999; Gillingham et al. 2003; Murray & Yule 2007; Roberts & White 2016; White et al. 2017). The use of variable rate application technology would make the application of lime, fertiliser, and herbicide to the Broadspur block at Mt Grand more economically sustainable.

Given that Al is not currently a limiting factor to pasture production at the site, although it is approaching 3 mg/kg, it may be possible to boost clover production by applying the SS30 fertiliser along with added micronutrients, such as Mo and B, to get a similar benefit to applying lime in relation to micronutrient availability. However, these applied micronutrients are likely to rapidly become unavailable over time due to the acidity of the soil, but are usually cheap to add to the fertiliser (Ravensdown 2019). The other potential benefits of the lime application, such as increased organic matter mineralisation and N cycling, would not be realised by doing this however.

Given that pasture production and clover growth was found to be most responsive to S at the experimental site, and likely in large areas of the South Island hill and high country, then the most profitable fertiliser to apply will be that which had the most S in it. So, depending on P levels and the degree of P limitation, there is potential for use of other S fertilisers, such as a lime-sulphur product, to get the best return for the amount of S applied. In superphosphate-based fertiliser, the highest level of S fortification available is 50% (Ravensdown 2019). Pelletized elemental S could also be used, especially in tight financial years given that within superphosphate P is the more expensive nutrient (Edmeades et al. 2016)

5.5 Conclusions

Sulphur was found to be more limiting to pasture production and quality than P at the experimental site. This is likely to be a widespread situation in the high and hill country of the South Island. The yield of NAC's and sown clovers was highly dependent on seasonal rainfall and applied treatments. Different plots within the experiment responded differently to the applied treatments, with some being more responsive to applied lime and others to the fertiliser. To raise soil pH at the site (initially pH 5.5) to the biological optimal range of 5.8-6.0, 3 t lime/ha was required. Applying lime alone had no effect on pasture growth or quality, given that S was the most limiting nutrient at the site. However, when applied with fertiliser, lime

increased clover growth and overall pasture production. However, the high application cost of applying lime is economically restrictive. Initial soil Al at the site was below the 3 mg/kg toxicity threshold, but had it been higher a greater response to liming would have been expected. The lime response that was observed is mostly likely due to increased micronutrient availability and organic matter mineralisation. The application of lime did not increase plant available P in the soil or plant P uptake, indicating no P-sparing effect occurred at the site. Applying the herbicide haloxyfop-P greatly increased clover growth and therefore pasture quality, and it could be a useful tool for increasing clover density. However, the cost of applying the herbicide is restrictive to its use. The use of variable rate application technology would greatly increase the economic viability of broad-scale application of lime, fertiliser and herbicide in the hill and high country.

Chapter 6

A study on the deep application of lime at three hill and high country farms

6.1 Introduction

Toxic levels of exchangeable aluminium (Al) occur extensively in the acid soils of the South Island hill and high country (Haynes & Ludecke 1981b; Edmeades et al. 1983; Moir & Moot 2010, 2014; Whitley et al. 2016). Al is particularly toxic to legumes which are relied upon in the hill and high country for nitrogen fixation and as high quality feed (Scott 2003). Al toxicity impedes root growth, damages root caps and root hairs, inhibits nodulation and reduces the ability of plants to extract water and nutrients from soil (Foy et al. 1978). It is prevalent in South Island hill and high country soils, especially in subsoil layers (Whitley et al. 2016), which limits access for deep rooting legumes to water deeper in the soil profile.

Al is considered to become toxic to legumes at 3 mg/kg soil (Edmeades et al. 1983). However, the susceptibility of different legumes to Al toxicity differs considerably (Wheeler et al. 1992; Moir et al. 2016b). Lucerne is a high desirable legume for use in South Island hill and high country as it is deep rooting and drought tolerant, and produces high quality feed (Brown et al. 2003; Anderson et al. 2014). However, it is highly susceptible to Al at concentrations of >3 mg/kg which restricts its use in these environments (Moot & Pollock 2014; Moir et al. 2016b). Research has identified other legumes, such as Russell lupins and *Lotus pedunculatus*, which have proved to be more tolerant of low pH and Al and have good potential for use in the South Island hill and high country (Haynes & Ludecke 1981a; Lowther et al. 1987; Scott 1989a; Edmeades et al. 1991b; Moot & Pollock 2014; Moir et al. 2016b). However, these species are less suited to grazing and are therefore less commonly sown.

Liming is required to reduce soil acidity and Al concentrations before Al sensitive crops, like lucerne, can be successfully grown. However, passive movement of lime down the soil profile is slow, especially in dryland environments (Farina & Channon 1988; Kirchhof et al. 1995; Moir & Moot 2010, 2014). Conventionally cultivating lime into soil is undesirable in hill and high country due to the high risk of soil erosion, the stony soil, and the expense considering the low productivity of the environment. Liming is also ineffective at reducing soil acidity below the depth that it is cultivated in to soil, usually no more than 15 cm deep (Sumner et al. 1986; Farina & Channon 1988; Coventry 1991).

Thus, one possibility to reduce the impact of Al toxicity on lucerne growth is to modify the soil below the 15 cm plough depth. To do this we developed a machine to directly inject lime into acid subsoil with minimal cultivation in order to improve the growth and persistence of deep rooting legumes. This machine

is the first of its kind in New Zealand but other examples have been built overseas (Wojta et al. 1955; Anderson & Hendrick 1983; Farina & Channon 1988; Coventry 1991; Nelson et al. 2012; Gazey & Davies 2017 unpublished). The machine pneumatically feeds lime from a hopper to two delivery ports on each of the eight height and angle adjustable, 30 cm spaced Kverneland® ripper tines. Optimise® pelleted lime was used in this experiment due to its consistency of particle size for steady calibration of the machine (CP Lime Solutions Ltd 2015). Al sensitive lucerne was sown as a bio-indicator of the effectiveness of the machine to reduce Al toxicity in soil. Al tolerant Russell lupins were sown to investigate their response to deep liming.

The aim of this research was to improve the growth and survival of legumes in acid high country soils with high concentrations of Al and low P fertility, through using the Flexiseeder® lime ripper to directly inject lime into the soil profile. The growth of the deep rooting legumes lucerne and Russell lupin was measured at four treated sites across three hill and high country farms; Omarama Station, Glenmore Station and The Dasher (two sites), over three growing seasons. French serradella (*Ornithopus sativa*) and *Lotus pedunculatus* were also grown at The Dasher experimental sites to investigate their agronomic potential there. Detailed sampling of the effects of the applied lime on soil pH and Al was carried out at the Omarama Station site.

The specific objective for the experiment was to determine the effect of deep lime placement on soil pH and aluminium, P availability and plant growth. The null hypothesis is that the chemical properties of the soil, and the growth of lucerne and Russell lupin plants will not be affected by the direct injection of lime deep into the soil profile.

6.2 Methodology

6.2.1 Site Descriptions

Omarama Station

Omarama Station is a 12,000 ha high country station located directly south of the Omarama township which winters 23,000 stock units. It ranges in altitude from 450 m asl, where the farm has extensive fluvial flats adjoining the Ahuriri River, to 1558 m asl at the summit of Mt St Cuthbert. The experimental site was located at the western end of the farm on the fluvial flats at 44˚30’23.60”S 169˚54’16.04”E and at 475 m asl. Prior to the experiment the site and surrounding flats were dominated by sheep sorrel (*Rumex acetosella*), heracium (*Pilosella officinarum*), vipers blugoss (*Echium vulgare*) and haresfoot clover (*Trifolium arvense*). In the year prior to beginning the experiment the site had been direct drilled with a Russell lupin-cocksfoot mix which had failed to establish. The Mackenzie Pallic Orthic Brown soil at the site (S-Map 2015a) varies in depth to the river gravels below, ranging from as deep as 40 cm to being

stony at the soil surface. The site has a known issue of toxic concentrations of Al in the subsoil that has prevented lucerne from being grown there.

Work to establish the experiment began in May 2015. Rainfall (figures 6.1, 6.2), air temperature and soil temperature at 10 cm depth (Figure 6.3) were measured and recorded using a Onset® HOBO rain gauge and temperature sensors (Onset Computer Corporation 2015), beginning in late October 2015 and these data are presented on a 1st July to 30th June production year basis. Weather data for 1st July to 31st October 2015 has been substituted for data collected at the nearby (<2 km's) NIWA Tara Hills automatic weather station (AWS) (NIWA 2018).

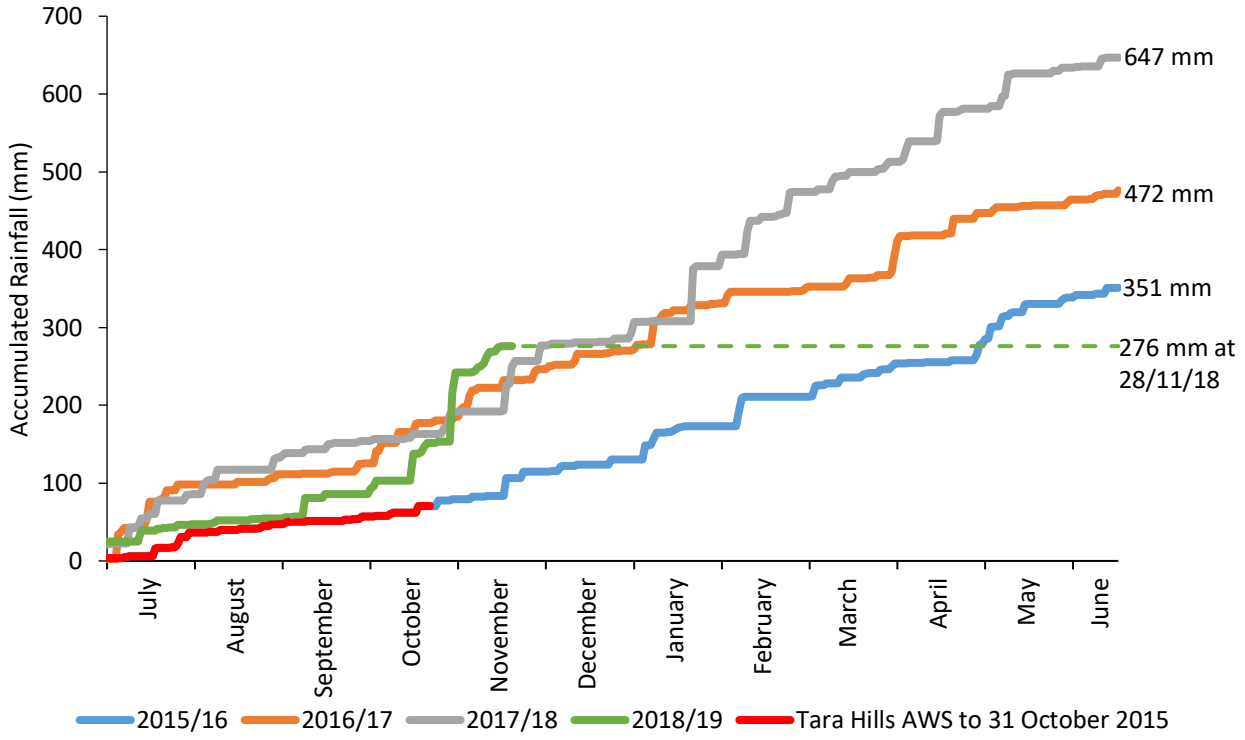


Figure 6.1 Accumulated annual rainfall at Omarama Station from 1st of July 2015 to the time of the final harvest on the 28th of November 2019.

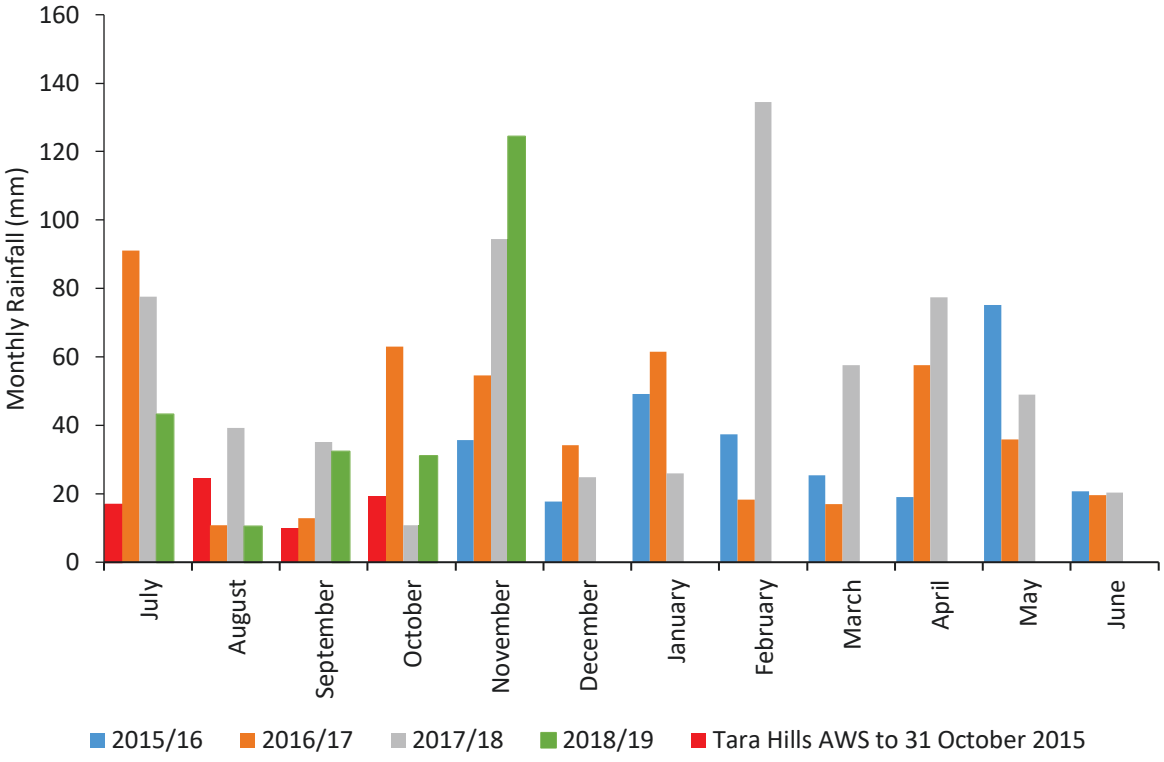


Figure 6.2 Monthly rainfall at Omarama Station from the 1st of July 2015 to the time of the final harvest on the 28th of November 2019.

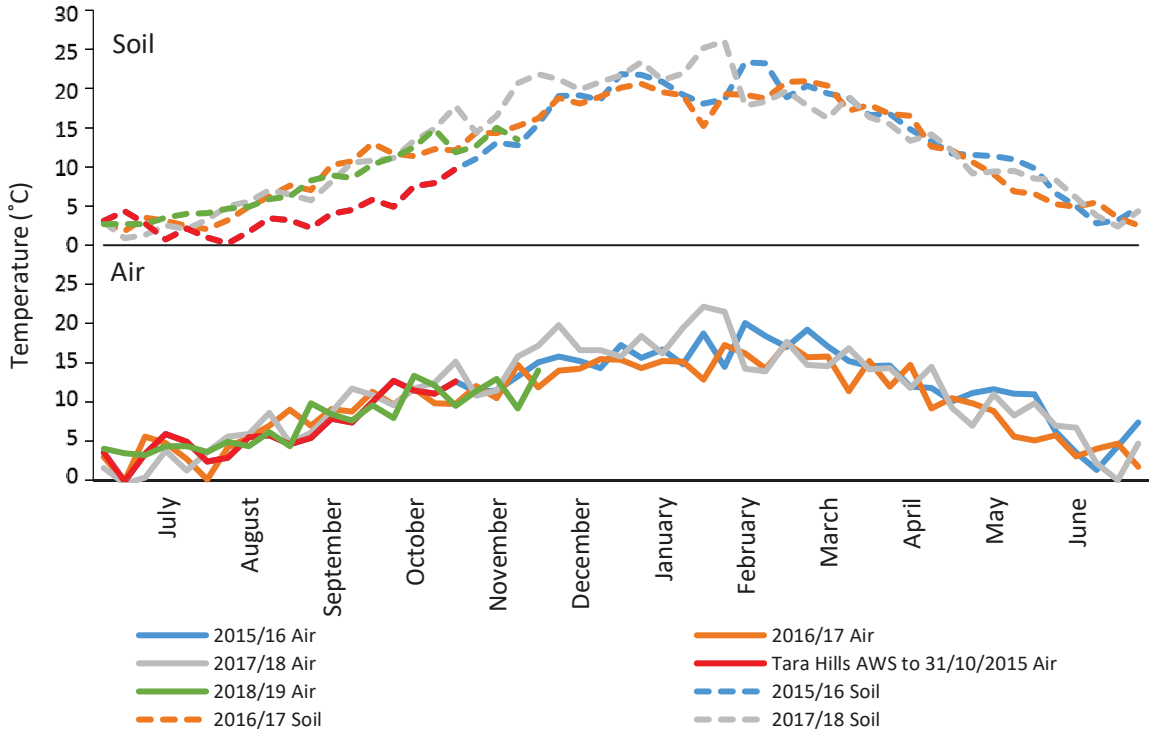


Figure 6.3 Weekly mean air and soil temperature at 10 cm depth at Omarama Station from the 1st of July 2015 to the time of the final harvest on the 28th of November 2019.

Frosts occurred at the experimental site from mid-April through to late-October of each year of the experiment. The maximum air temperature recorded at the site was 34.4˚C on the 29th of January 2018.

During the establishment of the experimental site, and before the weather station was installed the lowest temperature recorded at the nearby Tara Hills weather station was -21.0˚C on the 24th of June 2015. This region therefore experiences a more continental climate of temperature extremes than most of the more temperate, maritime climate of coastal New Zealand.

Glenmore Station

Glenmore Station is a 19,200 ha high country station located on the western side of Lake Tekapo at the northern edge of the Mackenzie Basin. It currently runs 10,000 merino sheep, 460 red deer and 400 Angus cattle. Glenmore Station rises from 710 m asl on the shore of Lake Tekapo to 2500 m asl in the Gammack Range. Work began at the experimental site in March 2015 which was located at 43˚54'47.58"S 170˚28'49.97"E and at 741 m asl. The soil at the site is a Tekapo Orthic Brown soil (S-Map 2015b) and comprises of mixed depth loess on top of glacial till. There is a vast area of cultivatable land at Glenmore Station that has been well fertilised in the past, but has not been cultivated and developed to its full potential from its current fescue tussock (*Festuca novae-zealandiae*) and browntop (*Agrostis capillaris*) dominant pasture with a thick thatch layer at the soil surface. One contributing factor to this is the Al toxicity throughout the soil profile across this land (Berenji 2015). This makes the direct injecting of lime deep into this soil a potential major benefit for land development and substantial production gains.

Rainfall (Figures 6.4, 6.5), air temperature and soil temperature at 10 cm depth (Figure 6.6) have been recorded at the site since December 2011 using a Onset® HOBO rain gauge and temperature sensors (Onset Computer Corporation 2015). Weather data is presented on a 1st July to 30th June production year basis, beginning in July 2015 until the end of June 2018.

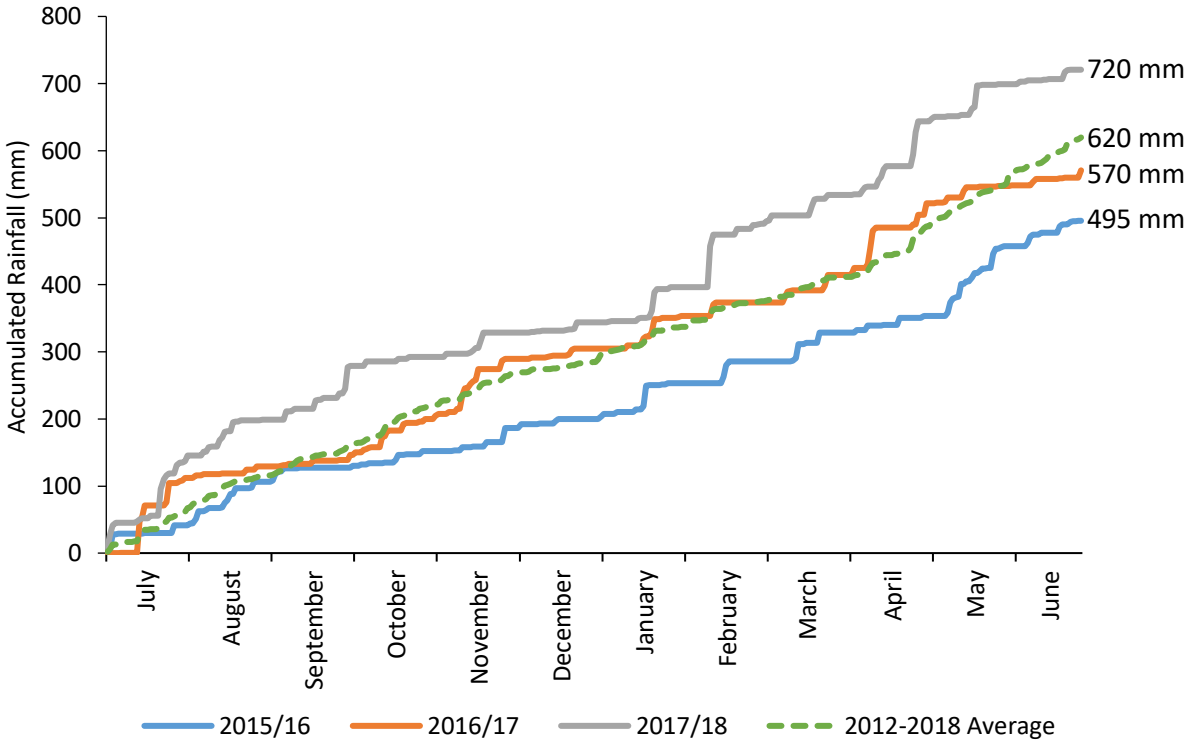


Figure 6.4 Accumulated rainfall at Glenmore Station from the 1st of July 2015 to the 30th of June 2018.

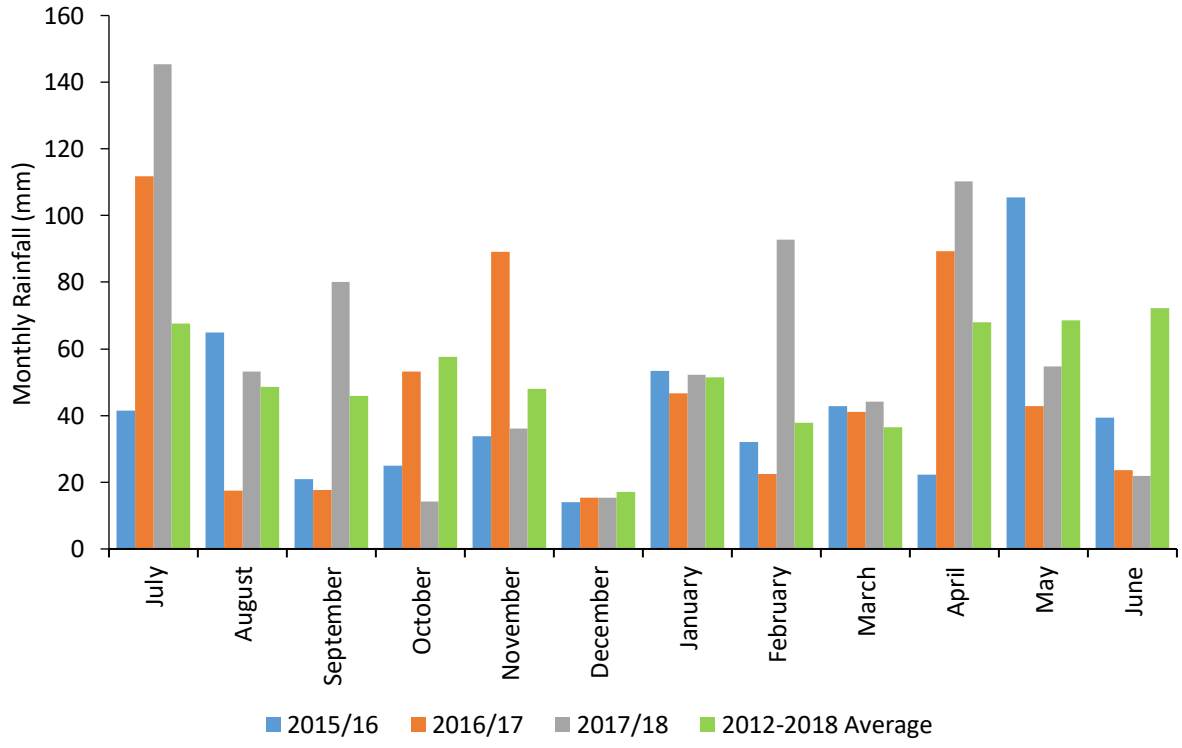


Figure 6.5 Monthly rainfall at Glenmore Station from the 1st of July 2015 to the 30th of June 2018.

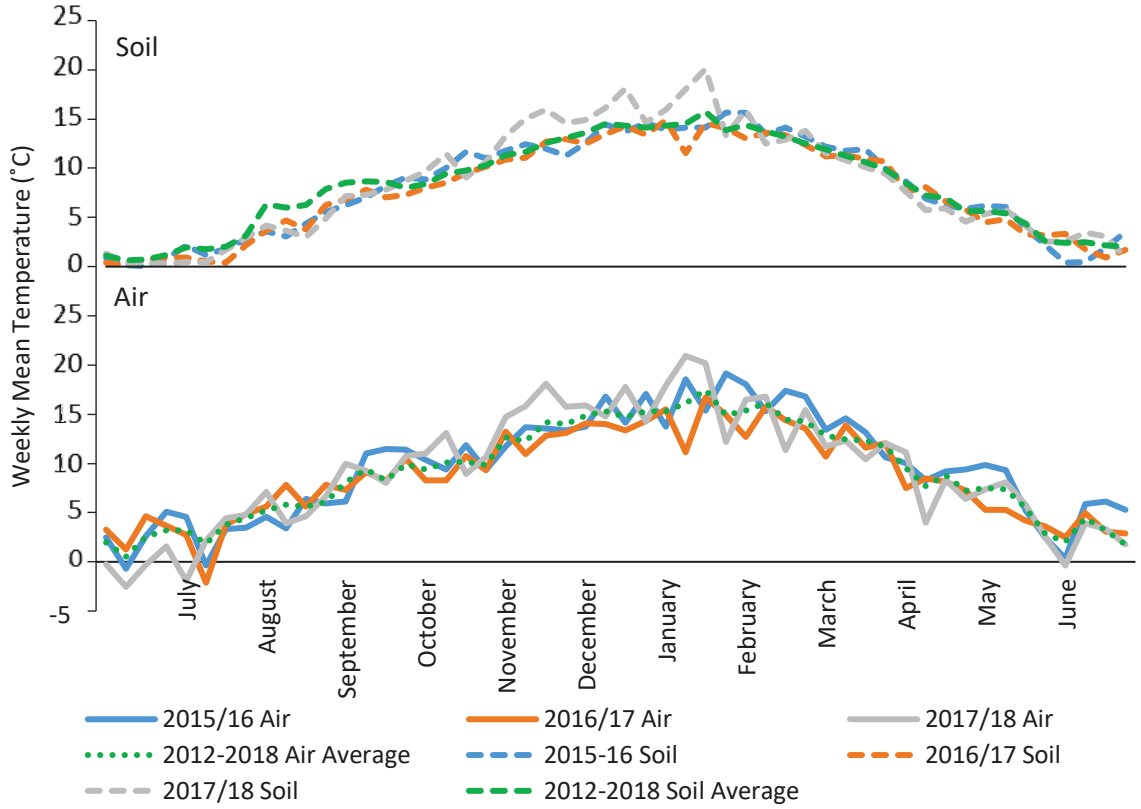


Figure 6.6 Mean weekly air and soil temperature at 10 cm depth at Glenmore Station from the 1st of July 2015 to the 30th of June 2018.

The Dasher

The Dasher is a 6224 ha hill country station situated in the Kakanui mountain range, 35 km inland from Oamaru, running 9500 stock units. The Dasher rises from 250 m asl at the eastern end of the property, to 1450 m asl at Mt Obi at the very western end of the property. The experiment was split over two sites at The Dasher; the "Paradise site" (TDP) in the aptly named Paradise paddock, and the "Packhorse site" (TDPH) in the aptly named Packhorse Lane paddock. The sites were located at 45˚09'50.00"S 170˚40'36.75"E and 45˚10'51.49"S 170˚39'52.60"E respectively, and at altitudes of 540 m and 572 m asl respectively. The soil at both sites is a Kakahu Acidic Firm Brown Soil (Land Information New Zealand 2003) formed from deep loess deposits over top of uplifted greywacke and schist bedrock. The Dasher was selected as an experimental site after the university was approached by the farm owner seeking advice on legumes for high Al soil. The TDP site was selected by the farmer as a paddock he wanted to develop from the current low quality browntop pasture, fescue tussocks and Scotch thistles (*Onopordum acanthium*). The TDPH site was selected due to the extreme concentrations of Al at the site (Table 6.2). The original cover at the TDPH site are large snow tussocks (*Chionochloa rigida*) and browntop pasture. The Dasher contrasts to the other two sites, as it has a wetter, more oceanic climate.

Located near the homestead at The Dasher, central to the two experimental sites, is an electronic rain gauge administered by the Otago Regional Council (Figures 6.7, 6.8). No air or soil temperature is recorded at The Dasher.

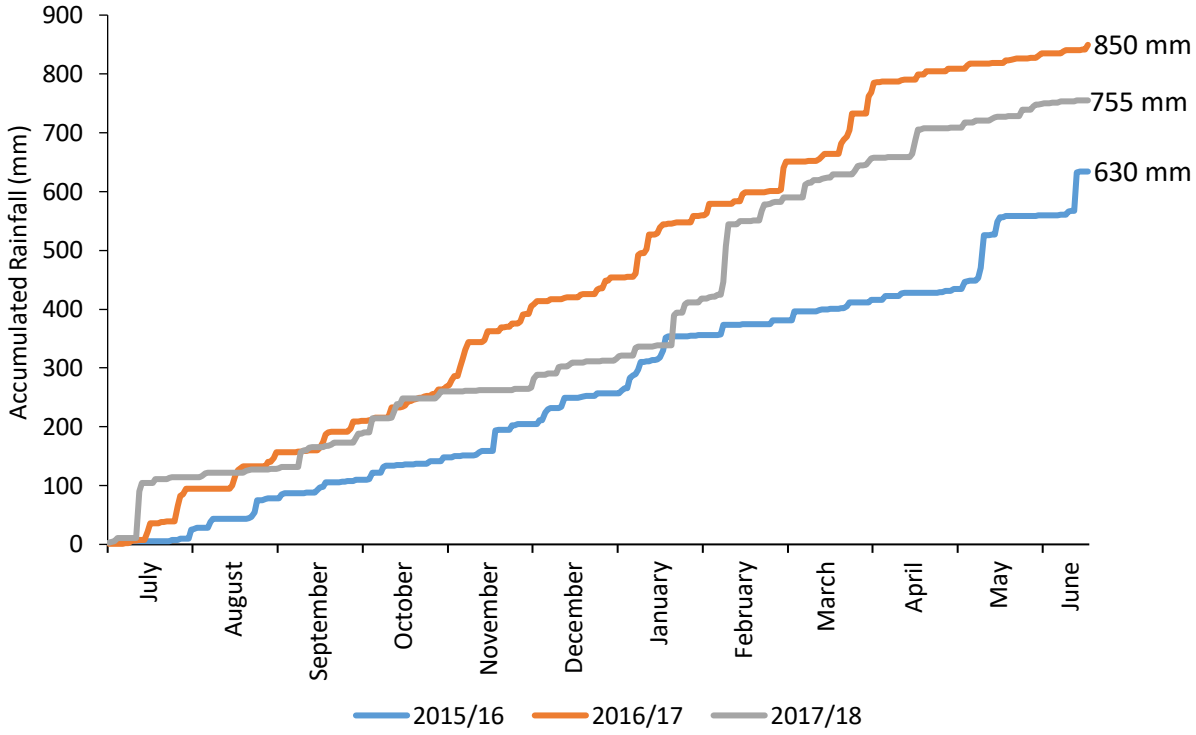


Figure 6.7 Accumulated annual rainfall at The Dasher over three years from the 1st of July 2015 to the 30th of June 2018.

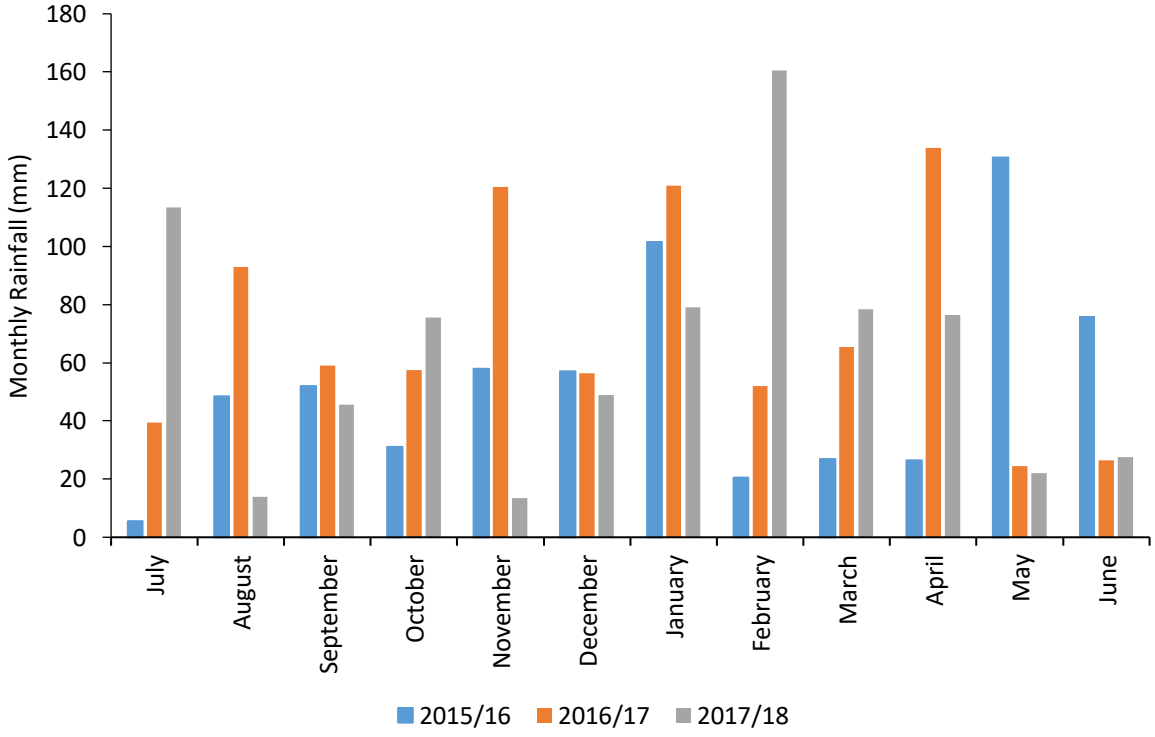


Figure 2.1.8. Monthly rainfall at The Dasher over three years from the 1st of July 2015 to the 30th of June 2018.

6.2.2 Initial soil chemical analysis results

Prior to conducting the experiment, soil core samples were taken to assist in site selection and to determine the severity of soil acidity and aluminium toxicity, and the level of soil fertility (Table 6.1). The core sampling occurred at the Glenmore (GM) site in March 2015 and at the Omarama (OM) site in early May 2015. The Paradise (TDP) and Packhorse (TDPH) sites at The Dasher were selected on past soil test results before further core sampling was conducted in December 2015.

Table 6.1 Initial topsoil chemical analysis test results at each site.

Site	Sampling Depth (cm)	Olsen P (µg/ml)	ASC (%)	SO_4^--S (mg/kg)	Org-S (mg/kg)	CEC (me/100g)	BS (%)	Carbon (%)	Nitrogen (%)	C/N ratio
OM	0-7.5	16	19	7	4	10	37	2.89	0.24	11.8
GM	0-7.5	32	30	26	8	13	36	5.53	0.44	12.5
TDP	0-7.5	14	35	8	15	28	66	7.43	0.58	12.9
TDPH	0-7.5	9	59	15	12	18	24	7.51	0.46	16.2

Soil fertility in the top 0-7.5 cm of the soil profile varied greatly between sites, and in particular importance to this experiment there were large differences in soil Olsen P and sulphur levels. Of the four sites, Olsen P was moderate at OM, but both SO_4^--S and Org-S levels were very low (<10 mg/kg). The GM soil had the greatest Olsen P and high concentrations of SO_4^--S but only moderate levels of Org-S. At both the 0-7.5 cm and the deeper sampling depths soil pH (H_2O) was very low at all of the sites (pH <5.3) (Table 6.2), and the acidity of the soil was further accentuated by the pH ($CaCl_2$) measurements. Soil Al was above the 3 mg/kg toxicity threshold for lucerne at depth at each site, and was particularly concentrated at the TDPH site.

Soil core sampling below 15 cm depth at the Omarama site was restricted by soil stoniness but was later tested when plots were excavated during the root sampling that took place in December 2016 and January 2018 (Section 2.7.2). At Glenmore, Berenji (2015) conducted a field trial 1.3 km NW of the experimental site and found pH (H_2O) and Al to be 5.1 and 7.4 mg/kg respectively in soil collected at 20 cm to 40 cm, and 5.1 and 8.9 mg/kg from 40 cm to 60 cm depth respectively, on the same soil type.

Table 6.2 Lime ripper experiment initial soil pH and exchangeable aluminium concentrations.

Site	Sampling Depth (cm)	pH (H_2O)	pH ($CaCl_2$)	Exch Al (mg/kg)
OM	0-7.5	5.3	4.6	1.5
	7.5-15	5.0	4.2	8.0
GM	0-7.5	4.7	4.2	5.9
	7.5-15	4.9	4.3	6.3
TDP	0-7.5	5.1	4.7	1.9
	7.5-15	4.8	4.2	5.1
	15-30	5.0	4.3	3.7
	30-45	4.9	4.2	7.5
TDPH	0-7.5	4.3	3.7	24.5
	7.5-15	4.3	3.7	37.2
	15-30	4.6	3.9	11.5
	30-45	4.9	4.3	27.4

6.2.3 The Flexiseeder Lime Ripper

In 2014, Lincoln University in conjunction with Flexiseeder Ltd and Geoff Gray Engineering Ltd, implemented the construction of the 'Flexiseeder lime ripper' which is designed to incorporate lime directly into subsurface soil horizons. The machine consists of a pneumatic lime delivery system incorporated upon a trailed Kverneland CLC ripper (Kverneland 2017) (Plate 6.1).

The ripper is designed for up to 40 cm deep ripping and consists of two rows of four reinforced and sprung 'C' tines, each with chisel tips. The spacing between each tine is 30 cm for an overall total machine width of 2.4 m (2.7 m operating width). Tine angle is adjustable, as is ripping depth by hydraulically adjusting wheel height. Attached to each tine are leaf springs that allow some flexibility for passing over stones.

The lime delivery system is built upon the ripper and consists of a pneumatic system driven by a power take off shaft (PTO). Pelleted lime is fed from a 500 L hopper by a metering wheel into a single venturi, which then splits into two ducts that rise up to two distribution heads to evenly deliver lime out from two delivery ports on each tine (Plate 6.1. a and b). The lower of the two ports is at a fixed depth on the tine and the upper port is height adjustable to alter lime delivery depth relative to the lower port.

To deliver the desired application rate of lime a floating, trailing metering wheel measures the speed at which the machine is travelling to determine the rate at which lime is fed from the hopper, based on a set

calibration entered into the machines computer. Optimise pelleted lime (CP Lime Solutions Ltd 2015) was used due to its regular particle size and ability to flow through the machine, compared with crushed agricultural lime which when tested did not flow consistently from the hopper or through the machine.

Plate 6.1 a. Rear view of the Flexiseeder lime ripper. b. Side view of the Flexiseeder lime ripper. c. Applying lime at the Omarama Station experimental site, 26th May 2015. d. Applying lime at the Glenmore Station experimental site 25th May 2015.

6.2.4 Experimental Design and Treatments

Each experimental site began as a split plot design with deep lime application rate as one treatment (main plots), perpendicularly overlaid with legume species as the second treatment (sub plots). At the beginning of the second year of the experiment at the Omarama and Glenmore sites, a fertiliser treatment was applied over the legume species treatment. This fertiliser treatment was applied at the onset of the experiment at The Dasher. For trial plans of each experiment site see Appendix E.

After spraying the experimental areas with herbicide, lime was ripped into soil at each site at depths of 5 cm and 25 cm simultaneously, see Appendix F for full details. The depth of lower application port of the Flexiseeder® lime ripper was set based on the depth of the stony soil layer at the OM site, and based on the ripping depth capacity of the machine. The upper port was set to apply lime just below the soil surface, to apply lime to the upper part of the soil profile down to the lower lime delivery port. At Omarama the

length of the main plots was 25 m, and at Glenmore and The Dasher they were 20 m. The lime application rates were 0 (control, to examine the effects of soil tillage alone), 500 kg/ha, 1000 kg/ha and 2000 kg/ha, each replicated five times (five blocks). At Omarama and The Dasher there was also a 1000 kg/ha surface applied lime treatment in which the machine was used to spread the pelleted lime without putting the tines in the soil (Plate 6.2). This treatment was added to contrast the effects of the deep placed lime over that of the surface applied lime. No surface applied lime treatment was applied at the Glenmore site because it had previously been assessed as ineffective there (Berenji 2015). Each lime treatment was laid side by side and replicated five times at each site in separate blocks with 6 m spacing between the blocks (Plate 6.4). At The Dasher the experiment was split over the two sites with two replicates at the Paradise site and three at the Packhorse site. Once the lime was applied each site was heavy-rolled to recompact the cultivated soil before sowing.

Plate 6.2 Using the Flexiseeder lime ripper to surface apply Optimise pelleted lime at the Omarama Station experimental site, 26th May 2015.

Following a winter fallow to allow sufficient time for the lime to dissolve in the soil, the plant species treatments were sown perpendicularly to the direction the lime was applied (Plate 6.3), to cross all of the lime treatments per block. The plants were sown with a 2.1 m wide Flexiseeder plot drill (15 cm coulter spacing) with two passes of the drill used per treatment to create lime by plant species plots of 2.5 by 4.2 m (Plate 6.4). At the Omarama and Glenmore sites 'Force 4' lucerne, Russell lupins, and two replicates of 'Rahu' ryecorn (*Secale cereale*) were sown in each block. The ryecorn was grown for one year as a 'break in crop', which is a common establishment practise in South Island high country to aid in preparing the seedbed and reducing the weed seed bank in soil when developing land (Anderson et al. 2014; Moot & Pollock 2014). This was replaced with more lucerne and Russell lupins in year two. 'Perun' festulolium (a meadow fescue, Italian ryegrass cross) was also grown at the Omarama site. The experiment at The

Dasher began one year later than the Omarama and Glenmore sites, so no ryecorn crop was grown there. Instead, Lotus *pedunculatus* and French serradella (*Ornithopus sativus*) were added as treatments in addition to the lucerne and Russell lupins. All plots received a surface application of urea at 40 kg/ha immediately following sowing to assist plant establishment.

Table 6.3 Plant sowing rates for the lime ripper experiment

Species	Sowing rate (kg/ha)	Germination (%)	Adjusted Sowing rate (kg/ha)	TSW (g)	Sowing Density (plants/m^2)
'Force 4' Lucerne	14	86	16.3	2.89	570
Russell lupins	12	90	13.3	39.3	34
'Rahu' Ryecorn	150	97	155	33.7	460
'Perun' Festulolium*	25	89	28.1	3.95	1420
Lotus pedunculatus**	7.5	90	8.3	0.72	1560
French serradella**	24	72	33.3	104	1050

*Only sown at the Omarama Station experimental site. **Only sown at The Dasher experimental sites.

Germination testing was carried out on all species by recording the germination of three replicates of 50 seeds, each on a wetted filter paper at 20˚C for 10 days. The results of the germination testing (Table 6.3) were then used to adjust the sowing rate of each species to sow the desired application rate of viable seed. Thousand seed weight (TSW) was measured by weighing three replicates of 50 seeds each, for each species.

Plate 6.3 Using the Flexiseeder plot drill to sow the Glenmore Station experimental site, 20th January 2016.

Before being sown (and germination tested) all of the Russell lupin seed was scarified in sulfuric acid (97%) for 20 minutes and then rinsed with water for equally as long, no more than three days before sowing (Berenji 2015). The seed was then inoculated the day before sowing with a slurry made from ground up root nodules collected from Russell lupin roots, deionised water and methyl cellulose. The slurry was allowed to dry before fine lime powder was used to remove excess moisture. The *Lotus pedunculatus* and French serradella were also inoculated in this manner using *Bradyrhizobium* ICMP 5798 (Andrews et al. 2015) and *Bradyrhizobium* WSM471 (Hartley et al. 2004), respectively.

At the beginning of the second spring of the experiment at the Omarama and Glenmore sites, and at the initial sowing of the two sites at The Dasher, the sown plant treatments were overlaid with a 'low' and 'high' fertiliser treatment. This was done by dividing the treatment lengthways between the two drill passes. The fertiliser was Ravensdown 'Sulphur Super 30' (7% P, 30% S) and applied at rates of 100 kg/ha and 400 kg/ha for the 'low' and 'high' fertiliser treatments respectively.

The treatments were laid out at the Omarama site according to Plate 6.4. The experiment plan shows the positioning of the lucerne and Russell lupins that were sown following the ryecorn crop (new lucerne and new lupins), and the original lucerne and Russell lupins sown at the beginning of the experiment (1 yr old lucerne and 1 yr old lupins). Experiment plans of the Glenmore and the two sites at The Dasher are in Appendix E.

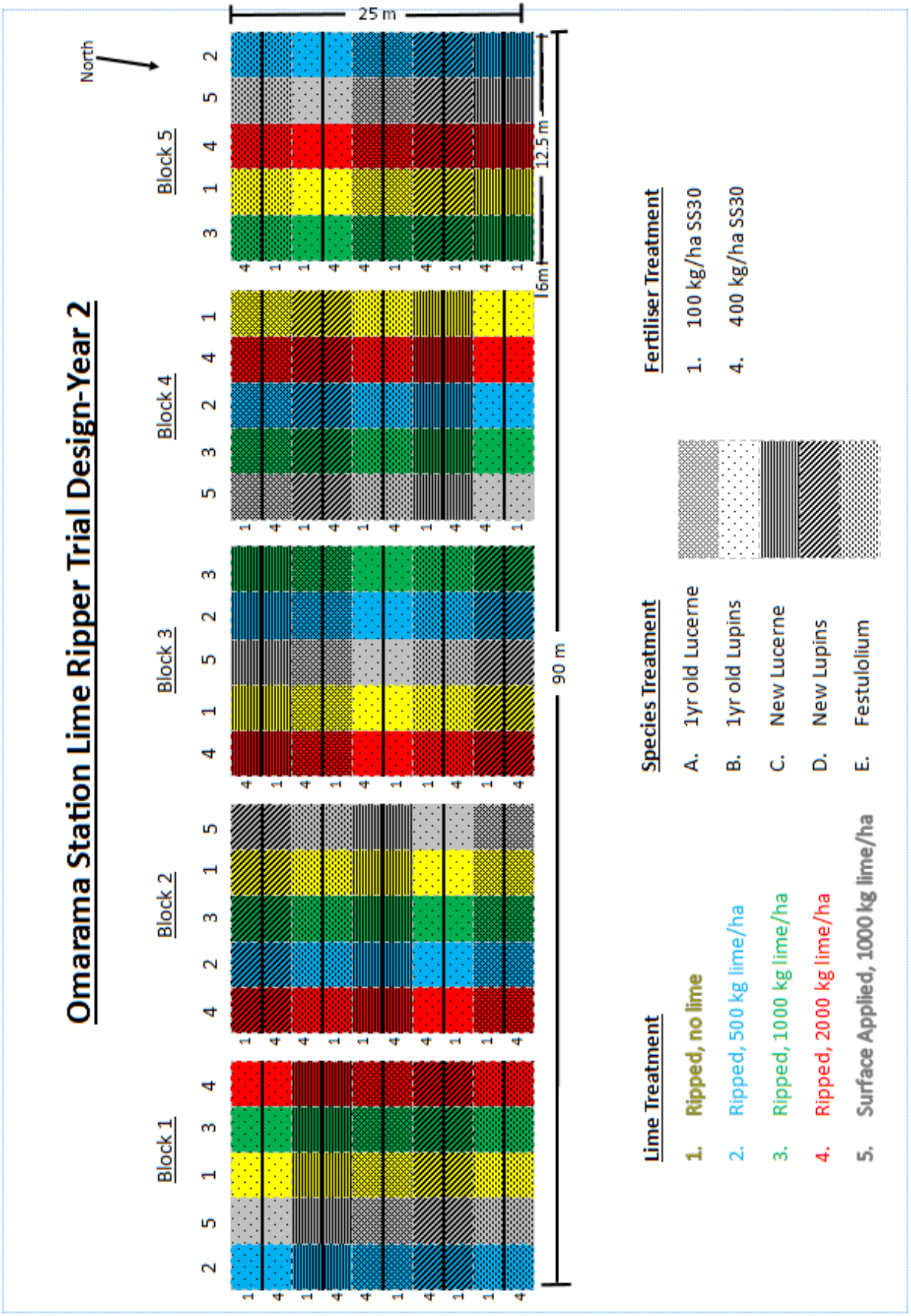


Plate 6.4 Omarama Station lime ripper experiment site plan.

6.2.5 Experimental Procedure and Measurements

Omarama Station

Having selected a suitable site based on soil uniformity and soil test results the experimental site was sprayed off with a combination of glyphosate, tribenuron methyl, MCPA, suflufenacil, and carfentrazone-ethyl, before the lime treatments were applied on the 26th of May 2015. For a full description of the methods used at each experimental site see Appendix F. Following the winter fallow the plant treatments were sown on the 9th of November 2015. In the first year of the experiment (July 2015-June 2016) three harvests of plant material were conducted in December 2015, and February and May 2016. At each harvest, measurements of plant height and plant density were recorded. At the beginning of the second year of the experiment (Plate 6.5), the plots that had initially been sown in the ryecorn 'break in crop' were harvested for a final time and sprayed off before being resown with more lucerne and Russell lupins on the 27th of October 2016.

Plate 6.5 The Omarama Station experimental site on the 20th September 2016.

The fertiliser treatments were applied to the plots on the 7th of November 2016. The only harvest of plant material for the second year of the experiment was on the 30th of November- 1st December. After this the site became very dry for the remainder of the growing season and no regrowth occurred (Figure 6.2). Given that the fertiliser treatment had only been applied three weeks before this harvest, the effects of the applied fertiliser on herbage yield were not measured at this harvest. Herbage samples collected from this harvest were investigated for any lime effects on their morphological and chemical composition. Herbicide applications were carried out at the end of the second production year and beginning of the third to control weeds in the plots. The third production year was the wettest (Figure 6.1) and so was the most productive growing season. Full site harvests of plant material occurred on the 28th-29th of November 2017 and on the 26th of April 2018, with an additional harvest of the lucerne plots on the 17th

of January 2018. A final harvest of plant material in the spring of the fourth year of the experiment was conducted on the 27th-28th of November 2018.

Overall, seven full harvests of plant material were carried out at the site over three full production years and a fourth spring, with additional ryecorn and lucerne harvests occurring in the second and third years respectively. For full experimental methods and parameters see Appendix F.

Root and Soil Sampling at Omarama Station

To investigate the effects of the five lime treatments on soil chemistry and root architecture on the 14th of December 2016, 40 cm wide by 50 cm deep trenches were dug adjacent to the original (November 2015 sown) lucerne and Russell lupin plots with a mini-excavator in all blocks (Plate 6.6. a and b). The location of these trenches are shown on the experimental plan in Appendix E. The walls of the trenches were squared off parallel with the outside drill row in each plot and all roots contained within a 1 m transect were carefully extracted (Plate 6.6.c). The collected root samples were washed, and up to 20 plants were measured for total root length and apparent rooting depth in the soil. Dry matter measurements of roots contained within 0-20 cm and 20-40 cm depths below the soil surface were made.

The distribution of the deep placed lime compared with the surface applied lime at depth in the soil was also investigated. To do this the walls of the trench where the root samples had been taken from were again squared off and soil samples were taken by inserting a standard 7.5 cm deep soil corer into the wall of the trench (Plate 6.6.d). Eleven individual soil core samples were collected every 10 cm along the same transect (0-100 cm) at depths of 10 and 20 cm below the soil surface. Each soil sample was individually air dried and 2 mm sieved before being analysed for pH (H_2O) and Al.

On the 17th-18th January 2018, lucerne and Russell lupin roots were again sampled using the same trenches as in December 2016. The samples investigated the effects of the lime treatments on root architecture and nodulation, and the cumulative effects of the lime treatments on soil chemistry at a range of depths. To do so the walls of the trenches were dug back into and root samples were again extracted from a 1 m transect from one drill row of each crop. Lucerne roots were sampled from all blocks and Russell lupin roots were sampled from Blocks 1, 3 and 5 only. The walls of the trenches were then again squared off and six soil core samples were taken every 20 cm along the 1 m transect (0-100 cm), these were bulked together at sampling depths of 0-7.5, 10, 15, 20, 25, and 30 cm below the soil surface. Soil sampling occurred in each block.

Plate 6.6 a. Digging a trench alongside lupin plots, 14th December 2016. b. A squared off trench wall adjacent to one lucerne drill row, 17th January 2018. c. Extracting lucerne roots, 14th December 2018. d. Taking soil core samples from the wall of a trench in a lucerne plot 15th December 2016.

The collected root samples were washed and all collected Russell lupin plants and 10 randomly selected lucerne plants from each sample were measured for length and scored for nodulation and branching, before dry matter biomass for 0-30 cm and >30 cm below the soil surface was recorded. The degree of both the nodulation and branching of the selected roots was visually scored on a 1 (no nodulation, or branching) to 5 (very highly nodulated, or branched) scale. The collected soil samples were air dried and 2 mm sieved before being analysed for pH (H_2O) and Al.

Full experimental methods and parameters regarding the root and soil sampling at Omarama Station are described in Appendix F.

Glenmore Station

This experimental site was located in a large block at Glenmore Station that had been sprayed out with glyphosate and metsulfuron prior to the beginning of the experiment in February 2015. It was intensively soil sampled before beginning the experiment. The lime treatments were applied at the site on the 26th of May 2015, followed by a long fallow to reduce any potential residue effects of the metsulfuron spray before the site was sown on the 20th of January 2016.

The first harvest of plant material occurred on the 29th of April 2016. Measurements of plant height and density were recorded at each harvest. Following the first harvest, the plots were over grazed by Merino sheep from which they did not recover. Subsequently they had to be sprayed out and resown, which occurred on the 26th of October 2016 (Plate 6.7.a). At this sowing the two ryecorn 'break in crop' treatments that had been sown in each block were replaced with an additional new lucerne and Russell lupin treatments in each block respectively, according to the trial plan in Appendix E. The 'high' and 'low' fertiliser treatments were then applied evenly by hand immediately after the plots were resown.

Plate 6.7 a. Resowing the Glenmore Station experimental site, 26th October 2016. b. Sampling Russell lupin plots at the Glenmore Station experimental site, 30th November 2017.

After resowing, harvests of plant material occurred on the 21st of March 2017, 30th of November 2017 (Plate 6.7.b), and for a final time on the 20th of April 2018.

Overall, four harvests of plant material were carried out at the Glenmore Station experimental site; one each in the first two production seasons and two in the third. Full experimental methods and parameters measured at the Glenmore site are presented in Appendix F.

The Dasher

The two experimental sites at The Dasher were added to the project one year later than those at Omarama Station and Glenmore Station. The two sites at The Dasher were deep soil sampled in November 2015 and then sprayed out with glufosinate-ammonium in January 2016. At the Packhorse site the original cover of thick, tall snow tussocks had to be removed by cultivating the site to 10-15 cm depth with offset discs before the tussocks were able to be pulled from the soil and removed from the site. This mild cultivation will have had a minimal effect on the results of the experiment. The lime was applied at both of the experimental sites at The Dasher on the 3rd of March 2016 (Plate 6.8.a), and the plots were sown on the 28th of October 2016. The fertiliser treatment was applied at the time of sowing. Plant establishment was measured at both sites on the 13th of December 2016. The first harvest of the Paradise site was on the 8-9th March 2017, while the first harvest of the colder, slower growing Packhorse site occurred on the 4-5th of May 2017 (Plate 6.8.b).

Plate 6.8 a. Applying lime with the Flexiseeder lime ripper to precultivated soil at the Packhorse site at The Dasher, 3rd March 2016. b. French serradella growing at the Packhorse site at The Dasher, 4th May 2017.

The second and third (final) harvests of the Paradise site occurred on the 10th of November 2017 and the 12th of January 2018, respectively. The Packhorse site failed to recover following an unexpected prolonged winter grazing in 2017, so was resown on the 16th of November 2017. However, the site failed to re-establish, so it was abandoned.

Full experimental methods and parameters measured at the two sites at The Dasher are in Appendix F.

6.2.6 Sample processing and chemical analysis

Herbage Samples

After each harvest collected herbage material was sorted into sown species and weeds at Lincoln University before both were weighed, then dried at 65˚C for 48 hours to determine their dry matter content. For large samples (>1500 g fresh material) the whole sample was weighed fresh and then subsampled. The dry weight of any weeds were excluded from the effect of the applied lime and fertiliser treatments on sown species herbage yield. Morphological separations into individual plant components (leaf, petiole, stem, flowers, seedpods, dead material, and weeds) of samples collected at OM in spring 2016, and in the spring of 2017 at GM, was done to investigate effects of lime treatment on plant morphological composition. Herbage material collected at OM in spring 2016, and root material from the December 2016 sampling, was finely ground and analysed for P, S and Mo content by digesting 0.2 g of ground sample material in 2.0 ml HNO_3 (69%) and 2.0 ml H_2O_2 (30%) in a microwave digester (CEM MARS Xpress, CEM Corporation, Matthews, NC, USA), followed by ICP-OES analysis (Varian 720, Varian Australia Pty Ltd, Melbourne, Australia) (Nolte 2003).

Soil Samples

Soil samples collected at the December 2016 and January 2018 trench sampling at OM were all air dried at 30˚C for 7 days before being passed through a 2 mm sieve. All of the individual core samples from the December 2016 sampling were analysed for pH but only the samples from Blocks 1, 3, and 5 were analysed for Al (Figure 6.8 and 6.9). At the January 2018 sampling all samples were analysed for both pH and Al. Soil pH was measured by mixing 10 g of soil in 25 ml de-ionised water and analysing with a pH probe (Blakemore et al. 1987) and Al by extracting 5 g soil with 10 ml 0.02 M $CaCl_2$ (Edmeades et al. 1983), followed by ICP-OES analysis.

6.2.7 Statistical Analysis

Before the application of the fertiliser treatment at the OM and GM sites herbage yield, weed yield, and root biomass, soil pH and Al concentrations at the OM site, were all analysed by one-way ANOVA followed by a Fishers LSD test using Genstat 16 for each plant species individually to investigate lime treatment effects due to the size of the variances between the species relative to their yields. For the plant nutrient uptake measurements at OM a two-way ANOVA. This was followed by a Fishers LSD test was used to determine the effects of lime treatment and plant species on nutrient concentrations. Following the fertiliser application at OM and GM, and from the onset of the experiment at TD, herbage yield was analysed by two-way ANOVA with lime treatment as main plots and fertiliser treatment as subplots, followed by a Fishers LSD test for each plant species. Any effects of the fertiliser treatment on root biomass or soil pH and Al were not considered when doing the trench sampling at OM. Following the two

rounds of trench sampling at OM, the Al concentrations in the lucerne plots of Block 2 were consistently <0.7 mg/kg at each depth and therefore not consistent with the high Al (>3 mg/kg) in the other blocks. This highlights the variability of the Mackenzie soil across the landscape, so all plant yield, root biomass and soil analysis results from Block 2 for the lucerne were excluded from the analysis.

6.3 Results

6.3.1 Effects of lime treatment on plant establishment and persistence

In the first year of the experiment at the OM site plant establishment, five weeks post-sowing of the lucerne, was higher ($P<0.05$) in the 2000 kg/ha direct injection application rate of lime (284±22.5 plants/m^2) than the 0, 500, and 1000 kg/ha application rates (217±25.8 plants/m^2 on average). For the 1000 kg/ha surface applied lime the lucerne establishment was 257±25.9 plants/m^2 and not different to the other application rates. The establishment of the Russell lupins, festulolium and ryecorn was not affected by lime application rate or method with 41.7±4.6 ($P=0.75$), 282±30.6 ($P=0.91$) and 386±16.5 plants/m^2 ($P=0.94$) establishing, respectively. Lucerne plant population remained steady at the site throughout the experiment, however the population of the Russell lupins decreased to an average of 5.8±0.9 plants/m^2 by the time of the final spring harvest in November 2018.

Initial plant establishment at the GM site in the first year was poor and patchy, probably due to poor drill coulter penetration because of the thick thatch on the soil. Following the site being resown in October 2016, plant establishment was more successful and no effects of lime or fertiliser application rate, or the ryecorn break-in crop on plant establishment was recorded. On average, lucerne establishment was 60.0±13.5 plants/m^2 ($P=0.93$) three weeks post-sowing, and Russell lupin establishment was 20.7±3.0 plants/m^2 ($P=0.10$). Lucerne density remained constant during the experiment but by November 2017 Russell lupin density had decreased to an average of 13.0±0.5 plants/m^2 ($P<0.001$).

At the two TD sites, plant establishment was unaffected by lime or fertiliser treatment. At the Paradise site plant establishment six weeks post sowing was 106±27.1 ($P=0.38$) for lucerne, 21.0±7.9 ($P=0.63$) for Russell lupin, 90.0±19.5 ($P=0.80$) for French serradella, and 190±48.5 plants/m^2 ($P=0.41$) for lotus. Plant establishment six weeks post sowing was similar at the Packhorse site with 80.6±19.8 ($P=0.16$) for lucerne, 21.8±3.4 ($P=0.32$) for Russell lupin, 113±14.0 ($P=0.23$) for French serradella, and 201±48.7 plants/m^2 ($P=0.41$) for lotus. Being an annual legume the French serradella only persisted at each site at TD for one year. Only the lotus maintained a consistent plant population density at both sites during the experiment. At the Paradise site lucerne density had reduced by almost half to 56.4±11.6 plants/m^2 and Russell lupins density was at 16.5±2.0 plants/m^2 by November 2017. Soil pH and Al conditions were so severe at the Packhorse site (Table 6.2) that despite germinating, the lucerne at the site had completely failed by the time of the first harvest there, six months after sowing. Unfortunately, uncontrollable grazing pressure

(coupled with soil and climate conditions) at the Packhorse site in the winter of 2017 resulted in the Russell lupins failing to persist into the 2017/18 growing season, consequently only one year (2016/17) of herbage yield data is available for the Packhorse site.

6.3.2 Annual herbage dry matter yields

Omarama Station

At the OM site dry matter (DM) yield was low in the first year as the plants established and no effects of the lime treatments were observed. The ryecorn yielded the greatest, with an average yield of 2030±116 kg/ha over all lime treatments (P=0.32), followed by the festulolium at 1470±169 kg/ha (P=0.48), the Russell lupins at 963±161 kg/ha (P=0.71), but only 322±58.8 kg/ha (P=0.99) for the lucerne.

The application of lime, both deep placed and surface applied (irrespective of application rate) increased the yield of lucerne (P<0.001) over that of the control in 2016/17 (Table 6.4). As the lucerne became more established the 1000 kg/ha and 2000 kg/ha deep application rates of lime increased lucerne yield compared with the control in both 2017/18 (P<0.001) and the spring of 2018 (P<0.001). In contrast the 1000 kg/ha surface applied lime treatment had no effect on lucerne yield in 2017/18 nor the spring of 2018. The second rotation of lucerne followed this same trend as it established. Only the 2000 kg/ha deep lime application rate increased its yield in 2017/18 (P=0.006), nearly doubling it, and both the 1000 kg/ha and 2000 kg/ha application rates of deep placed lime did in the spring of 2018 (P=0.008), roughly doubling and tripling the yield of this lucerne respectively. Again, the surface applied lime had no effect on the yield of the second rotation lucerne. In the spring of 2018, and generally throughout the experiment, the yield of the weeds in the lucerne plots was greatest in the surface applied lime plots compared with the ripped plots (P=0.03), and this was compounded by the high fertiliser application rate (P<0.001).

The 400 kg/ha fertiliser also increased the yield of both the lucerne (P=0.006) and festulolium (P<0.001) in 2017/18, each by 200 kg DM/ha. However the yield of the Russell lupins was unaffected. For the festulolium, yield decreased year on year to the point where plants were mostly dead by April 2018 and the plots were not sampled in the spring of 2018. No lime effects on the morphological composition of the lucerne (P=0.770) and Russell lupins (P=0.580) were measured at the November 2016 harvest.

Table 6.4 Mean annual dry matter yield of sown species per hectare (kg DM/ha) at Omarama Station (OM) in the second and third year, and fourth spring, of the experiment.

Year			Lucerne	Russell Lupin	Festulolium	2nd Rotation Lucerne	2nd Rotation Russell Lupin
2016/17	Lime Rate	0	456[b]	3760	951	-	-
		500	863[a]	3540	1240	-	-
		1000	792[a]	3730	1160	-	-
		2000	938[a]	4460	797	-	-
		1000 SA	903[a]	3000	1480	-	-
		SEM	62	642	230	-	-
		$LSD_{(5\%)}$	192	1360	688	-	-
		P value	***	NS	NS	-	-
2017/18	Lime Rate	0	750[c]	1690	388	919[b]	1170
		500	945[b]	2360	507	1150[b]	1460
		1000	1140[a]	2320	402	958[b]	1610
		2000	1270_a	1940	446	1810[a]	1320
		1000 SA	996[b]	1550	424	1060[b]	1300
		SEM	105	280	47	203	185
		$LSD_{(5\%)}$	215	814	136	426	531
	Fert Rate	100	907[b]	1900	337[b]	950[b]	1460
		400	1130[a]	2050	530[a]	1410[a]	1280
		SEM	66.4	177	30	128	117
		$LSD_{(5\%)}$	136	515	86	269	336
	P values	Lime	***	NS	NS	**	NS
		Fert	**	NS	***	**	NS
		Lime x Fert	NS	NS	NS	NS	NS
Spring 2018	Lime Rate	0	982[c]	5640	-	493[c]	-
		500	1760[b]	6620	-	744[bc]	-
		1000	2210[ab]	7810	-	1080[ab]	-
		2000	2890[a]	6520	-	1430[a]	-
		1000 SA	1470[b]	6260	-	569[bc]	-
		SEM	340	1220	-	264	-
		$LSD_{(5\%)}$	700	2560	-	541	-
	Fert Rate	100	1650	6670	-	756	-
		400	2070	6470	-	968	-
		SEM	215	771	-	167	-
		$LSD_{(5\%)}$	442	1620	-	342	-
	P values	Lime	***	NS	-	**	-
		Fert	0.063	NS	-	NS	-
		Lime x Fert	NS	NS	-	NS	-

Significance levels; * P<0.05, ** P<0.01, *** P<0.001, NS not significant. – No data.

The yield of the Russell lupins greatly exceeded that of the lucerne and the festulolium in each year and was not affected by lime or fertiliser application throughout the experiment. During the experiment the population density of the Russell lupins decreased from 41.7±6.4 plants/m across all treatments at the beginning of the experiment, to an average of 4.6±0.7 plants/m^2 by April 2018 before increasing slightly to 5.8±09 plants/m^2 by the spring of 2018. The ryecorn break-in crop had no effect on second rotation lucerne, and Russell lupin yield, which was not measured in the spring of 2018.

Glenmore Station

Due to the poor and patchy establishment at the GM site in 2015, the yield results for that year have been omitted. Table 6.5 shows dry matter yield results obtained in the second (2016/17) and third (2017/18) years following the resowing of the experimental site.

Table 6.5 Mean annual dry matter yield per hectare (kg DM/ha) at Glenmore Station (GM) in the second and third years of the experiment.

Year			Lucerne	Russell Lupin	2nd Rotation Lucerne	2nd Rotation Russell Lupin
2016/17	Lime Rate (kg/ha)	0	427	3440	1470	4370
		500	599	4820	1220	3860
		1000	470	3960	1360	4280
		2000	494	5390	1280	4560
		SEM	138	555	191	712
		$LSD_{(5\%)}$	418	1680	581	2160
	Fert Rate (kg/ha)	100	529	4650	1390	4190
		400	466	4160	1280	4340
		SEM	97	392	135	504
		$LSD_{(5\%)}$	295	1190	411	1530
	P values	Lime	NS	NS	NS	NS
		Fert	NS	NS	NS	NS
		Lime x Fert	NS	NS	NS	NS
2017/18	Lime Rate (kg/ha)	0	195[b]	9810	554	9950
		500	360[ab]	9530	991	8010
		1000	515[a]	10020	1190	10170
		2000	442[a]	9400	1220	9890
		SEM	75	1110	240	966
		$LSD_{(5\%)}$	228	3720	729	3230
	Fert Rate (kg/ha)	100	393	8206[b]	988	9690
		400	363	11180[a]	987	9320
		SEM	53	787	170	683
		$LSD_{(5\%)}$	161	2630	515	2280
	P values	Lime	*	NS	NS	NS
		Fert	NS	*	NS	NS
		Lime x Fert	NS	NS	NS	NS

Significance levels; * P<0.05, NS not significant

The direct injection of lime at the GM site resulted in an increase (P<0.005) in lucerne yield over that of the control (0 kg/ha) in the third year of the experiment. A two-tailed t-test showed that the yield of the second rotation lucerne (that sown after the ryecorn break-in crop) was significantly greater in both the second (P<0.001) and third year (P<0.001) than that of the plots sown directly into lucerne from the beginning of the experiment. However, the lucerne struggled to successfully establish at the site with the average yield being only 378±75.0 kg/ha, compared with 9690±1110 kg/ha for the Russell lupins in 2017/18. As for the OM site, Russell lupins were unaffected by lime and fertiliser application at GM.

The Dasher

At TD herbage yield was greater at the Paradise site (Table 6.6) than at the higher, more acidic Packhorse site (Table 6.7). In 2016/17, at the Paradise site the French serradella yielded the greatest, followed by the lotus. Both were unaffected by the application of lime and fertiliser. The lucerne was the lowest yielding but responded to the deep application of lime, irrespective of application rate (P=0.031). Lucerne yield was greatest in the 1000 kg/ha deep lime treatment (1580 kg/ha). The deep application of lime (P=0.010), and the higher rate of fertiliser (P<0.001), also increased the yield of the Russell lupins. This was the only example of Russell lupins responding to deep placed lime during the experiment across all sites. The Russell lupin yield was low in both the ripped control and surface applied lime plots which indicates it was a deep placed lime effect, and not just a cultivation effect, that contributed to the improved growth.

Table 6.6 Mean annual dry matter yield (kg DM/ha) of the sown legumes at the Paradise site at The Dasher (TDP).

Year			Lucerne	Russell Lupin	French Serradella	Lotus
2016/17	Lime Rate	0	324[c]	964[c]	6470	2050
		500	1210[ab]	2950[a]	5760	1540
		1000	1580[a]	1720[b]	6290	3340
		2000	951[b]	1640[b]	4990	2500
		1000 SA	735[b]	845[c]	4600	3310
		SEM	237	291	1260	654
		$LSD_{(5\%)}$	536	659	2860	1480
	Fert Rate	100	634	1010[b]	5300	2774
		400	862	1880[a]	5940	2320
		SEM	150	184	797	414
		$LSD_{(5\%)}$	339	417	1800	936
	P values	Lime	*	**	NS	NS
		Fert	NS	***	NS	NS
		Lime x Fert	NS	NS	NS	NS
2017/18	Lime Rate	0	374	694	-	1140
		500	509	506	-	1070
		1000	416	537	-	1580
		2000	538	308	-	1360
		1000 SA	437	368	-	1550
		SEM	215	244	-	280
		$LSD_{(5\%)}$	487	552	-	633
	Fert Rate	100	421	360	-	1140
		400	489	605	-	1530
		SEM	136	154	-	177
		$LSD_{(5\%)}$	308	349	-	400
	P values	Lime	NS	NS	-	NS
		Fert	NS	NS	-	0.054
		Lime x Fert	NS	NS	-	NS

Significance levels; * P<0.05, ** P<0.01, *** P<0.001, NS not significant

By 2017/18 the Paradise site had become weed infested and this limited the yield of the lucerne and Russell lupins, and to a lesser extent the lotus, causing large variability to sown species yields. The lime and fertiliser treatment effects on the yield of the Russell lupins were not observed in the second year of the experiment at the Paradise site as was observed in the first year. Being an annual legume the French serradella only persisted for one year, but in that year it showed good potential for use at TD, being the highest yielding species at both sites.

Table 6.7 Mean annual dry matter yield (kg DM/ha) of the legumes sown at the Packhorse site at The Dasher (TDPH).

Year			Lucerne	Russell Lupin	French Serradella	Lotus
2016/17	Lime Rate	0	0	203	665	361
		500	0	194	848	280
		1000	0	146	725	328
		2000	0	137	873	386
		1000 SA	0	173	1123	378
		SEM	-	44	231	47
		$LSD_{(5\%)}$	-	93	485	100
	Fert Rate	100	0	119[b]	611[b]	286[b]
		400	0	222[a]	1080[a]	407[a]
		SEM	-	28	146	30
		$LSD_{(5\%)}$	-	59	307	63
	P values	Lime	-	NS	NS	NS
		Fert	-	**	**	***
		Lime x Fert	-	NS	NS	NS

Significance levels; ** P<0.01, *** P<0.001, NS not significant

All species struggled to establish and grow at the Packhorse site. The lucerne failed by the time of the first and only harvest of herbage material at the site, six months after it was sown. No effects of the applied lime were observed for any of the surviving species but all responded to the higher application rate of fertiliser. The lotus was the only species to persist into the second year of the experiment, forming a thin mat on the soil surface, but the yield was so low it could not be effectively measured.

6.3.3 Root biomass, branching and nodulation at the Omarama Station site

Plant root biomass to a depth of 30 cm (Table 6.8), degree of branching, and plant nodulation of lucerne and Russell lupins were not affected by lime application at the OM site in December 2016 or January 2018.

Table 6.8 Mean root biomass per hectare to 30 cm depth at Omarama Station in the second and third years of the experiment.

Date			Lucerne	Russell Lupin
December 2016	Lime Rate (kg/ha)	0	1030	3190
		500	1100	2630
		1000	941	3100
		2000	1840	1750
		1000 SA	1010	2480
		SEM	298	841
		$LSD_{(5\%)}$	892	2520
		P value	NS	NS
January 2018	Lime Rate (kg/ha)	0	1670	7950
		500	1780	3490
		1000	1910	5260
		2000	1570	6520
		1000 SA	1800	7020
		SEM	336	3210
		$LSD_{(5\%)}$	716	7250
		P value	NS	NS

Significance levels: NS not significant

Despite no differences in total root biomass, visual observations showed more laterally growing lucerne roots in the control (0 kg/ha lime) and surface applied lime plots than in the deep-placed limed plots in both years (Plate 6.9).

In January 2018, lime treatments had no effect on the degree of branching (P=0.55) or root nodulation (P=0.91) of either the lucerne or Russell lupins. The level of branching on a visual score of 1 (single taproot) to 5 (heavily branched) for both the lucerne and Russell lupins was 2.6±0.2 and 2.7±0.4 across all lime treatments, respectively. For nodulation on a 1 (not nodulated) to 5 (heavily nodulated) visual assessment scale, the lucerne scored 1.1±0.2 across all lime treatments compared with the Russell lupins at 3.8±0.2. The impact of the fertiliser treatment was not assessed when the roots were sampled. More than 95% of the sampled root biomass was contained within 30 cm depth of the soil surface. Despite the reduction in Russell lupin plant population, the root biomass increased between December 2016 and January 2018 as the roots became larger and woodier. Large variation in the Russell lupin root biomass was observed in the January 2018 sampling event due to the limited number of blocks sampled and low plant population in each plot.

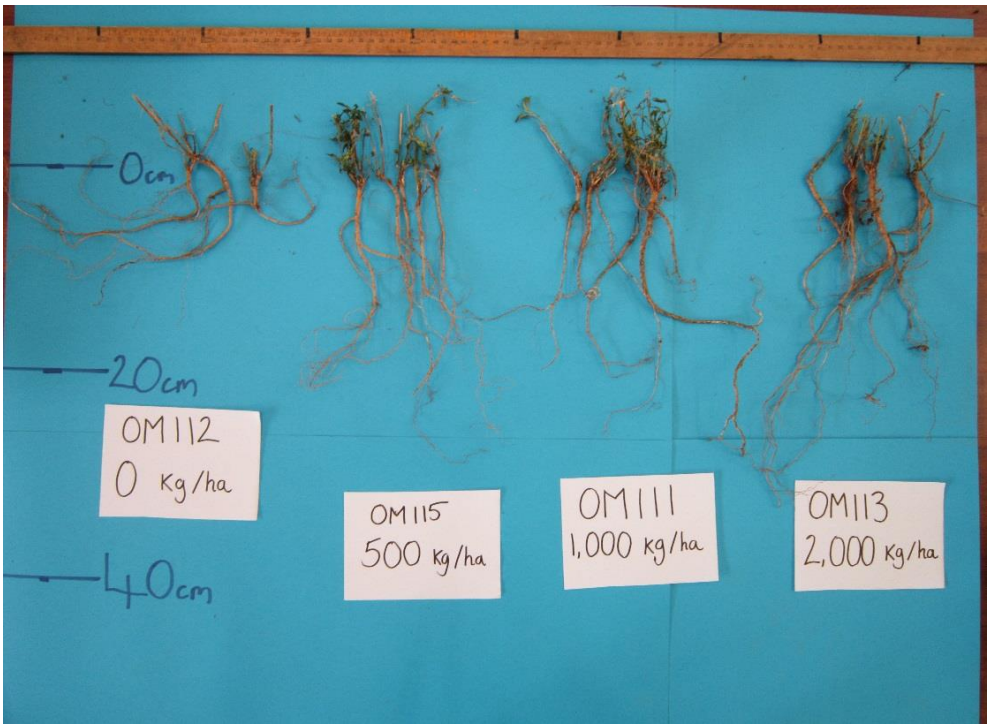


Plate 6.9 The effects of deep placed lime on the rooting depth of lucerne, December 2016, 13 months after sowing.

6.3.4 Plant shoot and root nutrient concentrations at the Omarama Station site.

Plant shoot and root nutrient concentrations (mg/kg dry matter) from the November 2016 harvest of plant material, and from the December 2016 root sampling, are shown in Tables 6.9 and 6.10. Lucerne, Russell lupin and festulolium shoot material was analysed for nutrient concentrations but only lucerne and Russell lupin root samples were sampled in December 2016.

There were differences in the concentrations and total amounts of nutrients taken up between the different plant species. In the lucerne, lime had no effect on the concentration of P and S in the plant tissue. However, when considering the total nutrient uptake both P ($P=0.037$) and S ($P=0.014$) uptakes were greater in limed plots (both deep placed and surface applied), irrespective of application rate. The 1000 kg/ha surface applied lime treatment increased the concentration of Mo in lucerne tissue ($P=0.023$), and increased the total uptake of Mo by both the lucerne ($P=0.002$) and the festulolium ($P<0.001$).

Table 6.9 Nutrient concentrations (mg/kg DM) in plant herbage material and total nutrient uptake (kg/ha) at the Omarama Station (OM) site, November 2016.

Species			P uptake		S uptake		Mo uptake	
			mg/kg	kg/ha	mg/kg	kg/ha	mg/kg	g/ha
Lucerne	Lime Rate (kg/ha)	0	1530	0.76[b]	2200	1.10[b]	0.38[b]	0.44[b]
		500	1370	1.31[a]	2260	2.09[a]	0.37[b]	0.40[b]
		1000	1420	1.30[a]	2170	1.97[a]	0.32[b]	0.40[b]
		2000	1290	1.26[a]	1850	1.80[a]	0.41[b]	0.38[b]
		1000 SA	1320	1.32[a]	2300	2.35[a]	1.42[a]	1.39[a]
		SEM	153	0.19	244	0.32	0.34	0.24
		$LSD_{(5\%)}$	324	0.40	517	0.68	0.71	0.51
		P value	NS	*	NS	*	*	**
Russell Lupin	Lime Rate (kg/ha)	0	2320	9.73	1580	6.30	0.39	1.78
		500	2270	7.91	1600	5.44	0.33	1.24
		1000	2340	7.35	1630	5.33	0.32	1.11
		2000	2300	10.4	1650	7.30	0.36	1.65
		1000 SA	2420	7.04	1650	4.85	0.57	1.71
		SEM	173	1.63	94.0	1.15	0.09	0.41
		$LSD_{(5\%)}$	367	3.46	199	2.43	0.18	0.86
		P value	NS	NS	NS	NS	0.077	NS
Festulolium	Lime Rate (kg/ha)	0	1940	1.81	1640	1.62	1.46	1.31[b]
		500	1790	2.15	1440	1.69	0.91	1.01[b]
		1000	1970	2.33	1660	1.84	0.88	1.07[b]
		2000	2050	1.64	1920	1.59	1.55	1.28[b]
		1000 SA	1850	2.48	1540	2.04	1.75	2.10[a]
		SEM	169	0.44	276	0.41	0.27	0.22
		$LSD_{(5\%)}$	359	0.93	585	0.86	0.58	0.47
		P value	NS	NS	NS	NS	NS	***

Significance levels; * P<0.05, ** P<0.01, *** P<0.001, NS not significant

When comparing between the different species the Russell lupins extracted more P compared with the lucerne and festulolium (P<0.001). In comparison the lucerne contained a greater amount of S than both the Russell lupins and festulolium (P<0.001). Despite this more S deficiency symptoms were observed in the lucerne, and this is reflected in their positive yield response to the added fertiliser (Table 6.4).

Compared with the shoot nutrient content (Table 6.9), the roots of the lucerne and Russell lupins contained the same concentration of P, and Russell lupin root S and Mo content were greater than that of the lucerne roots, whereas the opposite was observed in the plant shoots. When coupled with the high root yields of the Russell lupins (Table 6.8) their root uptake or each nutrient was much greater than that of the lucerne. Only the uptake of Mo by lucerne roots was affected by lime treatment with the highest uptake of Mo again occurring in the 1000 kg/ha surface applied lime plots.

Table 6.10 Nutrient concentrations (mg/kg DM) in plant root material, and total nutrient uptake by roots (kg/ha) at the Omarama Station (OM) site, December 2016.

Species			P uptake		S uptake		Mo uptake	
			mg/kg	kg/ha	mg/kg	kg/ha	mg/kg	g/ha
Lucerne	Lime Rate (kg/ha)	0	1790	1.70	1170	1.13	0.75[b]	0.64[b]
		500	1560	1.92	1150	1.29	0.72[b]	0.91[b]
		1000	1450	1.67	1010	1.14	0.67[b]	0.73[b]
		2000	1470	2.26	1100	1.69	0.61[b]	0.87[b]
		1000 SA	1680	1.89	1170	1.46	1.64[a]	2.20[a]
		SEM	150	0.78	79.8	0.55	0.13	0.42
		$LSD_{(5\%)}$	317	1.71	169	1.19	0.27	0.92
		P Value	NS	NS	NS	NS	***	*
Russell Lupin	Lime Rate (kg/ha)	0	1700	5.30	1880	7.07	1.14	3.43
		500	1870	4.45	2250	6.40	1.06	2.73
		1000	1910	5.21	1600	5.21	1.05	2.78
		2000	1900	3.39	2240	3.73	1.43	2.43
		1000 SA	1770	4.02	2340	5.84	0.92	2.16
		SEM	141	2.00	300	2.50	0.21	0.89
		$LSD_{(5\%)}$	300	4.25	635	5.30	0.45	1.89
		P value	NS	NS	NS	NS	NS	NS

Significance levels; * P<0.05, *** P<0.001, NS not significant

6.3.5 Soil pH and Al at the Omarama Station site in December 2016 and January 2018

At the December 2016 soil sampling, Al concentration decreased exponentially as pH increased at 10 (Figure 6.8) and 20 cm depth (Figure 6.9). For the deep-placed lime treatments, there was a wide range in pH and Al concentrations within treatments and Al toxicity was only alleviated at certain points within each of the 1 m sampling transects. Some undissolved lime pellets were still observed in the soil.

The point at which Al was reduced to below the 3.0 mg/kg toxicity threshold was pH 5.6 at both sampling depths. At the 10 cm sampling depth no effects of lime on soil pH (P=0.43) or Al (P=0.14) were measured. Al was only reduced to below the 3.0 mg/kg toxicity threshold at several points across the sampling transects in the deep placed lime treatments, but remained high (>12 mg/kg) at other sampling points. At the 20 cm sampling depth the highest levels of Al (>14 mg/kg) occurred at pH ≤5.2 in the control (0 kg/ha) treatment. At this depth soil pH was increased (P=0.010) and Al was reduced (P<0.005) by each of the deep-placed lime treatments compared with the control and surface applied lime treatments. Of the deep placed lime treatments the 2000 kg/ha treatment had the most individual sampling points with Al below the 3.0 mg/kg toxicity threshold.

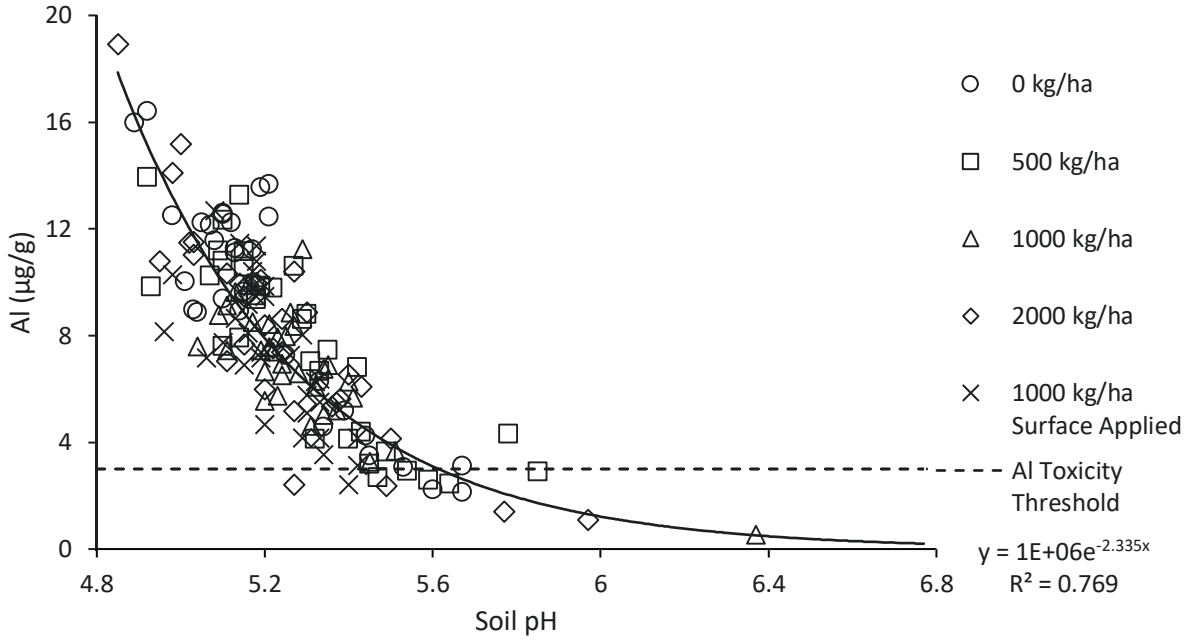


Figure 6.8 Lime treatment effects on soil pH and Al concentration at 10 cm depth, December 2016.

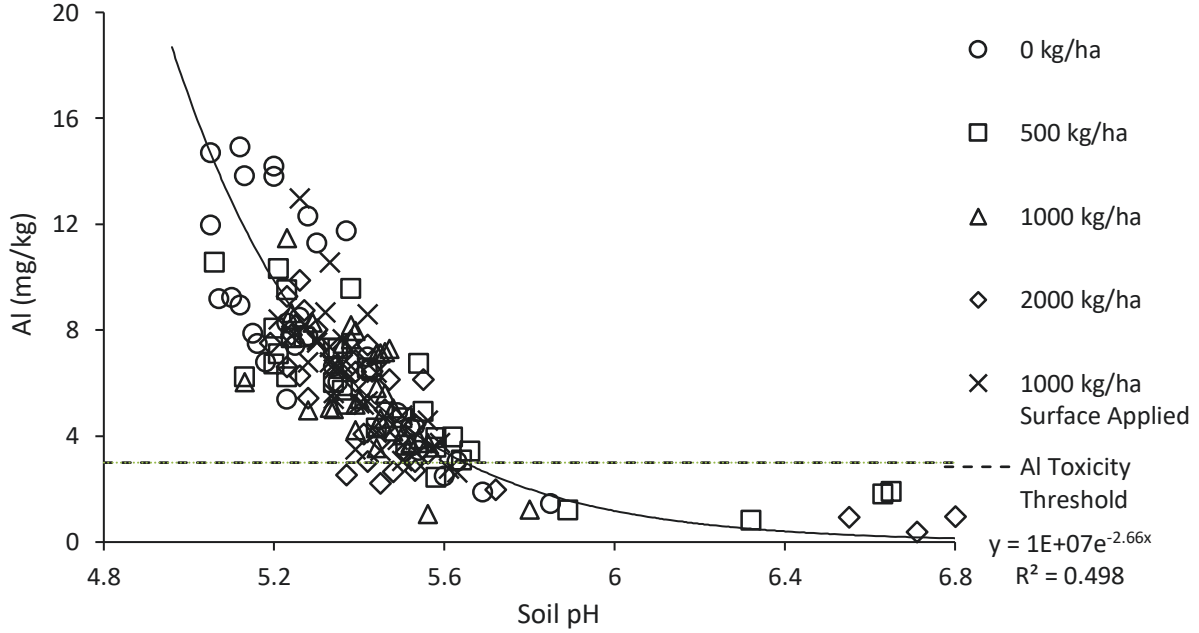


Figure 6.9 Lime treatment effects on soil pH and Al concentration at 20 cm depth, December 2016.

In January 2018, the greatest cumulative effect of the deep-placed lime occurred below 20 cm (Figure 6.10). For example, at 25 cm depth the 2000 kg/ha deep placed lime treatment increased soil pH to 6.0±0.3 and reduced Al to 0.73±0.21 mg/kg compared with the control treatment (pH 5.1±0.02, and 6.16±0.65 mg/kg respectively).

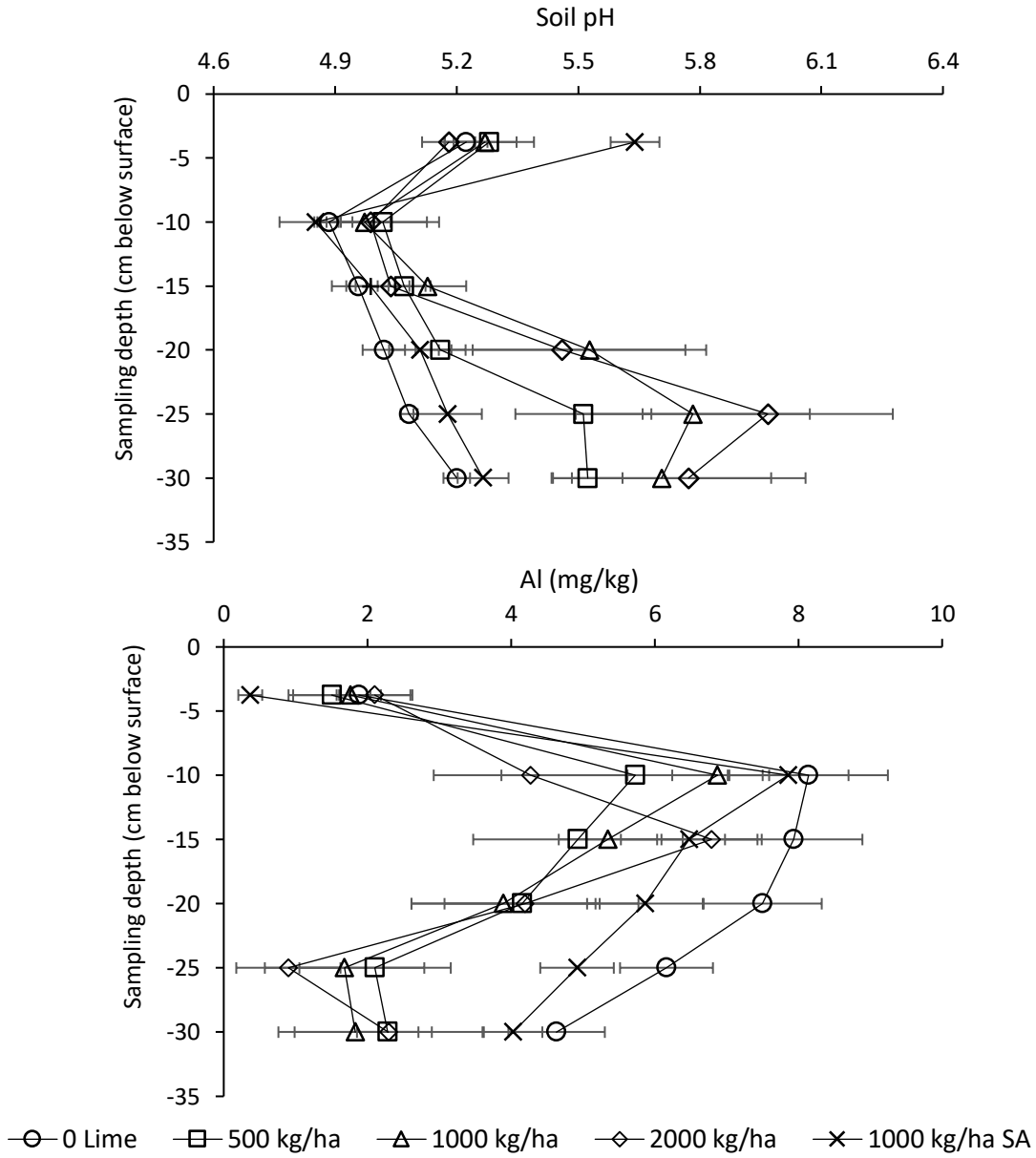


Figure 6.10 The cumulative effects of lime treatments on soil pH (top) and Al (bottom) at depth, January 2018.

The deep placement of lime, irrespective of application rate, was successful at increasing soil pH ($P<0.05$) and reducing Al ($P<0.001$) to below the 3.0 mg/kg toxicity threshold for lucerne at depths of 25 and 30 cm. At depths of less than 25 cm the deep-placed lime had no effect on soil pH and Al compared to the control. The 500 kg/ha deep-placed lime application rate was sufficient to successfully reduce soil Al to below 3.0 mg/kg, but was not as effective at increasing soil pH as the two higher application rates. The 1000 kg/ha surface- applied (SA) lime treatment increased soil pH ($P<0.001$) and reduced Al ($P<0.001$) in the top 7.5 cm of the soil profile, but had no effect below this depth.

6.4 Discussion

These experiments aimed to determine if subsoil acidity and Al toxicity could be reduced with deep lime placement using the Flexiseeder® lime ripper. This was successfully achieved at the OM site below 20 cm in the soil profile (Figure 6.10) with the lime delivered from the 25 cm deep lower port of the machine. Lucerne, being the selected biological indicator for Al toxicity, responded positively to the deep placed lime and yield increased year on year during the experiment at OM as the lucerne slowly became established. Sim (2014) showed that lucerne partitions a high ratio of its resources underground until it has built up its root biomass to 4-5 t DM/ha. Given that lucerne root yield, when measured in December 2018, was in the vicinity of 1.8 t DM/ha across the deep placed lime treatments it can be expected it will take more time, possibly another 3-4 years (Sim 2014; Moot 2018), before the full beneficial effects, and outcomes of this deep placed lime experiment on lucerne shoot yield are fully realised at OM.

However, the lime delivered from the upper port (5-10 cm below the soil surface) was not effective at reducing acidity and Al toxicity in the soil profile above 25 cm depth (Fig 6.10). Compared with the lower delivery port, which injects lime directly downwards deep into the void behind the foot of the ripper tine, the upper port is angled back slightly. Therefore, the soil may have been closing back in around the tine by the time the lime was ejected from the port, limiting the vertical distribution of the lime pellets. Having this port angled straight downwards in line with the ripper tine may have made the machine more effective at liming this upper part of the soil profile. Further experiments would be required to test this. Other possible solutions for increasing the effectiveness of the machine above 25 cm could include adjusting the application depths of the two delivery ports, or adding more delivery ports to the machine. A similar machine built by Nelson *et al.* (2012) had four delivery ports behind each ripper tine and the machine built by Farina & Channon (1988) had six. For further development and commercialisation, adding more ports would seem an obvious modification because where the lime was successfully placed at depth, the Al toxicity was alleviated.

When considering the lateral distribution of the deep-placed lime at the OM site, the machine created bands of soil where pH was increased and Al reduced. However, between these bands the soil was not effectively limed (Figure 6.8 and 6.9). Therefore, the actual application rate of the deep placed lime within the bands will effectively be much higher than the effective (t/ha) application rates. Given the 30 cm spacing between the ripper tines, therefore roughly three tines per metre, the actual application rate within the limed bands of soil may be as much as three times the effective (t/ha) application rate. By creating these bands down the profile behind the ripper tines, lucerne roots were able to grow down deeper into the soil at the OM site, which increased yields. Within these bands lucerne roots would have had increased access to soil water and nutrients, but due to unfavourable conditions in the wide inter-band areas will also have led to greater inter-plant competition for water and nutrients within the limed

bands (Farina & Channon 1988). This was evident in some plots where greater lucerne growth was visible in strips across the plots (Plate 6.10). To reduce this issue the spacing between the ripper tines could be narrowed, or multiple passes of the machine through the soil could be made.

Plate 6.10 a. An original lucerne plot treated with 2000 kg/ha of deep placed lime, February 2018. To the left of this plot is a plot treated with no lime and to the right the next plot has had 1000 kg/ha of lime surface applied. b. The same plot with the apparent strips of greater growth marked in blue.

A limitation to the experiment was the lack of in-depth root and soil sampling at the GM and TD site. At the OM site when applying the lime, the ripper created a thorough tilth of the soil between the ripper tines (Plate 6.1.c), but the pelleted lime was not evenly spread throughout the soil (Figure 6.10). Compared to OM, at the GM site and the Paradise site at TD the ripper tines tended to more slice through the heavier soil and did not create much tilth. This lack of tilth between ripper tines probably restricted lateral distribution of the lime and limited its spread to within the slot cut through the soil by the ripper tine. The higher rainfall at these sites, compared with OM, may have assisted in spreading the applied lime through the soil, but overall, the liming effect at GM and TD is likely to have been more limited to the bands of soil the ripper tines immediately passed through. As the Packhorse site had been mildly top worked prior to ripping, it is difficult to comment on how much tilth the ripper created between tines deeper in the profile, but giving the heavy soil there it can be expected little tilth was created and the lime stayed within the bands of soil that the ripper tines passed through.

Despite the poor cumulative effect of the deep-placed lime at reducing Al above 25 cm depth at the OM site, the yield of lucerne was significantly increased over that of the control treatment in the 2016/2017 production year, and both the control and the 1000 kg/ha surface applied lime treatments in 2017/2018 and the spring of 2018. Due to the variability of the alluvial Mackenzie soil across the landscape, and at the site, an anomaly occurred in the Block 2 lucerne plots (2015 sown) of the OM experimental site in which there was deeper topsoil and non-toxic levels (<0.7 mg/kg) of Al throughout the soil profile. This resulted in unimpeded (high) lucerne growth in these plots, leading to the lucerne yield and soil test data from Block 2 being excluded from statistical analysis of the results. Assuming all of the rainfall and

available soil water in these plots was used by the lucerne, then it can be assumed that the yield of the lucerne in these plots was the maximum attainable yield at the site for the given seasons, and given levels of soil fertility. In 2016/17 this was 1300±216 kg DM/ha, in 2017/18 it was 2700±126 kg DM/ha, in the spring of 2018 it yielded 5120±215 kg DM/ha. Although the soil depth and moisture holding capacity in these plots will have contributed to these high yields, if we assume that the lucerne in the other plots was still able to utilise all of the available rainfall, and soil fertility levels were constant across the site, then the average yield of the 2015 sown lucerne in the highest yielding deep placed lime plots in the other blocks was 72%, 47%, and 56% of the maximum yield attained in Block 2 in 2016/17, 2017/18, and the spring of 2018, respectively. This limitation can be attributed to the remaining toxic levels of Al in the upper part of the soil profile (>20 cm deep) impeding lucerne growth and causing it to fail to successfully nodulate (Berenji et al. 2017). Berenji et al. (2017) were able to show the potential growth response of lucerne to effectively limed acid high country soils, increasing yield more than 18-fold from only 270 kg DM/ha to more than 5000 kg DM/ha, without any addition of fertiliser nutrients. This was achieved when soil Al was reduced from 8 mg/kg to >1 mg/kg, and soil pH was increased from 5.5 to 6.5 using 4 t lime/ha. Modifying the machine to deliver more lime to the upper part of the profile, coupled with surface applied lime may improve the growth and nodulation of the lucerne.

The root biomass measurements for the lucerne at the OM site showed no difference among the lime treatments. However, the control and surface-applied lime treatment plots were observed to contain a greater proportion of laterally growing roots than the deep-placed lime treated plots, which contained a greater number of deeper, vertical growing roots. Had the deep-placed lime been more effective in the top 20 cm of the profile, the yield and nodulation of the lucerne may have been greater. Implementing the modifications and application methods suggested could improve the efficiency and economic viability of using the machine to develop stands of lucerne in hill and high country with high Al soils.

The low rainfall and consequent soil moisture limitation at OM will have limited plant growth, fertiliser nutrient utilisation, and the dissolution of the deep placed lime. Undissolved lime pellets were observed in the soil profile there during the root and soil sampling in January 2018, almost three years post application. Therefore, it may take considerable time to see the full effect of the applied lime on soil pH and Al concentrations, and plant yields, at the site. This also corresponds to ongoing maturation of the lucerne over time. It is likely that directly injecting lime would be more successful in areas with higher rainfall, where lime pellets dissolve more rapidly, such as at the GM and TD sites. At the GM site higher rainfall resulted in high Russell lupin yields (up to 10 t DM/ha/yr), which were unresponsive to the deep lime application. However, the deep lime application was not successful in producing a viable crop of lucerne at GM, probably because of poor distribution of the applied lime in the soil. These findings were emulated by Berenji (2018) at GM. The TD site was selected specifically for its high rainfall, but in the

second year of the experiment the Paradise site became weed infested, especially with Scotch thistles, and the soil acidity and level of Al toxicity at the Packhorse site was just so severe, that consequently all sown legumes struggled to survive there.

The large pellet size of the pelleted lime may have been a limitation to the rate at which they dissolved in the dry conditions at the OM site, despite being comprised of finely ground lime (CP Lime Solutions Ltd 2015). Studies by Higgins et al. (2012) and Lentz et al. (2010) have shown that despite the added cost of pelleted lime, it is no more effective at increasing soil pH and reducing Al than the same quantity of standard agricultural lime. No known studies have investigated the solubility of lime pellets made from constituted finely ground lime in dryland conditions. Modifying the lime ripper to apply standard agricultural lime, without blocking, may make deep placed lime technology more economically viable.

It is recommended that deep lime application should be coupled with surface lime application, as the surface applied lime treatment at the OM site was shown to be the most effective of the applied lime treatments at increasing soil Mo availability and plant uptake, when measured in November 2016. Mo availability is highly dependent on soil pH (McLaren & Cameron 1996; Kaiser et al. 2005) and this result indicates that the greatest plant uptake of Mo occurred from the top 7.5 cm of the soil profile. Without the surface application of lime is potentially limiting to plant growth and N fixation at the site (Kaiser et al. 2005; Rutkowska et al. 2017). Lime application did not increase the concentration of P or S in plant tissue, but did increase the overall total uptake of each by the lucerne as a function of increased lucerne yield. Despite the measured yield responses to the applied fertiliser throughout the experiment at each site, there were no recorded confounding interaction effects between the applied lime and fertiliser treatments on plant yield. Therefore, the application of lime had no effect on the utilisation of the applied fertiliser. This was consistent with results from a similar study by Edmeades et al. (1989). At GM the lucerne did not respond to applied fertiliser, but at OM where the lucerne responded to the deep placed lime it did respond to applied fertiliser, indicating that Al toxicity was the limiting factor to lucerne growth over P or S deficiency. Corresponding to this, the Al tolerant Russell lupins at GM and TD did respond to the added fertiliser.

The yield of the festulolium at the OM site decreased as the experiment went on. This was most likely due to the development of severe nitrogen deficiency. This highlighted the importance of growing deep rooting, nitrogen fixing legumes in these dryland environments. Overall, the Russell lupins were the most successful plant at the site. It is likely the lupin density decreased due to the plants being unable to withstand intense mowing of the plots following herbage yield measurements (Moot & Pollock 2014). In a lax grazing system the lupin plant population would be expected to self-govern and higher yields could have been achieved at the site if the plots had not been mown (Moot & Pollock 2014; Berenji et al. 2018). Unlike lucerne and festulolium, the Russell lupins were not affected by either the lime or the fertiliser

treatments at any site, and they were able to successfully and prolifically nodulate in unlimed soil at OM and this can be expected throughout the high country (Black et al. 2015). With the high spatial variability of the top soil depth at OM there are traces of deep, low Al soil across the landscape in which lucerne is able to flourish, as seen in Block 2 of this experiment. Coupled with the measured response to the deep placed lime there, of the four sites tested this site has the best potential for development with deep placed lime, especially following fertilisation, improvements to the machine, and additional surface application of lime, to grow lucerne on a broad scale.

The Russell lupins thrived at GM, even without lime and fertiliser, and the results of this experiment concur with the findings of Berenji et al. (2018) and Moot and Pollock (2014) that Russell lupins have the greatest agronomic potential compared with other tested legumes in this harsh dryland environment. A broad scale development of subdivision and direct drilling of a Russell lupin-cocksfoot-Caucasian clover pasture mix, following a ryecorn break-in crop, is currently underway and proving to be highly successful at Glenmore Station (Berenji et al. 2018).

At TD, the Russell lupins struggled to establish and the French serradella was the highest yielding legume, while the lotus was the most persistent. Both the Russell lupins and French serradella were unaffected by the deep lime application. This highlights the potential for use of these species at the site, and the requirement for further research into identifying legumes and developing site-specific legume based pasture systems that are best suited to the acid soil, low fertility, South Island high country. The two sites at TD were selected for the experiment due to their very low pH and high Al concentrations, along with the higher rainfall there compared to the OM and GM sites. Given the results of this experiment there, and the heavier soils and higher rainfall reducing the risk of erosion from cultivation there, simply cultivating lime into the upper part of the soil profile to develop grass-clover pastures is likely to be the most efficient agronomic solution for developing land at TD.

6.5 Conclusions

The deep application of lime was successful at reducing soil acidity and Al toxicity at 20-30 cm depth at the OM site. The 500 kg/ha application rate was sufficient to reduce Al to below the 3.0 mg/kg toxicity threshold for lucerne. The higher application rates were more effective at increasing soil pH. The deep application of lime significantly increased the growth of lucerne at the OM and GM sites. However, the machine was not fully successful at eliminating Al toxicity at 7.5 to ≤20 cm depth at the OM site, and this is likely to have still been a limitation to the maximum potential growth, and nodulation, of the lucerne at the site. Adding more lime delivery ports between the current upper and lower ports on the machine could improve the distribution of lime in this mid-zone. Lime should also be surface applied along with the deep placement. At the OM and GM sites, the Russell lupins were the most successful species and

were unaffected by the application of lime, highlighting their potential for use in acidic, low fertility, hill and high country environments. At TD the best performing species was French serradella. This experiment highlights the importance of applying lime, but also demonstrates how difficult it is to modify subsoil pH and Al conditions in dryland environments. The experiment also showed that further research is required to identify legume species, and for hill and high country farmers to grow species, that are tolerant of these conditions without the need for economically unviable applications of lime and fertiliser.

Chapter 7

General Discussion

The most limiting nutrient to pasture production in hill and high country soils is N (Lambert et al. 2003; Stevens et al. 2014). To overcome this limitation hill and high country farmers rely on legumes as the main source of N through the process of biological N fixation. Legumes are also a high quality stock feed. For optimal growth legumes require higher levels of P and S fertility than grasses (Caradus 1980; Haynes & Williams 1993b), but they are also more susceptible to, and restricted by, soil acidity and Al toxicity, which is a widespread and growing limitation in hill and high country soils (Edmeades et al. 1984a; Moir et al. 2000; Moir & Moot 2010; Whitley et al. 2016). Most hill and high country soils have been regularly fertilised with superphosphate in the past, but soil P and S concentrations remain low, as does the utilisation of applied fertiliser P and S (Ledgard et al. 1991). In acid soils, applied P is rapidly adsorbed by Fe and Al hydroxides, rendering it unavailable to plants, and over time it accumulates in organic forms (Haynes 1984; Parfitt et al. 1989; Pierzynki et al. 2005). Lime is required to alleviate soil acidity and aluminium toxicity, but due to the economics of aerial application very little of the hill and high country of the South Island has been sufficiently limed (Edmeades et al. 1984b; Craighead 2005; Moir & Moot 2010). There has been much debate and uncertainty on what effects liming acid soils has on the availability of soil P and the potential for it to be increased (Haynes 1982), and no such studies have been conducted on South Island hill and high country soils.

There is large potential for widespread development and increased production in South Island hill and high country. To improve the productivity and profitability of South Island hill and high country soil fertility and legume production needs to be increased. Therefore, the aim of this PhD project was to investigate ways in which this could be achieved through selective inputs of lime and fertiliser, and legume species selection. It was hypothesized that liming these previously unlimed, acid South Island hill and high country soils would increase legume growth and abundance by alleviating soil acidity and Al toxicity, and by increasing soil P availability. By doing so the requirements for inputs of fertiliser P could potentially be reduced. It was uncertain however if an increase in P availability due to liming would be due to a release of 'locked up' plant unavailable P to available form, and/or if it would be due to improved soil pH and Al conditions allowing for greater plant root growth and access to existing available soil P.

To quantify the effects of liming on P availability in South Island hill and high country soils an understanding of the speciation and amounts of P in these soils was required. To do so, a field survey (Chapter 3) of 19 hill and high country soils with different fertiliser histories were sampled and analysed for P fractions and basic soil chemistry. The specific objective was to investigate P and S availability in

these soils and to relate this back to fertiliser history and soil acidity. It was hypothesised that P accumulation in these soils would be able to be related to soil acidity and past fertiliser history by comparing the accumulation of P at 0-7.5 cm depth to P in the 7.5-15 cm deep soil. However, without knowing the specific fertiliser history of each site the accumulation of P in soil was unable to be determined. The amounts and proportion of P in each P fraction was also not correlated with soil acidity or concentrations of exchangeable Al. In overseas studies by Herlihy and McGrath (2007), and Redel et al. (2016), concentrations of oxalate extractable Al and Fe, soil clay content and organic carbon content were shown to be drivers of fertiliser P fixation in acid soils. Apart from the latter, these factors were not measured in the sampled soils in this study. In this study it was found that S is likely to be the major limiting nutrient to legumes in these hill and high country soils, and that only a very small proportion (7.2±0.45%) of the P contained in these soils is plant available. However, the project identified that these soils contain large quantities of moderately labile NaOH extractable Pi (13.3±0.91% of total P) and Po (45.2±1.74%) of total P, which may have the potential to become plant available if these soils are limed.

To investigate if liming will increase soil P availability, soils collected from each of the field experiment sites (Chapters 5 and 6) were treated with lime and additional P and exhaustively cropped in a glasshouse experiment (Chapter 4) using a modified Stanford and DeMent (1957) technique. The objective for this experiment was to quantify P availability and extraction in response to this liming using two legume species as bio-indicators. The aim was to determine whether liming these soils increased P availability and create a 'P-sparing effect', or whether liming simply created more favourable soil conditions to allow for greater plant uptake of P. It was hypothesised that liming these acid soils would cause the former, but instead the results proved the outcomes to be the latter. This result has widespread implications for hill and high country as it indicates that sorbed P from past fertiliser applications is unlikely to become plant available due to liming, and further fertiliser applications will be required to maintain nutrient availability. In the experiment, increasing soil pH by liming increased plant growth, shoot P concentration, plant P uptake, and utilisation of soil P up to an optimal pH for each of the two species. However, as pH increased beyond this optimal pH each of these variables declined, indicating the observed results were due to a plant physiological response to liming. For Russell lupins this optimal pH was found to be 5.5-6.5, and for *Lotus pedunculatus* it was pH 6.2-6.8. The utilisation of applied P in each soil was low and was not improved by liming as expected. Soil Olsen P was proven to be a poor measurement of P availability in these soils, and unfortunately soil P fractionation was unable to be performed following the experiment to investigate the fate of this unutilised P, and how liming and plant growth affected other forms of P in these soils.

As P utilisation in each soil during this relatively short term (407 day) experiment was less than the initial amount of plant available P contained in each, it can be presumed without performing P fractionation,

that little P contained in the large pools of moderately labile P fractions was made plant available by liming. However, mobilisation of this P to plant available forms may be a slow process and could be affected by liming over the long term, and should be investigated. This could be done by scaling up this experiment, or by investigating P fractions and plant growth in past hill and high country lime application field trials, such as those performed by Moir and Moot (2010, 2014), Berenji et al. (2018), and Black et al. (Black et al. 2014a).

Alongside this glasshouse experiment (Chapter 4) the effects of liming acid hill and high country soils were investigated in the field on an existing pasture comprising of sown and adventive annual legumes and several low quality grass species at Mt Grand Station (Chapter 5). As with a large proportion of the South Island hill and high country, the experimental site at Mt Grand had previously been well fertilised (Power et al. 2006; Maxwell et al. 2014, 2016). However, Olsen P concentrations remained suboptimal (Edmeades et al. 2016; Roberts & White 2016). Despite this, it was again shown that S was the most limiting nutrient to pasture production at the site. Total pasture yield ($P=0.003$), clover yield ($P<0.001$), the clover content in the sward ($P<0.001$), and pasture shoot S concentration ($P<0.001$) were all increased by the application of the S component of applied 'sulphur super 30' fertiliser. In contrast, although Olsen P was increased by the P component of this fertiliser, there were no measured increases in pasture yield or P uptake related to applications of fertiliser P in the experiment.

Compared with the glasshouse experiment, P fractionation was able to be performed on treated soils two years in to this experiment at Mt Grand Station. These results confirmed those from the glasshouse experiment, whereby plant available P was not increased by liming at this site. This indicates that no 'P-sparing effect' occurred and instead the observed responses to liming, and to fertiliser when applied conjointly with lime, were due to other factors such as micronutrient availability, possibly greater N fixation, and beneficial lime driven changes in other soil properties (Wheeler & O'Connor 1998). In this experiment greater pasture growth responses to applied lime may have occurred if initial Al concentrations at the site had exceeded the 3 mg/kg toxicity threshold. As well as not showing an increase in plant available P, the P fractionation results showed that liming caused increased amounts of P to be bound to Ca in the HCl Pi fraction. This was most notable in plots where fertiliser P was applied. The high application rate of lime also caused an increase ($P=0.016$) in soil residual P content when applied with fertiliser P. This was possibly due to the formation of Al-P precipitates from fertiliser P as the result of liming and neutralisation of exchangeable Al. Therefore, a major finding of this study is that it can be recommended not to conjointly apply P fertiliser when liming acid soils with high concentrations of exchangeable Al.

Economic analysis showed that it is costly to apply lime, so intensive consideration when devising lime application strategies is required. At the Mt Grand Station experimental site 3 t lime/ha was required to

increase soil pH to the biological optimum of 5.8-6.3 soils (Edmeades et al. 1984a; Edmeades et al. 1984b; Edmeades et al. 1985; O'Connor et al. 2007). However, the economic optimum will in reality be lower than the biological optimum (Edmeades et al. 2016) and this will be site specific. Due to high application costs it can be recommended that when aerially liming hill and high country soils it is important to identify and focus on areas where liming will be most beneficial and economically sensible, making use of modern variable rate application technology. Lime should be applied to these areas at sufficient quantities to completely alleviate Al toxicity and to increase soil pH to the economic optimum. This is instead of blanket applying low rates of lime over large areas that will be buffered by soils and are only going to have small effects on soil chemistry and pasture production. Due to the formation of Ca-P precipitates caused by excess Ca, and the potential for the formation of Al-P precipitates where initial exchangeable Al concentrations are high, P fertiliser should not be applied in conjunction with liming these hill and high country soils. A possible method of applying lime to selected areas where soil acidity is a major limitation to legume production would be to forgo a single application of P fertiliser and instead use the budgeted money for this fertiliser to sufficiently lime these areas. As S is less effectively stored in soil than P, S should also be applied with this lime if economically viable. This could be done by mixing elemental S with lime, or should one be developed, the use of a lime-S fertiliser product. Further research is required on other South Island hill and high country sites with differing soil types, climates, fertiliser histories and initial P, S and Al concentrations to investigate the wider potential productivity, sustainability and economic benefits for the farming community resulting from liming these soils.

The work conducted at Mt Grand Station in this PhD project supports the work by Maxwell et al. (2013, 2014, 2016) of the productive potential of naturalised annual clovers (NAC's) in South Island hill and high country. It was fortunate that there was a large pre-existing population of NAC's at the commencement of the experiment. Careful management is required though to ensure persistence of naturalised and sown annual legume content in pasture swards (Maxwell et al. 2016). However, as it stands, it is currently difficult to source annual clover seed, other than sub clover seed, in New Zealand (Maxwell 2013; Monk et al. 2016). Opportunity exists for the extension of research into the use of annual legumes in hill and high country, and for the expansion of the market for their seed in New Zealand.

The use of a selective grass herbicide was also shown to be an effective tool for increasing pasture quality by allowing these annual legumes to thrive and persist. It has been shown that using selective grass herbicides can be useful for establishing newly applied legume seed and increasing the size of soil seedbanks (Casey et al. 2000; Hepp et al. 2003). However, it could possibly also have detrimental impacts such as where the initial seedbank is very low and a loss of total pasture is recorded, or if the herbicide is applied going in to a dry year which limits plant production. Results from this experiment also show the increase in pasture weed content in the following season post-spraying. More research is required to

design and manage hill and high country pasture systems that incorporate the one-off or regular applications of selective grass herbicides to encourage N fixation by legumes and ensure that these systems remain sustainable and economic.

In an alternative aspect to increasing hill and high country legume production, a prototype machine for directly injecting lime deep into the soil profile was tested at four hill and high country sites (Chapter 6). The objective was to determine how deep application of lime with this machine effected subsoil pH, Al concentrations and the growth of deep rooting legumes. The machine was only successful at doing this at one of the four sites, where the friability of the soil allowed the applied lime pellets to mix with the soil beyond the point of injection by the machine. This was at the Omarama Station site, where subsoil pH was increased and Al toxicity alleviated at depths of 20-30 cm. This resulted in increased persistence and growth of Al sensitive lucerne. In contrast, Al tolerant Russell lupins yielded much greater than the lucerne at this, and the Glenmore Station site. French serradella and *Lotus pedunculatus* were the most successful species at the other two sites at The Dasher, and their growth was unaffected by the deep application of lime with this machine. Although the deep application of lime shows promise as a method for increasing legume production in hill and high country soils, significant further research and development of the machine is required to scale up and improve this technology, to make it more effective and economically justifiable. Primarily this research needs to first focus the ability of the machine to use standard crushed agricultural lime, and to more evenly distribute this lime through the soil profile.

Continued research is also required to identify and develop management strategies for utilising novel acid tolerant legumes that can survive and thrive in these acid hill and high country environments. Current research on developing Russell lupin and Caucasian clover based systems shows promise (Black et al. 2014b; Berenji et al. 2018), but there is greater scope for the introduction of other acid tolerant species, such as other varieties of lupins and serradella species. Further research should also be conducted to identify and fit species that utilise greater amounts of fertiliser P and can mobilise non-available P in soil into hill and high country pastures (Menezes-Blackburn et al. 2017).

7.1 Conclusions and recommendations

Overall, the main findings and conclusions of this PhD project are that despite South Island hill and high country soils have generally been well fertilised in the past, and containing large amounts of P, only a small proportion of this P is plant available and liming should not be relied upon to increase P availability in these soils. Instead, plant P responses to liming were due to increased ability of plants to access existing available P and to utilise applied fertiliser P in soil. However, over-liming can be detrimental to P availability. In these soils, S was found to most often be the main limiting nutrient to legume production and is therefore of greater importance to be regularly applied than P. With ongoing soil acidification liming

hill and high country soils will be required to sustain legume survival and production in pasture swards. This project identified ways in which hill and high country farmers can greatly increase soil fertility and pasture production, using combinations of legumes, lime, fertiliser and herbicide. From this project it can be recommended to hill and high country farmers that:

- When aerially applying lime, areas where it will be most beneficial and rewarding should be selected and focussed on, rather than blanket applying low rates of lime over large areas.

- When applying lime to these selected areas, apply sufficient quantities of lime to completely alleviate Al toxicity and to increase pH to or near the site specific economic optimum.

- As it is not regularly applied, the cost of aerially applying a single large application of lime can be covered by forgoing a regular application of P fertiliser.

- P should not be applied with lime, especially where soil Al concentrations are high (>3 mg/kg) as precipitation of applied P with neutralised Al, and applied Ca, can occur, making it plant unavailable.

- As S is more prone to leaching, and is more often the most limiting nutrient to legume production, it should be applied along with lime where possible. Regular inputs of S fertiliser should be maintained for sustained legume production.

- The use of grass herbicide is valuable tool for increasing pasture legume content where there is a good seedbank. Care is needed when the clover seedbank is small, but if managed correctly this can be a good tool for increasing pasture clover content going forward.

- The deep application of lime can be beneficial for alleviating subsoil acidity and Al toxicity. However, there are legumes available that are tolerant of these conditions and more effort should be made to adopt these legumes into farming systems before trying to extensively modify the soil environment.

Appendix A: Phosphorus Fractionation Methodology

A.1 Phosphorus fractionation procedure

To determine the concentration of P in each fraction sequentially extract soil according to steps 1-6 below. Methodology developed by Hedley et al. (1982), Olsen and Sommers (1982), Condron et al. (1996), and Condron and Newman (2011).

1. NH_4Cl Pi

Weigh 0.5g of oven dry, finely ground soil into a 15 mL centrifuge tube and add 10 ml of 1M NH_4Cl. Shake for 16 hours on and end-over-end (33 rpm) shaker before centrifuging at 4000 rpm for 15 min. Carefully remove the supernatant from the tube without disturbing the soil for Pi analysis using the Murphy and Riley (1962) method of analysis for P in acidic extract (below).

2. $NaHCO_3$ Pi and Po

Using the same soil add 10 ml of 0.5M $NaHCO_3$ that has been adjusted to pH to 8.5 with NaOH and again shake for 16 hours on and end-over-end (33 rpm) shaker. Centrifuge at 4000 rpm for 15 min. After having carefully removing the supernatant add 5 ml of 0.5M NaCl to soil in the centrifuge tube without mixing it. Centrifuge again at 4000 rpm for 5 min to remove any remaining $NaHCO_3$ P in the soil before carefully removing the supernatant. Add this collected supernatant to the previously extracted supernatant. Analyse the combined supernatants for Pi according to the method of Dick and Tabatabai (1977) for Pi analysis in alkaline extracts (below), and for total P according to auto-clave digestion method (below). $NaHCO_3$ Po can be calculated by the difference between Total $NaHCO_3$ P and $NaHCO_3$ Pi.

3. NaOH I Pi and Po

Now add 10 ml of 0.1M NaOH to the soil and shake for 16 hours on and end-over-end (33 rpm) shaker. Centrifuge at 4000 rpm for 15 min before collecting the supernatant without disturbing the soil. Add 5 ml of 0.5M NaCl to the soil in the centrifuge tube without mixing it and again centrifuge at 4000 rpm for 5 min before carefully removing the supernatant without disturbing the soil. Combine the supernatants and analyse for Pi according to the method of Dick and Tabatabai (1977), and for total P according to auto-clave digestion method. NaOH Po can be calculated by the difference between Total NaOH P and NaOH Pi.

4. HCl Pi

As immediately above but instead now add 10 ml of 1M HCl to the soil. Analyse the combined supernatants for P according to the Murphy and Riley (1962) method.

5. NaOH II Pi and Po

Repeat the process for the first NaOH extraction, above.

6. Residual P

Having completed all of the extractions above, dry the remaining soil in a drying over a 50˚C. Finely grind the dried soil and weigh 0.1g into a glass digestion tube. According to the method of Olsen and Sommers (1982) for total P analysis of residual soil add 1 ml of concentrated H_2SO_4 and cap the digestion tube with a reflux funnel. Place the digestion tube on a digestion block and gradually ramp the temperature (+5˚C/min) up to 225˚C and hold this temperature for 1 hour before removing the sample and allowing it to cool. Remove the reflux funnel and add 2ml of concentrated H_2O_2 then replace the funnel on the digestion tube. On the digestion block again gradually ramp the temperature (+5˚C/min) up to 135˚C and hold this temperature for a further 1 hour. If the sample remains cloudy or dirty after this time due to the presence of remaining organic matter add another 1 ml of concentrated H_2O_2 and again hold the temperature at 135˚C for 1 hour, repeat if necessary. Once the sample becomes clear ramp the temperature to 150˚C and hold this temperature for 30 mins to ensure all of the H_2O_2 becomes fully degraded. Allow the sample to cool before diluting it up to 40 ml with DI water. Analyse the sample for P using the Murphy and Riley (1962) method.

A.2 Murphy and Riley (1962) method of determining P concentration in acid soil extracts

Pipette an aliquot of known volume (approximately 1 ml) of acid soil extract to be analysed into a 35 ml vial and make up to 3 ml with DI water. Add one drop of p-Nitrophenol indicator and neutralise the solution with 10 M NaOH. Add 0.5 ml of solution B reagent and let rest for 30 minutes before reading the absorbance on a UV-Vis spectrophotometer with the wavelength set to 882 nm. The colour will be stable for 3-4 hours.

The concentration of P in the given fraction (mg P/kg) is calculated by: concentration of P in extract (mg/l) x dilution of extract x (volume of extract (ml) / mass of soil (g)).

Solution B reagent: dissolve 1.356g of ascorbic acid ($C_6H_8O_6$, MW 176.12 g/mol) in 100 ml of solution A reagent in a 100 ml volumetric flask. Solution B must be prepared fresh before use.

Solution A reagent: dissolve 15.35 g of ammonium molybdate ($(NH_4)_6Mo_7O_{24}.4H_2O$, MW 1235.9 g/mol) in approximately 200 ml of DI water in a 500 ml Beaker. Dissolve 0.2743 g of potassium antimony ($K(SbO)C_4H_4O_6$, MW 324.9 g/mol) in approximately 100 ml of DI water in a separate 250 ml Beaker. Put

approximately 300 ml of DI water in a 1000 ml beaker and very slowly add 178 ml of concentrated sulphuric acid (H_2SO_4, 95-98%). Once the acid solution has cooled transfer it to a 1000 ml volumetric flask and add the solutions of ammonium molybdate and potassium antimony. Adjust the volume to 1000 ml with DI water.

A.3 Dick and Tabatabai (Dick & Tabatabai 1977) method, with modifications by He and Honeycutt (2005), for determining inorganic P in alkaline soil extracts

Pipette 2 mL of the alkaline extract to be analysed into a 35 mL vial and add to it 2.5 ml of solution A, immediately followed by 0.5 mL of solution B. After a standardized length of time (e.g. 45 seconds) add 1.25 mL of solution C. It is highly important to add this solution C with this set interval to maintain the reproducibility and sensitivity of the method. After 15 minutes read the absorbance using a UV-Vis spectrophotometer set to the wavelength 850 *n*m (He & Honeycutt 2005), although the colour will be stable for 24 hours.

The concentration of P in the given fraction (mg P/kg) is then calculated by: concentration of P in extract (mg/l) x dilution of extract x (volume of extract (ml) / mass of soil (g)).

Solution A: dissolve 8.80 g of ascorbic acid ($C_6H_8O_6$, MW 176.12 g) and 41.0 g of trichloroacetic acid (Cl_3CCOOH, MW 163.4 g) in a 500 ml volumetric flask containing approximately 400 ml of DI water. Once dissolved make up the volume to 500 ml with more DI water. This solution must always be prepared fresh before use.

Solution B: dissolve 6.20 g of ammonium molybdate ($(NH_4)_6Mo_7O_{24}.4H_2O$, MW 1235.9 g/mol) in a 500 ml volumetric flask containing approximately 400 ml of DI water. Once dissolved make up the volume to 500 ml with more DI water.

Solution C: in a 1000 ml beaker containing at least 800 ml DI water dissolve 29.4 g of trisodium citrate ($Na_3C_6H_5O_7$ MW 294.1 g) while stirring with a magnetic stirrer. Once this has fully dissolved add in 26.0 g of sodium arsenite ($NaAsO_2$, MW 129.9 g). Once this has then fully dissolved add in 50 ml of glacial acetic acid (CH_3COOH 99%, MW 60.1 g/mol). Transfer the content of the beaker to a 1000 ml volumetric flask and adjust the volume up to 1000 ml with more DI water.

A.4 Auto-clave digestion of alkaline soil extracts for determining total P according to USEPA (1983) incorporating the modifications of do Nascimento et al. (2015)

Pipette a 2 ml aliquot of the alkaline soil extract into a digestion tube with a screw cap. Add 10 ml of 7.5% ammonium persulfate ($(NH_4)_2S_2O_8$, MW 228.2 g/mol) followed by 1 ml of 50% sulphuric acid (H_2SO_4, MW 98.1 g/mol). Screw on the cap and autoclave at 121°C and 103 kPa for 120-240 minutes, or until complete digestion has occurred. After digestion allow to cool before making the volume of the extract up to 20 ml with DI water in a 20 ml volumetric flask. Use the Murphy and Riley (1962) (above) to determine P concentration in the extract.

Appendix B: Supplementary Results for Chapter 3

Table B.1. Concentration of P in each P fraction at 0-7.5 cm depth.

Sample Name	Soil Code	P Concentrations 0-7.5 cm (mg P/kg)											
		NH_4Cl Pi	$NaHCO_3$ Pi	$NaHCO_3$ Po	Available P	NaOH I Pi	NaOH I Po	HCl Pi	NaOH II Pi	NaOH II Po	Residual P	Total Pi	Total Po	Total P
Moles worth Station	**MO**	0.35	30.0	73.6	103.9	186.0	571.4	29.3	43.4	121.1	128.7	289.1	766.0	**1183.8**
Glenmore Station	**GM**	1.14	37.7	113.2	152.0	146.3	729.7	187.4	52.4	167.8	134.8	425.0	1010.7	**1570.5**
Omarama Station	**OM**	0.17	12.6	7.3	20.1	83.6	176.5	102.0	50.1	56.8	98.0	248.5	240.6	**587.1**
Ben Dhu Station	**BD**	0.19	21.1	21.3	42.6	135.4	313.1	36.8	51.3	99.7	128.9	244.8	434.1	**807.8**
Lindis Peaks Station	**LP**	0.24	18.8	37.3	56.3	96.8	313.8	67.0	43.0	70.9	105.0	225.7	422.1	**752.8**
Glenfoyle Station	**GF**	0.35	21.1	33.2	54.6	136.2	422.9	43.4	78.6	123.9	152.5	279.6	580.1	**1012.1**
Mt Grand Station	**MG**	0.76	19.0	58.5	78.3	88.8	457.8	36.7	59.9	108.4	168.3	205.2	624.7	**998.1**
Sawdon Station 1	**SW1**	1.48	64.2	36.4	102.1	211.3	552.9	229.6	54.3	107.2	141.3	560.9	696.6	**1398.8**
Sawdon Station 2	**SW2**	1.44	93.1	56.9	151.5	299.5	414.6	280.8	56.2	54.5	84.8	730.9	526.0	**1341.8**
Simon's Hill Station 1	**SH1**	1.02	25.6	39.5	66.2	103.9	549.5	182.7	65.4	153.2	163.0	378.7	742.3	**1284.0**
Simon's Hill Station 1	**SH2**	0.50	18.0	34.4	52.9	96.3	567.4	165.0	54.9	118.9	161.0	334.7	720.7	**1216.4**
The Dasher	**TD**	0.35	20.0	35.5	55.9	107.4	364.3	18.6	68.9	96.2	204.4	215.3	496.1	**915.8**
Waikora Station	**WK**	1.03	34.3	17.8	53.2	136.6	213.0	79.0	60.3	65.1	116.2	311.2	295.9	**723.3**
Ida Valley Station 1	**IV1**	0.73	26.2	59.1	85.9	129.5	372.6	57.9	81.1	82.8	202.6	295.4	514.5	**1012.5**
Ida Valley Station 2	**IV2**	0.85	31.6	33.8	66.3	146.6	315.9	147.9	64.1	105.5	183.7	391.1	455.2	**1029.9**
Glenmore Ripper Site	**GMR**	1.09	61.7	44.7	107.4	164.5	470.6	224.5	46.4	97.6	93.1	498.2	612.8	**1204.0**
Omarama Ripper Site	**OMR**	0.40	32.3	18.6	51.3	138.0	214.8	129.5	57.8	69.0	103.9	357.9	302.4	**764.2**
The Dasher Paradise Sit	**TDP**	0.44	18.4	50.8	69.7	90.5	369.1	7.0	52.0	141.3	186.6	168.3	561.2	**916.1**
The Dasher PH Lane Site	**TDPH**	0.48	13.1	58.8	72.4	71.6	379.6	3.3	33.8	110.5	141.6	122.2	548.9	**812.8**

Table B.2. Concentration of P in each P fraction at 7.5-15 cm depth.

Sample Name	Soil Code	P Concentrations 7.5-15 cm (mg P/kg)											
		NH_4Cl Pi	$NaHCO_3$ Pi	$NaHCO_3$ Po	Available P	NaOH I Pi	NaOH I Po	HCl Pi	NaOH II Pi	NaOH II Po	Residual P	Total Pi	Total Po	Total P
Molesworth Station	**MO**	0.25	41.7	40.6	82.6	202.3	555.9	302.8	54.7	59.7	94.2	601.8	656.1	**1352.2**
Glenmore Station	**GM**	0.17	24.5	39.1	63.7	131.6	702.3	184.3	49.3	98.4	122.3	389.9	839.8	**1352.0**
Omarama Station	**OM**	0.29	16.0	20.8	37.1	95.5	571.2	186.1	65.8	102.0	141.5	363.7	694.0	**1199.2**
Ben Dhu Station	**BD**	0.30	11.5	30.7	42.4	88.7	608.9	141.7	56.9	83.3	131.8	299.1	722.8	**1153.8**
Lindis Peaks Station	**LP**	0.22	10.2	21.6	32.0	78.5	294.6	7.6	61.0	64.2	165.5	157.5	380.5	**703.5**
Glenfoyle Station	**GF**	0.36	19.4	16.1	35.9	123.9	266.1	53.0	58.3	63.7	124.4	255.1	345.8	**725.3**
Mt Grand Station	**MG**	0.20	17.2	24.5	41.9	126.4	321.6	40.6	79.7	92.3	162.6	264.1	438.4	**865.1**
Sawdon Station 1	**SW1**	0.12	12.9	9.4	22.5	117.7	312.4	118.2	74.7	72.4	144.8	323.6	394.3	**862.7**
Sawdon Station 2	**SW2**	0.09	8.0	35.6	43.7	96.3	635.7	17.2	34.5	129.4	120.3	156.1	800.7	**1077.0**
Simon's Hill Station 1	**SH1**	0.19	17.5	67.2	84.9	105.9	722.7	180.4	49.8	122.0	102.3	353.7	911.8	**1367.9**
Simon's Hill Station 1	**SH2**	0.10	9.0	21.6	30.7	71.0	218.5	81.3	51.3	52.8	92.8	212.8	292.8	**598.4**
The Dasher	**TD**	0.17	7.6	13.0	20.7	95.9	311.3	27.0	51.2	87.0	134.5	182.0	411.3	**727.7**
Waikora Station	**WK**	0.19	14.6	45.1	59.9	87.7	333.8	57.0	42.2	75.7	112.6	201.7	454.5	**768.8**
Ida Valley Station 1	**IV1**	0.14	13.5	28.0	41.7	112.3	430.8	36.8	77.7	110.9	147.4	240.5	569.7	**957.5**
Ida Valley Station 2	**IV2**	0.11	6.7	19.1	25.9	56.4	428.2	26.4	56.3	98.3	139.2	145.9	545.6	**830.6**
Glenmore Ripper Site	**GMR**	0.12	13.9	37.9	52.0	76.6	660.7	192.5	46.2	84.6	79.1	329.3	783.2	**1191.6**
Omarama Ripper Site	**OMR**	0.19	18.8	10.1	29.1	108.2	213.1	104.7	55.4	64.6	99.4	287.3	287.8	**674.6**
The Dasher Paradise Sit	**TDP**	0.24	10.2	43.3	53.8	62.9	342.7	3.5	47.2	95.5	154.7	124.1	481.5	**760.3**
The Dasher PH Lane Site	**TDPH**	0.16	9.2	44.0	53.3	57.4	332.7	2.4	29.8	80.3	116.3	98.9	457.0	**672.1**

Table B.3. Concentration of P in each P fraction as a proportion of Total P at 0-7.5 cm depth.

Sample Name	Soil Code	P concentrations as a proportion of Total P in soil at 0-7.5 cm (% of Total P)											
		NH_4Cl Pi	$NaHCO_3$ Pi	$NaHCO_3$ Po	Available P	NaOH I Pi	NaOH I Po	HCl Pi	NaOH II Pi	NaOH II Po	Residual P	Total Pi	Total Po	Total P
Molesworth Station	**MO**	0.03	2.54	6.21	8.78	15.72	48.27	2.47	3.67	10.23	10.87	24.42	64.71	**100**
Glenmore Station	**GM**	0.07	2.40	7.21	9.68	9.32	46.47	11.93	3.34	10.68	8.58	27.06	64.36	**100**
Omarama Station	**OM**	0.03	2.15	1.24	3.43	14.25	30.07	17.37	8.53	9.67	16.69	42.33	40.98	**100**
Ben Dhu Station	**BD**	0.02	2.61	2.64	5.28	16.77	38.76	4.55	6.36	12.34	15.95	30.31	53.74	**100**
Lindis Peaks Station	**LP**	0.03	2.49	4.96	7.48	12.86	41.68	8.89	5.71	9.42	13.95	29.98	56.06	**100**
Glenfoyle Station	**GF**	0.03	2.08	3.28	5.40	13.46	41.79	4.29	7.76	12.25	15.07	27.62	57.31	**100**
Mt Grand Station	**MG**	0.08	1.91	5.86	7.84	8.89	45.87	3.68	6.00	10.86	16.86	20.56	62.59	**100**
Sawdon Station 1	**SW1**	0.11	4.59	2.60	7.30	15.11	39.53	16.41	3.88	7.67	10.10	40.10	49.80	**100**
Sawdon Station 2	**SW2**	0.11	6.94	4.24	11.29	22.32	30.90	20.93	4.18	4.06	6.32	54.48	39.20	**100**
Simon's Hill Station 1	**SH1**	0.08	2.00	3.08	5.15	8.09	42.80	14.23	5.10	11.94	12.69	29.50	57.81	**100**
Simon's Hill Station 1	**SH2**	0.04	1.48	2.83	4.35	7.92	46.64	13.57	4.52	9.77	13.23	27.52	59.25	**100**
The Dasher	**TD**	0.04	2.19	3.88	6.10	11.72	39.78	2.03	7.53	10.51	22.32	23.51	54.17	**100**
Waikora Station	**WK**	0.14	4.74	2.47	7.35	18.89	29.45	10.92	8.33	9.00	16.06	43.03	40.91	**100**
Ida Valley Station 1	**IV1**	0.07	2.58	5.83	8.49	12.79	36.80	5.72	8.01	8.18	20.01	29.18	50.81	**100**
Ida Valley Station 2	**IV2**	0.08	3.07	3.28	6.43	14.23	30.67	14.36	6.23	10.25	17.83	37.97	44.20	**100**
Glenmore Ripper Site	**GMR**	0.09	5.12	3.71	8.92	13.66	39.08	18.65	3.85	8.11	7.73	41.37	50.90	**100**
Omarama Ripper Site	**OMR**	0.05	4.23	2.44	6.72	18.05	28.10	16.94	7.56	9.03	13.60	46.83	39.57	**100**
The Dasher Paradise Sit	**TDP**	0.05	2.01	5.54	7.60	9.87	40.29	0.76	5.67	15.43	20.37	18.37	61.26	**100**
The Dasher PH Lane Site	**TDPH**	0.06	1.61	7.24	8.90	8.80	46.71	0.41	4.16	13.59	17.43	15.04	67.54	**100**

Table B.4. Concentration of P in each P fraction as a proportion of Total P at 7.5-15 cm depth.

Sample Name	Soil Code	P concentrations as a proportion of Total P in soil at 7.5-15 cm (% of Total P)											
		NH_4Cl Pi	$NaHCO_3$ Pi	$NaHCO_3$ Po	Available P	NaOH I Pi	NaOH I Po	HCl Pi	NaOH II Pi	NaOH II Po	Residual P	Total Pi	Total Po	Total P
Molesworth Station	**MO**	0.02	3.09	3.00	6.11	14.96	41.11	22.39	4.05	4.42	6.97	44.51	48.53	**100**
Glenmore Station	**GM**	0.01	1.81	2.89	4.71	9.73	51.95	13.63	3.65	7.28	9.05	28.84	62.12	**100**
Omarama Station	**OM**	0.02	1.33	1.74	3.09	7.96	47.63	15.52	5.49	8.51	11.80	30.33	57.87	**100**
Ben Dhu Station	**BD**	0.03	0.99	2.66	3.68	7.69	52.78	12.28	4.93	7.22	11.43	25.92	62.65	**100**
Lindis Peaks Station	**LP**	0.03	1.44	3.08	4.55	11.16	41.88	1.08	8.67	9.12	23.53	22.38	54.08	**100**
Glenfoyle Station	**GF**	0.05	2.68	2.22	4.95	17.09	36.68	7.31	8.04	8.78	17.15	35.17	47.68	**100**
Mt Grand Station	**MG**	0.02	1.99	2.83	4.84	14.61	37.18	4.69	9.21	10.67	18.79	30.53	50.68	**100**
Sawdon Station 1	**SW1**	0.01	1.50	1.09	2.60	13.64	36.21	13.71	8.66	8.40	16.79	37.51	45.70	**100**
Sawdon Station 2	**SW2**	0.01	0.75	3.30	4.05	8.94	59.02	1.59	3.20	12.02	11.17	14.49	74.34	**100**
Simon's Hill Station 1	**SH1**	0.01	1.28	4.91	6.21	7.74	52.83	13.19	3.64	8.92	7.48	25.86	66.66	**100**
Simon's Hill Station 1	**SH2**	0.02	1.51	3.61	5.13	11.86	36.51	13.59	8.57	8.82	15.51	35.55	48.93	**100**
The Dasher	**TD**	0.02	1.04	1.78	2.85	13.18	42.78	3.72	7.03	11.95	18.48	25.00	56.52	**100**
Waikora Station	**WK**	0.02	1.90	5.87	7.79	11.41	43.41	7.41	5.49	9.84	14.64	26.24	59.12	**100**
Ida Valley Station 1	**IV1**	0.01	1.41	2.93	4.35	11.73	44.99	3.85	8.12	11.58	15.39	25.11	59.50	**100**
Ida Valley Station 2	**IV2**	0.01	0.81	2.30	3.12	6.79	51.55	3.18	6.77	11.84	16.75	17.57	65.68	**100**
Glenmore Ripper Site	**GMR**	0.01	1.17	3.18	4.36	6.42	55.45	16.15	3.88	7.10	6.64	27.64	65.73	**100**
Omarama Ripper Site	**OMR**	0.03	2.79	1.49	4.31	16.04	31.60	15.53	8.21	9.58	14.74	42.59	42.67	**100**
The Dasher Paradise Sit	**TDP**	0.03	1.34	5.70	7.07	8.28	45.08	0.46	6.21	12.56	20.35	16.32	63.34	**100**
The Dasher PH Lane Site	**TDPH**	0.02	1.37	6.54	7.94	8.53	49.49	0.36	4.43	11.95	17.30	14.71	67.99	**100**

Table B.5. Pearson's correlation matrix for soil P fractionation and chemical data at 0-7.5 cm depth.

Pearsons correlation matrix for soil P fractionation and chemical data at 0-7.5 cm depth

NH_4Cl Pi	1	-																												
$NaHCO_3$ Pi	2	0.80	-																											
$NaHCO_3$ Po	3	0.33	0.18	-																										
Total Available P	4	0.54	0.45	0.60	-																									
NaOH I Pi	5	0.63	0.92	0.19	0.37	-																								
NaOH I Po	6	0.44	0.24	0.75	0.42	0.23	-																							
HCl Pi	7	0.77	0.79	0.08	0.15	0.64	0.35	-																						
NaOH II Pi	8	0.11	-0.03	-0.15	-0.26	0.05	-0.06	-0.01	-																					
NaOH II Po	9	0.10	-0.23	0.57	0.18	-0.23	0.76	-0.05	0.02	-																				
Residual P	10	-0.13	-0.45	0.15	-0.03	-0.40	0.17	-0.42	0.55	0.47	-																			
Total Pi	11	0.80	0.94	0.13	0.27	0.87	0.32	0.93	0.09	-0.15	-0.41	-																		
Total Po	12	0.39	0.17	0.80	0.43	0.16	0.99	0.27	-0.06	0.82	0.23	0.24	-																	
Total P	13	0.71	0.58	0.66	0.45	0.55	0.90	0.65	0.08	0.57	0.07	0.67	0.87	-																
$pH_{(H2O)}$	14	0.14	0.12	-0.54	-0.36	0.15	-0.29	0.26	0.37	-0.26	0.10	0.25	-0.34	-0.08	-															
$pH_{(CaCl2)}$	15	0.12	0.03	-0.40	-0.22	-0.04	-0.22	0.19	0.28	-0.13	0.19	0.12	-0.25	-0.08	0.89	-														
Exch Al	16	-0.20	-0.17	0.43	0.32	-0.10	0.21	-0.35	-0.46	0.23	-0.07	-0.30	0.26	0.00	-0.87	-0.86	-													
Olsen P	17	0.74	0.98	0.25	0.43	0.93	0.25	0.73	-0.07	-0.24	-0.43	0.90	0.18	0.57	0.07	-0.01	-0.12	-												
SO_4-S	18	0.34	0.45	0.43	0.58	0.23	0.25	0.34	-0.39	0.00	-0.30	0.31	0.25	0.31	-0.48	-0.22	0.26	0.45	-											
Org-S	19	-0.07	-0.14	0.63	0.52	-0.22	0.30	-0.30	-0.36	0.46	0.21	-0.30	0.39	0.13	-0.58	-0.25	0.46	-0.07	0.56	-										
ASC	20	-0.34	-0.23	0.46	0.26	-0.14	0.26	-0.47	-0.45	0.38	0.08	-0.40	0.32	0.02	-0.80	-0.73	0.88	-0.15	0.27	0.62	-									
C%	21	-0.01	-0.19	0.71	0.37	-0.22	0.53	-0.26	-0.14	0.72	0.45	-0.27	0.62	0.35	-0.58	-0.40	0.50	-0.15	0.30	0.76	0.70	-								
N%	22	0.08	-0.19	0.74	0.37	-0.25	0.62	-0.18	-0.01	0.80	0.54	-0.22	0.70	0.45	-0.46	-0.25	0.31	-0.18	0.28	0.73	0.50	0.96	-							
C:N Ratio	23	-0.19	0.08	0.10	0.05	0.17	-0.10	-0.22	-0.39	-0.07	-0.18	-0.09	-0.07	-0.13	-0.48	-0.60	0.63	0.15	0.10	0.19	0.74	0.37	0.09	-						
CEC	24	0.08	-0.20	0.39	0.28	-0.26	0.44	-0.21	0.07	0.66	0.70	-0.24	0.50	0.32	0.01	0.26	-0.02	-0.16	0.05	0.60	0.26	0.65	0.71	-0.04	-					
BS%	25	0.20	-0.08	-0.08	-0.11	-0.16	0.18	0.14	0.34	0.25	0.50	0.04	0.17	0.21	0.69	0.86	-0.72	-0.10	-0.20	-0.06	-0.55	-0.06	0.13	-0.61	0.59	-				
Bulk Density	26	0.03	0.28	-0.57	-0.31	0.29	-0.61	0.29	0.26	-0.82	-0.40	0.33	-0.68	-0.35	0.57	0.45	-0.63	0.24	-0.11	-0.63	-0.74	-0.82	-0.76	-0.33	-0.66	0.07	-			
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26			

Table B.6. Pearson's correlation matrix for soil P fractionation as proportion of Total P and chemical data at 0-7.5 cm depth.

Pearsons correlation matrix for soil P fractionation as proportion of Total P and chemical data at 0-7.5 cm depth

		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26
NH_4Cl Pi	1	-																									
$NaHCO_3$ Pi	2	0.66	-																								
$NaHCO_3$ Po	3	-0.06	-0.27	-																							
Total Available P	4	0.45	0.51	0.69	-																						
NaOH I Pi	5	0.30	0.80	-0.40	0.24	-																					
NaOH I Po	6	-0.35	-0.59	0.62	0.11	-0.71	-																				
HCl Pi	7	0.45	0.65	-0.55	0.00	0.40	-0.54	-																			
NaOH II Pi	8	-0.13	-0.14	-0.47	-0.52	0.22	-0.58	-0.13	-																		
NaOH II Po	9	-0.48	-0.77	0.26	-0.35	-0.63	0.45	-0.71	0.07	-																	
Residual P	10	-0.32	-0.58	0.01	-0.42	-0.31	-0.08	-0.64	0.67	0.55	-																
Total Pi	11	0.48	0.84	-0.62	0.08	0.79	-0.80	0.87	0.15	-0.79	-0.50	-															
Total Po	12	-0.39	-0.69	0.71	0.12	-0.76	0.96	-0.69	-0.49	0.64	0.09	-0.91	-														
Total P	13	-	-	-	-	-	-	-	-	-	-	-	-	-													
$pH_{(H2O)}$	14	0.21	0.28	-0.64	-0.36	0.36	-0.55	0.38	0.35	-0.30	0.09	0.48	-0.60	-	-												
$pH_{(CaCl2)}$	15	0.17	0.19	-0.40	-0.22	0.15	-0.42	0.28	0.27	-0.19	0.15	0.31	-0.43	-	0.89	-											
Exch Al	16	-0.22	-0.27	0.58	0.32	-0.22	0.50	-0.45	-0.37	0.32	-0.04	-0.47	0.56	-	-0.87	-0.86	-										
Olsen P	17	0.55	0.69	-0.10	0.43	0.38	-0.10	0.48	-0.49	-0.57	-0.61	0.47	-0.25	-	0.07	-0.01	-0.12	-									
SO_4-S	18	0.21	0.28	0.41	0.58	-0.13	0.21	0.17	-0.55	-0.19	-0.39	0.01	0.18	-	-0.48	-0.22	0.26	0.45	-								
Org-S	19	-0.13	-0.25	0.79	0.52	-0.43	0.47	-0.48	-0.40	0.45	0.10	-0.57	0.61	-	-0.58	-0.25	0.46	-0.07	0.56	-							
ASC	20	-0.43	-0.38	0.61	0.26	-0.30	0.61	-0.64	-0.39	0.53	0.09	-0.65	0.70	-	-0.80	-0.73	0.88	-0.15	0.27	0.62	-						
C%	21	-0.16	-0.37	0.73	0.37	-0.53	0.57	-0.50	-0.41	0.50	0.12	-0.65	0.68	-	-0.58	-0.40	0.50	-0.15	0.30	0.76	0.70	-					
N%	22	-0.10	-0.37	0.72	0.37	-0.59	0.56	-0.42	-0.39	0.44	0.09	-0.61	0.66	-	-0.46	-0.25	0.31	-0.18	0.28	0.73	0.50	0.96	-				
C:N Ratio	23	-0.23	-0.08	0.13	0.05	0.06	0.17	-0.34	-0.18	0.30	0.09	-0.24	0.23	-	-0.48	-0.60	0.63	0.15	0.10	0.19	0.74	0.37	0.09	-			
CEC	24	-0.06	-0.24	0.52	0.28	-0.41	0.35	-0.40	-0.26	0.35	0.24	-0.49	0.45	-	0.01	0.26	-0.02	-0.16	0.05	0.60	0.26	0.65	0.71	-0.04	-		
BS%	25	0.13	-0.05	-0.09	-0.11	-0.20	-0.04	0.10	0.05	-0.06	0.15	-0.01	-0.06	-	0.69	0.86	-0.72	-0.10	-0.20	-0.06	-0.55	-0.06	0.13	-0.61	0.59	-	
Bulk Density	26	0.18	0.40	-0.68	-0.31	0.53	-0.68	0.51	0.51	-0.56	0.00	0.67	-0.77	-	0.57	0.45	-0.63	0.24	-0.11	-0.63	-0.74	-0.82	-0.76	-0.33	-0.66	0.07	-

Table B.7. Pearson's correlation matrix for soil P fractionation and chemical data at 7.5-15 cm depth.

NH_4Cl Pi	1	-														
$NaHCO_3$ Pi	2	0.38	-													
$NaHCO_3$ Po	3	-0.03	0.21	-												
Total Available P	4	0.15	0.61	0.90	-											
NaOH I Pi	5	0.30	0.90	0.01	0.40	-										
NaOH I Po	6	-0.03	0.28	0.59	0.60	0.21	-									
HCl Pi	7	0.21	0.76	0.25	0.54	0.63	0.60	-								
NaOH II Pi	8	0.18	0.15	-0.51	-0.35	0.35	-0.20	0.08	-							
NaOH II Po	9	-0.26	-0.25	0.42	0.23	-0.14	0.63	-0.10	-0.08	-						
Residual P	10	0.19	-0.34	-0.33	-0.41	-0.15	-0.27	-0.54	0.54	0.21	-					
Total Pi	11	0.28	0.88	0.14	0.50	0.83	0.48	0.95	0.28	-0.14	-0.39	-				
Total Po	12	-0.05	0.23	0.65	0.62	0.17	0.99	0.53	-0.23	0.69	-0.24	0.42	-			
Total P	13	0.11	0.55	0.52	0.66	0.50	0.94	0.78	0.01	0.47	-0.26	0.73	0.92	-		
$pH_{(H2O)}$	14	0.50	0.31	-0.42	-0.21	0.37	-0.05	0.38	0.39	-0.42	0.05	0.44	-0.13	0.11	-	
$pH_{(CaCl2)}$	15	0.60	0.37	-0.27	-0.05	0.38	0.18	0.49	0.34	-0.27	0.12	0.52	0.11	0.33	0.91	-
Exch Al	16	-0.36	-0.29	0.26	0.08	-0.33	-0.08	-0.39	-0.47	0.21	-0.13	-0.45	-0.03	-0.24	-0.83	-0.82
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15

Table B.8. Pearson's correlation matrix for soil P fractionation as proportion of Total P and chemical data at 0-7.5 cm depth.

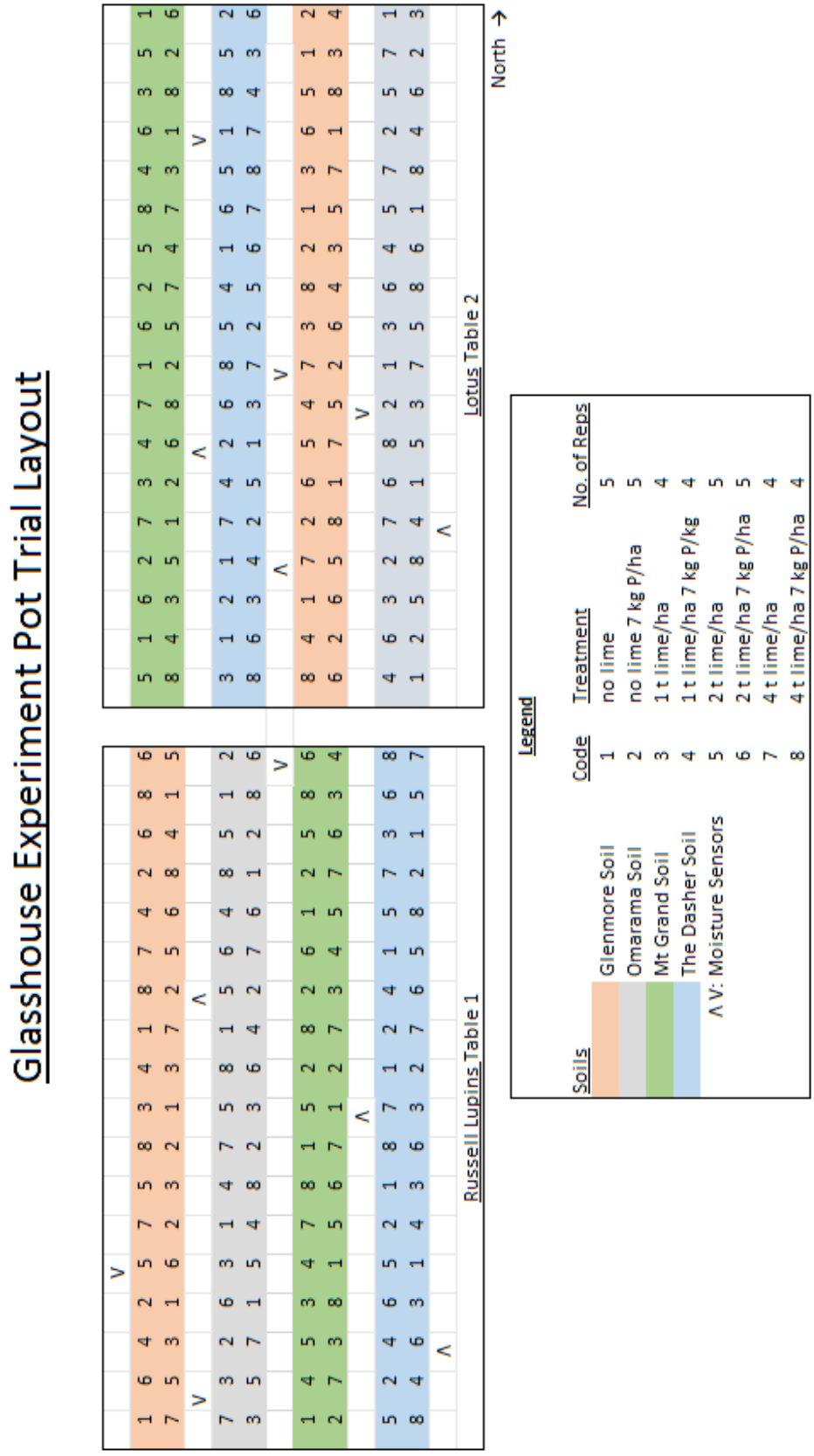
NH_4Cl Pi	1	-														
$NaHCO_3$ Pi	2	0.45	-													
$NaHCO_3$ Po	3	0.01	-0.13	-												
Total Available P	4	0.20	0.29	0.91	-											
NaOH I Pi	5	0.47	0.78	-0.38	-0.04	-										
NaOH I Po	6	-0.51	-0.67	0.30	0.01	-0.85	-									
HCl Pi	7	-0.21	0.46	-0.36	-0.15	0.16	-0.15	-								
NaOH II Pi	8	0.41	0.22	-0.40	-0.29	0.59	-0.81	-0.23	-							
NaOH II Po	9	0.05	-0.48	0.28	0.07	-0.15	0.08	-0.87	0.24	-						
Residual P	10	0.52	-0.08	0.02	-0.01	0.28	-0.50	-0.72	0.75	0.61	-					
Total Pi	11	0.15	0.77	-0.52	-0.18	0.70	-0.68	0.80	0.30	-0.70	-0.27	-				
Total Po	12	-0.43	-0.73	0.51	0.18	-0.85	0.94	-0.41	-0.71	0.36	-0.28	-0.85	-			
Total P	13	-	-	-	-	-	-	-	-	-	-	-	-	-		
$pH_{(H2O)}$	14	0.32	0.26	-0.57	-0.44	0.31	-0.31	0.43	0.25	-0.55	-0.04	0.52	-0.50	-	-	
$pH_{(CaCl2)}$	15	0.29	0.18	-0.54	-0.44	0.11	-0.08	0.43	0.03	-0.60	-0.14	0.39	-0.31	-	0.91	-
Exch Al	16	-0.14	-0.19	0.52	0.42	-0.18	0.23	-0.42	-0.22	0.47	0.09	-0.45	0.40	-	-0.83	-0.82
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15

Table B.9. PCA vector loadings for Principal Components 1-3.

	Vector Loadings		
Variable	**PC1**	**PC2**	**PC3**
NH_4Cl Pi	-0.06	0.07	0.36
$NaHCO_3$ Pi	-0.12	-0.05	0.36
$NaHCO_3$ Po	0.22	-0.02	0.24
NaOH I Pi	-0.12	-0.07	0.32
NaOH I Po	0.17	0.06	0.28
HCl Pi	-0.15	0.03	0.34
NaOH II Pi	-0.08	0.17	-0.02
NaOH II Po	0.25	0.12	0.10
Residual P	0.16	0.23	-0.10
Total P	0.08	0.07	0.37
$pH_{(H2O)}$	-0.21	0.24	-0.03
$pH_{(CaCl2)}$	-0.14	0.28	-0.03
Exch Al	0.21	-0.24	-0.02
Olsen P	-0.10	-0.06	0.35
SO_4-S	0.08	-0.11	0.22
Org-S	0.27	-0.03	0.04
ASC	0.26	-0.19	-0.03
C%	0.31	0	0.08
N%	0.29	0.07	0.10
C:N Ratio	0.11	-0.22	-0.01
CEC	0.24	0.21	0.04
BS%	-0.01	0.33	0.02
Ca (me/100g)	0.11	0.29	0.04
Mg (me/100g)	0.11	0.30	0.01
K (me/100g)	0.14	0.26	0.02
Na (me/100g)	0.26	0.05	0.10
Bulk Density	-0.31	0	-0.04

Appendix C: Supplementary Methods for Chapter 4

Plate C.1. Trial layout for exhaustive pot experiment



Appendix D: Supplementary Results for Chapter 4

D.1 Regression equations and correlation coefficients for graphed results

Figure 4.3.1. The effect of incorporated lime on soil pH_{H2O} in each soil at the conclusion of the experiment.

Soil	Regression Equation	r^2
GM	$y = -0.20x^2 + 1.5176x + 4.77$	0.99
OM	$y = -0.29x^2 + 1.70x + 5.52$	0.96
MG	$y = -0.28x^2 + 1.72x + 5.22$	0.99
TD	$y = -0.15x^2 + 1.36x + 4.53$	0.98

Figure 4.3.2. Soil Al concentration (mg Al/kg soil) in relation to soil pH_{H2O} in; a: the GM soil, b: the OM soil, c: the MG soil, and d: the TD soil.

Soil	Species x P	Regression Equation	r^2
GM (a)	Lupin	$y = 242x^{-3.83}$	0.98
	Lotus	$y = 5030x^{-4.32}$	0.95
OM (b)	Lupin	$y = 166x^{-2.19}$	0.81
	Lotus	$y = 0.28x^{0.90}$	0.06
MG (c)	Lupin	$y = 172x^{-2.47}$	0.50
	Lotus	$y = 52.3x^{-1.59}$	0.65
TD (d)	Lupin	$y = 20600x^{-4.91}$	0.85
	Lotus	$y = 26900x^{-5.11}$	0.79

Figure 4.3.4. The effect of soil pH on Russell lupin and Lotus shoot yield (g DM/pot) in; a: the GM soil, b: the OM soil, c: the MG soil, and d: the TD soil.

Soil	Species x P	Regression Equation	r^2
GM (a)	Lupin no P	$y = -0.69x^2 + 8.21x - 20.2$	0.90
	Lupin +P	$y = -0.54x^2 + 7.22x - 19.9$	0.99
	Lotus no P	$y = -0.37x^2 + 4.58x - 9.92$	0.99
	Lotus +P	$y = -0.64x^2 + 7.60x - 17.9$	0.98
OM (b)	Lupin no P	$y = -0.80x^2 + 9.34x - 23.2$	0.97
	Lupin +P	$y = -0.75x^2 + 9.47x - 25.8$	0.83
	Lotus no P	$y = -0.44x^2 + 6.02x - 17.7$	0.93
	Lotus +P	$y = -0.53x^2 + 7.00x - 19.43$	0.99
MG (c)	Lupin no P	$y = -0.96x^2 + 11.6x - 30.9$	0.97
	Lupin +P	$y = -0.75x^2 + 9.20x - 24.28$	0.82
	Lotus no P	$y = -0.05x + 3.72$	0.36
	Lotus +P	$y = -0.33x^2 + 3.93x - 7.10$	0.78
TD (d)	Lupin no P	$y = -0.59x^2 + 7.09x - 18.9$	0.94
	Lupin +P	$y = -0.65x^2 + 7.74x - 19.4$	0.97
	Lotus no P	$y = -0.51x^2 + 6.15x - 14.8$	0.77
	Lotus +P	$y = -0.48x^2 + 5.95x - 14.55$	0.64

Figure 4.3.5. The effect of soil pH on Russell lupin and Lotus root yield (g DM/pot) in; a: the GM soil, b: the OM soil, c: the MG soil, and d: the TD soil.

Soil	Species x P	Regression Equation	r^2
GM (a)	Lupin no P	$y = -0.22x^2 + 2.37x - 4.65$	0.99
	Lupin +P	$y = -0.14x^2 + 1.78x - 3.89$	0.84
	Lotus no P	$y = -0.09x^2 + 0.92x - 0.19$	0.99
	Lotus +P	$y = -0.11x^2 + 1.18x - 0.97$	0.99
OM (b)	Lupin no P	$y = -0.52x^2 + 6.38x - 17.5$	0.98
	Lupin +P	$y = -0.22x^2 + 2.66x - 6.34$	0.49
	Lotus no P	$y = 0.14x + 0.55$	0.88
	Lotus +P	$y = 0.38x^2 - 5.17x + 19.3$	0.96
MG (c)	Lupin no P	$y = -0.62x^2 + 7.68x - 22.0$	0.91
	Lupin +P	$y = 0.07x + 0.90$	0.07
	Lotus no P	$y = -0.15x + 2.78$	0.97
	Lotus +P	$y = -0.19x^2 + 2.22x - 4.19$	0.69
TD (d)	Lupin no P	$y = -0.46x^2 + 5.60x - 15.7$	0.98
	Lupin +P	$y = -0.35x^2 + 4.10x - 10.2$	0.95
	Lotus no P	$y = -0.53x^2 + 6.55x - 17.5$	0.99
	Lotus +P	$y = 0.03x^2 - 0.41x + 3.62$	0.86

Figure 4.3.6. The effect of soil pH on Russell lupin and Lotus shoot P concentration (mg P/kg DM) averaged over the six harvests of shoot material in; a: the GM soil, b: the OM soil, c: the MG soil, and d: the TD soil.

Soil	Species x P	Regression Equation	r^2
GM (a)	Lupin no P	$y = -118x^2 + 1412x - 3170$	0.89
	Lupin +P	$y = -60.0x^2 + 717x - 1051$	0.99
	Lotus no P	$y = -51.5x^2 + 672x - 1241$	0.98
	Lotus +P	$y = -128x^2 + 1543x - 3549$	0.88
OM (b)	Lupin no P	$y = -133x^2 + 1633x - 4076$	0.93
	Lupin +P	$y = -61.2x^2 + 761x - 1389$	0.63
	Lotus no P	$y = -69.9x^2 + 927x - 2319$	0.93
	Lotus +P	$y = -21.7x^2 + 266x - 10.7$	0.99
MG (c)	Lupin no P	$y = -23.5x^2 + 222x + 367$	0.97
	Lupin +P	$y = -173x^2 + 2065x - 4911$	0.97
	Lotus no P	$y = -174x^2 + 2232x - 6148$	0.82
	Lotus +P	$y = -129x^2 + 1640x - 4211$	0.99
TD (d)	Lupin no P	$y = -29.5x^2 + 304x - 43.5$	0.90
	Lupin +P	$y = -67.1x^2 + 754x - 1166$	0.79
	Lotus no P	$y = -43.8x^2 + 522x - 786$	0.65
	Lotus +P	$y = -25.1x^2 + 241x + 330$	0.99

Figure 4.3.7. The effect of soil pH on Russell lupin and Lotus total shoot P uptake (mg P/pot) in; a: the GM soil, b: the OM soil, c: the MG soil, and d: the TD soil.

Soil	Species x P	Regression Equation	r^2
GM (a)	Lupin no P	$y = -0.96x^2 + 11.4x - 29.9$	0.92
	Lupin +P	$y = -0.71x^2 + 9.03x - 24.2$	0.99
	Lotus no P	$y = -0.54x^2 + 6.79x - 17.4$	0.99
	Lotus +P	$y = -1.15x^2 + 13.8x - 36.1$	0.97
OM (b)	Lupin no P	$y = -0.70x^2 + 8.12x - 20.5$	0.96
	Lupin +P	$y = -0.84x^2 + 10.5x - 28.8$	0.99
	Lotus no P	$y = -0.51x^2 + 6.81x - 20.8$	0.93
	Lotus +P	$y = -0.29x^2 + 3.41x - 6.77$	0.99
MG (c)	Lupin no P	$y = -0.82x^2 + 9.80x - 25.8$	0.99
	Lupin +P	$y = -1.40x^2 + 16.8x - 45.3$	0.97
	Lotus no P	$y = -0.52x^2 + 6.61x - 17.8$	0.98
	Lotus +P	$y = -0.82x^2 + 10.2x - 27.1$	0.96
TD (d)	Lupin no P	$y = -0.46x^2 + 5.49x - 14.6$	0.88
	Lupin +P	$y = -0.71x^2 + 8.35x - 21.4$	0.94
	Lotus no P	$y = -0.48x^2 + 5.74x - 14.4$	0.99
	Lotus +P	$y = -0.38x^2 + 4.37x - 9.28$	0.65

Figure 4.3.8. The effect of soil pH on Russell lupin and Lotus total P uptake (mg P/pot) in; a: the GM soil, b: the OM soil, c: the MG soil, and d: the TD soil.

Soil	Species x P	Regression Equation	r^2
GM (a)	Lupin no P	$y = -1.25x^2 + 14.9x - 38.4$	0.95
	Lupin +P	$y = -0.92x^2 + 11.8x - 32.0$	0.99
	Lotus no P	$y = -0.93x^2 + 11.6x - 30.5$	0.99
	Lotus +P	$y = -1.56x^2 + 18.8x - 49.4$	0.95
OM (b)	Lupin no P	$y = -0.99x^2 + 11.6x - 30.0$	0.96
	Lupin +P	$y = -1.08x^2 + 13.4x - 36.1$	0.98
	Lotus no P	$y = -0.51x^2 + 7.01x - 21.1$	0.99
	Lotus +P	$y = -0.33x^2 + 3.80x - 6.63$	0.97
MG (c)	Lupin no P	$y = -1.16x^2 + 14.0x - 38.0$	0.94
	Lupin +P	$y = -1.49x^2 + 17.9x - 47.2$	0.90
	Lotus no P	$y = -0.47x^2 + 6.06x - 15.1$	0.89
	Lotus +P	$y = -0.98x^2 + 12.2x - 32.3$	0.97
TD (d)	Lupin no P	$y = -0.62x^2 + 7.42x - 19.9$	0.91
	Lupin +P	$y = -0.99x^2 + 11.6x - 29.5$	0.99
	Lotus no P	$y = -0.85x^2 + 10.0x - 25.7$	0.89
	Lotus +P	$y = -0.35x^2 + 3.92x - 6.21$	0.70

Figure 4.3.9. The effect of soil pH on utilisation (%) of initial plant available P by Russell lupin and Lotus in; a: the GM soil, b: the OM soil, c: the MG soil, and d: the TD soil.

Soil	Species x P	Regression Equation	r^2
GM (a)	Lupin no P	$y = -13.3x^2 + 157x - 406$	0.95
	Lupin +P	$y = -6.65x^2 + 85.2x - 230$	0.99
	Lotus no P	$y = -9.87x^2 + 123x - 324$	0.99
	Lotus +P	$y = -11.2x^2 + 135x - 355$	0.95
OM (b)	Lupin no P	$y = -26.6x^2 + 312x - 803$	0.96
	Lupin +P	$y = -13.1x^2 + 162x - 439$	0.98
	Lotus no P	$y = -13.8x^2 + 188x - 566$	0.99
	Lotus +P	$y = -3.98x^2 + 46.2x - 80.5$	0.97
MG (c)	Lupin no P	$y = -22.8x^2 + 275x - 748$	0.94
	Lupin +P	$y = -15.6x^2 + 186x - 493$	0.90
	Lotus no P	$y = -9.97x^2 + 127x - 318$	0.76
	Lotus +P	$y = -10.2x^2 + 128x - 337$	0.97
TD (d)	Lupin no P	$y = -13.6x^2 + 164x - 440$	0.91
	Lupin +P	$y = -10.9x^2 + 128x - 327$	0.99
	Lotus no P	$y = -14.0x^2 + 168x - 417$	0.96
	Lotus +P	$y = -3.91x^2 + 43.4x - 68.8$	0.70

Figure 4.3.10. The effect of soil pH on soil Olsen P in Russell lupin and Lotus pots at the conclusion of the experiment in; a: the GM soil, b: the OM soil, c: the MG soil, and d: the TD soil.

Soil	Species x P	Regression Equation	r^2
GM (a)	Lupin no P	$y = 9.40x^2 - 104x + 274$	0.99
	Lupin +P	$y = 8.86x^2 - 103x + 301$	0.99
	Lotus no P	$y = 4.36x^2 - 43.9x + 92.1$	0.99
	Lotus +P	$y = 4.32x^2 - 42.1x + 92.1$	0.97
OM (b)	Lupin no P	$y = 1.98x^2 - 20.5x + 50.4$	0.45
	Lupin +P	$y = 4.25x^2 - 49.4x + 143$	0.97
	Lotus no P	$y = -1.35x^2 + 20.4x - 77.3$	0.54
	Lotus +P	$y = 1.29x - 6.19$	0.07
MG (c)	Lupin no P	$y = 4.22x - 20.7$	0.95
	Lupin +P	$y = 9.06x^2 - 107x + 316$	0.80
	Lotus no P	$y = 0.83x - 4.11$	0.92
	Lotus +P	$y = 5.76x^2 - 69.9x + 205$	0.78
TD (d)	Lupin no P	$y = 1.74x^2 - 17.0x + 40.5$	0.84
	Lupin +P	$y = 3.84x^2 - 39.2x + 100$	0.99
	Lotus no P	$y = 5.03x - 26.7$	0.84
	Lotus +P	$y = 2.72x^2 - 27.4x + 67.4$	0.98

Appendix E: Supplementary Methods of Chapter 6

Plate E.1. Omarama Station lime ripper experiment site plan detailing location of soil sampling trenches.

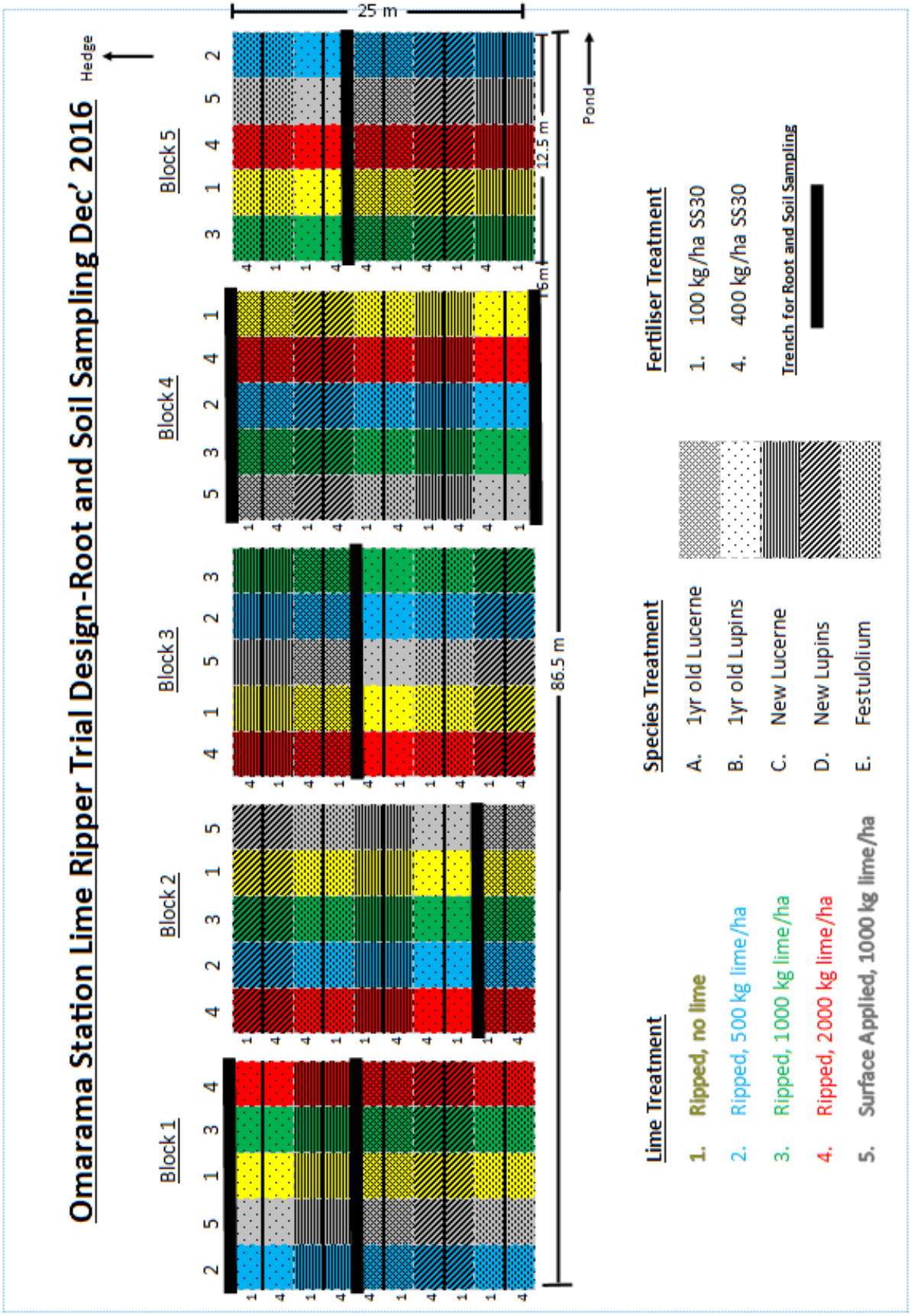


Plate E.2. Glenmore Station lime ripper experiment site plan

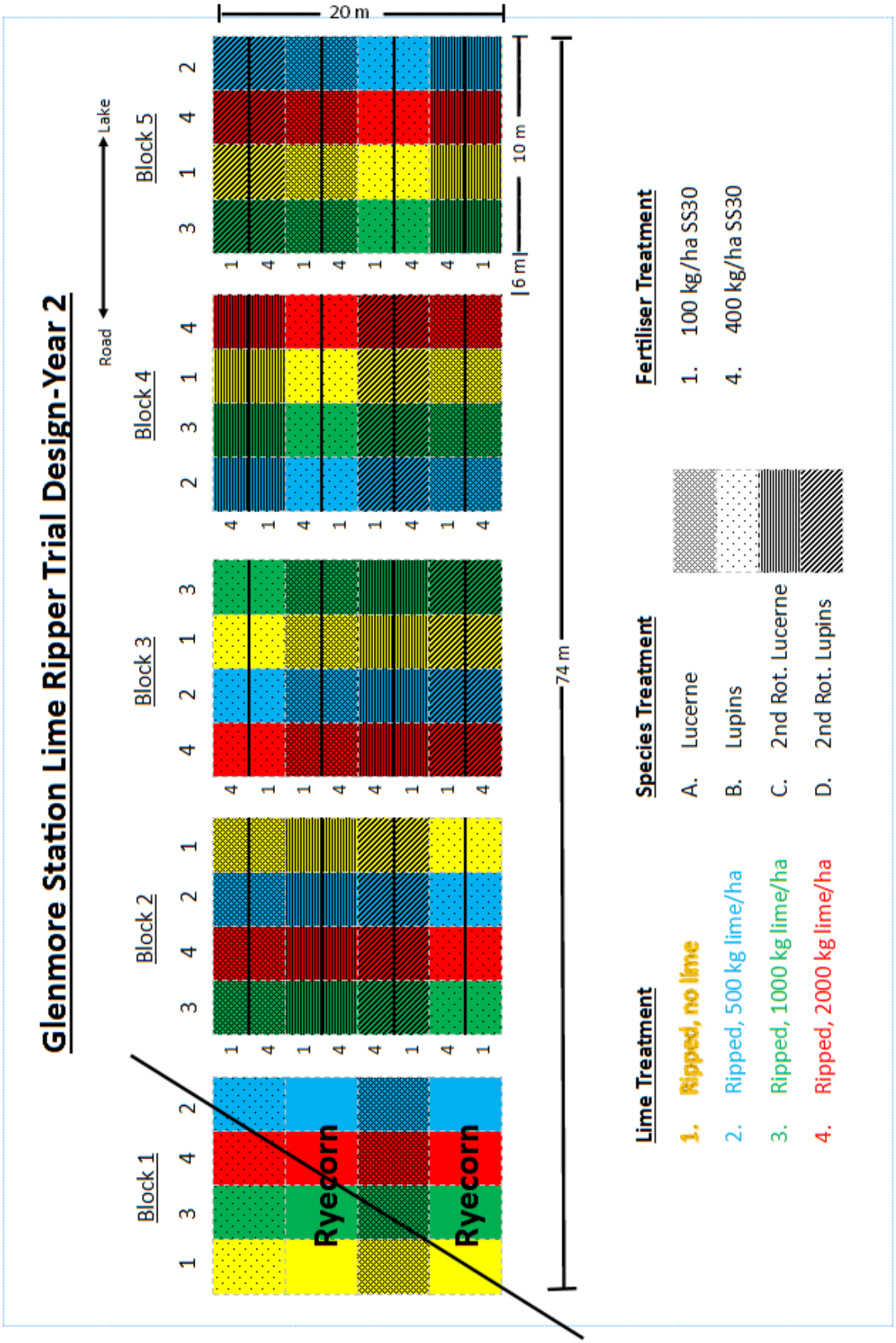
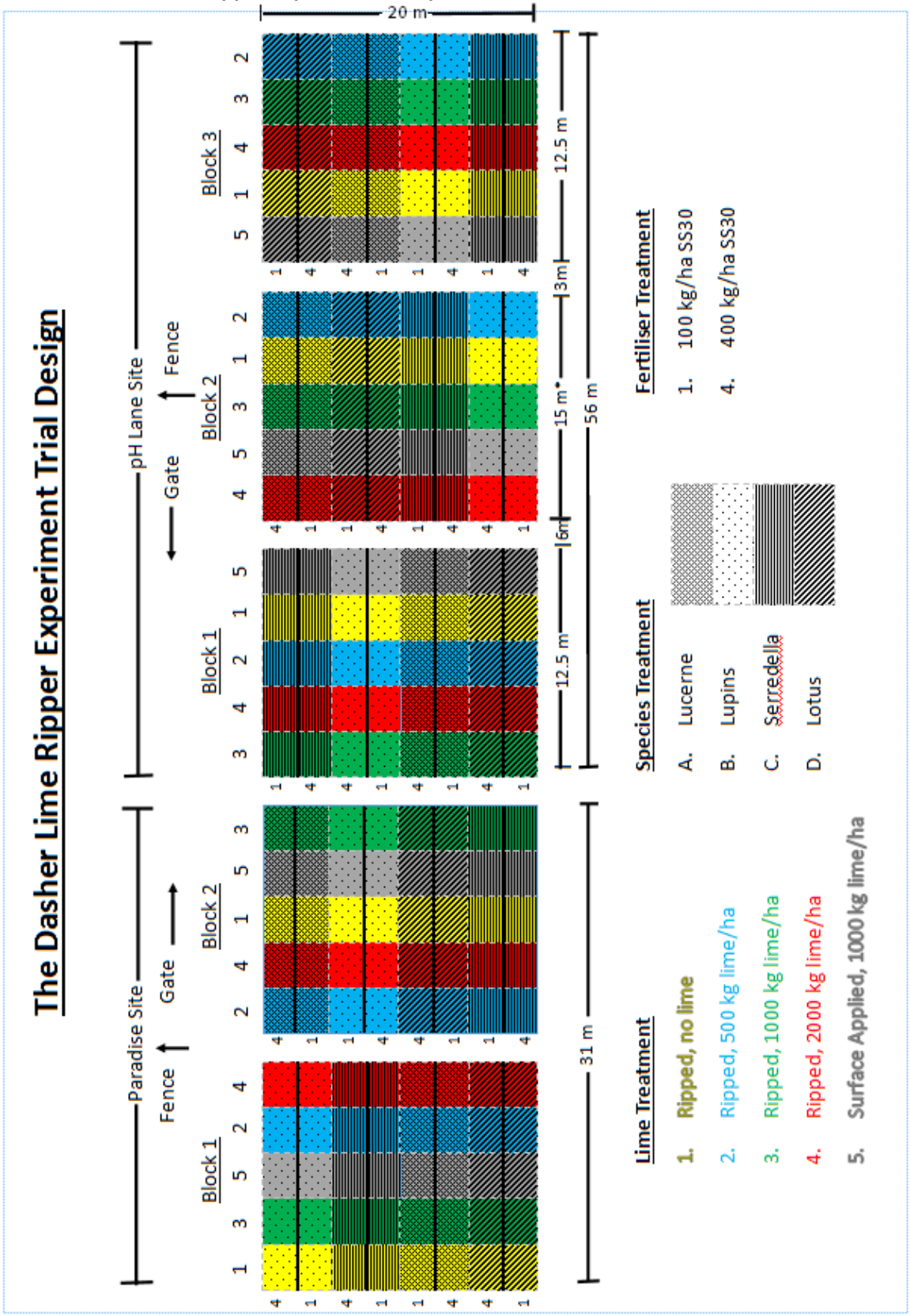


Plate E.3 The Dasher lime ripper experiment site plans



Appendix F: Supplementary Methods for Chapter 6

F.1 Detailed experiment methods for the Omarama Station experimental site.

Year	Event and procedure
2014/15	8th May: Site sprayed with a combination of herbicides to prepare the site for lime application and sowing. The herbicide mix contained 6 l/ha glyphosate (470 g/l), 40 g/ha tribenuron methyl (Sharpen), 3 l/ha MCPA, 25 g/ha suflufenacil, 70 m/ha carfentrazone-ethyl (Hammer), and 300 ml/ha organo-silicon penetrant in water applied at a rate of 200 l/ha. 26th May: Lime applied in 25 m long rips at rates of 0, 500, 1000, 2000 kg/ha and 1000 kg/ha surface applied replicated 5 times.
2015/16	28th Oct: Weather station installed. 9th Nov: All plots sown. 40 kg/ha Urea spread over all plots. 14-16th Dec: First Harvest. Two 0.2 m^2 quadrats used per plot covering 4 drill rows each. Quadrats placed two drill rows in from the edge of the plots. All plants counted. Eight plant height measurements per quadrat. All plots harvested to 3 cm height. Youngest lupin leaf left. 28-29th Jan: Plant count and height measurements taken. 0.2 m^2 quadrats used. All plants counted. 8 height measurements per quadrat. 16-17th Feb: Harvest 2. As per harvest 1. 4-6th May: Harvest 3. Two 0.2 m^2 quadrats per plot used for lucerne, ryecorn and festulolium. Two 0.4 m^2 quadrats per plot used for the lupins. All lucerne and lupins plants counted. 2 out of 4 drill rows of ryecorn and festulolium counted per quadrat. 8 height measurements per quadrat. All plants harvested to 3 cm height. Youngest lupin leaf left.
2016/17	19-20th Sept: Final ryecorn harvest. Lucerne and lupins: Two 0.2 m^2 quadrats per plot, all plants counted and 8 height measurements per quadrat. Festulolium: Height measurements only. Ryecorn: Two 0.2 m^2 quadrats per plot, plants counted in 2/4 drill rows and 8 height measurements per quadrat. Harvested to 3 cm height then sprayed off with glyphosate (360 g/l, 150 ml in 15 l water). 27th Oct: Sowing for year 2. The original ryecorn plots were sown with more lucerne and Russell lupins according to the 'Omarama Station Lime ripper Trial Design-Year 2' experimental plan. Urea was spread at 40 kg/ha on the newly sown plots to aid plant establishment. 7th Nov: Fertiliser treatment application. Ravensdown 'Sulphur Super 30' (7% P, 30% S) was hand spread on plots according to the 'Omarama Station Lime ripper Trial Design-Year 2' experimental plan. 30th Nov-1st Dec: Harvest 4 Recently applied fertiliser treatments were disregarded. Lupins: 8 height measurements per pot followed by a 1.15 m wide, 4.2 m long swath cut to 10 cm height through each plot with a sicklebar mower. Cut material was bulked and weighed in the field. A 2 kg subsample was taken with 500 g used to determine % DM and 1 kg used for morphological separation. Lucerne: Two 0.2 m^2 quadrats per plot. 8 height measurements per quadrat and all plants counted. Harvested to 5 cm height and morphologically separated. Festulolium: Two 0.2 m^2 quadrats per plot. 8 height measurements per quadrat. Harvested to 3 cm height. All harvested plots were then mown to 5-10 cm height. Newly sown lucerne and lupins: One 0.2 m^2 quadrat per plot accounting for fertiliser treatment. 8 height measurements per quadrat and all plants counted. 14th-16th Dec: Root and soil sampling 1. 40 cm wide, 50 cm deep trenches were dug adjacent to the original lucerne and Russell lupin plots with a mini-excavator according to the experimental plan in Appendix E. The walls of the trenches were squared off parallel with a drill row of the crop in each plot and plants from a 1 m transect were carefully excavated from one drill row in each plot from the wall of the trench. The wall of the trench was then again squared off and soil samples were taken by inserting a standard 7.5 cm deep soil corer into the wall of the trench.

	Eleven individual soil core samples were collected every 10 cm along the same transect (0-100 cm) at depths of 10 cm and 20 cm below the soil surface. The collected root samples were washed and up to 20 plants were measured for total root length and apparent rooting depth in the soil. Dry matter biomass measurements of roots contained within 0-20 cm and 20-40 cm depths below the soil surface. The collected soil samples were individually air dried and 2 mm sieved before being analysed for pH (H_2O) and Exch Al. 23rd May: Plant height measurements and site tidy up. All plots: One 0.2 m^2 quadrat per plot, 4 plant height measurements taken. All plants counted in newly sown lupin plots only. The lucerne, lupin and festulolium plots that were harvested on 30th Nov-1st Dec had become very weedy so were mown to 5 cm height to remove weed seedhead. The lucerne and lupin plots were then sprayed with the grass selective herbicide haloxyfop-P (37.5 ml, 150 ml spray oil, in 45 l water).
2017/18	11th Sept: Lucerne weed spray. The original lucerne plots (those sown at the beginning of the experiment) were sprayed with hexazinone (16 g in 15 l water) to remove all weeds. 28th-29th Nov: Harvest 5 All lupins: One 1 m^2 quadrat per plot. Plants counted in field. Harvested to 10 cm height. Original lucerne: One 0.5 m^2 quadrat per plot. New lucerne: One 0.2 m^2 quadrat per plot. All lucerne harvested to 5 cm height. All branches counted after harvest. Festulolium: One 0.2 m^2 quadrat per plot. Harvested to 3 cm height. All plots: Four plant height measurements per quadrat. 17th-18th Jan: Root and soil sampling 2, and lucerne harvest All lucerne: One 0.2 m^2 quadrat per plot. 8 height measurements per plot and plants harvested to 5 cm height. Lucerne and Russell lupin roots were again sampled from the same trenches as in December 2016. Lucerne roots were sampled from blocks 1-4 and Russell lupin roots were sampled from blocks 1 and 5 only. The walls of the trenches were dug back into and root samples were again extracted from a 1 m transect from one drill row of each crop. The wall of the trenches was then again squared off and six soil core samples were taken every 20 cm along the 1 m transect (0-100 cm) and bulked at sampling depths of 10, 15, 20, 25, and 30 cm below the soil surface. Soil samples were collected from each of the five blocks. The collected root samples were washed and 10 plants (where possible) from each sample were randomly selected and measured for length and scored on a 1-5 scale for nodulation (1-not nodulated, 5-heavily nodulated including on taproot) and branching (1-single taproot, 5-heavily branched with short crown) before biomass for 0-30 cm and >30 cm below the soil surface was recorded. The collected soil samples were air dried and 2 mm sieved before being analysed for pH (H_2O) and Exch Al. 26th Apr: Harvest 6 All lupins: One 0.5 m^2 quadrat per plot. All plants in quadrat counted and measured for height. Harvested to 7 cm height. All lucerne: One 0.5 m^2 quadrat per plot. Eight height measurements per quadrat. Harvested to 3 cm height ensuring to leave some young leaves on plant.
Spring 2018	27th-28th November: Lucerne and Russell lupin herbage sampling. All lucerne: One 0.5 m^2 quadrat per plot. Four plant heights taken from random plants per quadrat. Harvested to 5 cm height. Whole collected sample sorted into weeds and lucerne before being dried. Lupins: One 1 m^2 quadrat used in Blocks 1, 3, and 5 only for the 2015 sown lupins. Whole sample weighed before taking a 500 g subsample for drying to determine total DM yield.

F.2 Detailed experiment methods for the Glenmore Station experimental site.

Year	Event and Procedure
2014/15	February: The block in which the experimental site is located was sprayed with 6 l/ha glyphosate (360 g/l) and 100 g/ha metsulfuron. 25 March: The experimental site was selected and extensively soil core sampled at depths of 0-7.cm and 7.5-15 cm. 25th-26th May: Lime application. Lime was applied in 20 m long rips at simultaneous depths of 5 and 25 cm at rates of 0, 500, 1000, and 2000 kg/ha. Replicated 5 times. The site was heavy rolled following lime application.
2015/16	20th Dec: Site sprayed. The site was sprayed with 1 l of glyphosate (360 g/l) in 80 l water. 20th Jan: All plots sown. Following a long fallow to reduce any residue effects of the metsulfuron spray the plots were sown with lucerne, Russell lupins and two replicates of ryecorn per block. Urea was spread at 40 kg/ha over all plots post sowing. 27th Jan: A netting fence was constructed around the experimental site. 17th Feb: Plant establishment counts and height measurements. All plants were counted in two 0.2 m^2 quadrats per plot. 8 height measurements were taken from each quadrat. Plant establishment at the site had been poor. 29th April: Harvest 1. Lucerne and lupins: Two 0.2 m^2 quadrats per plot. All plants counted and 8 height measurements per plot. Harvested to 5 cm height, youngest lupin leaf left. Ryecorn: 8 plant heights measurements per plot. A 1.15 m wide, 4.2 m long swath was cut through the plots at 5 cm height, the cut material was bulked and weighed in the field and a subsample was then taken for drying and determining % DM. All plots were grazed after being harvested.
2016/17	10th Oct: Having been excessively grazed the experimental site was sprayed out with 800 ml of Buster (glufosinate-ammonium, 200 g/l) in 80 l of water. The ryecorn plots were additionally sprayed with 450 ml of glyphosate (360 g/l) in 45 l of water. 22-23rd Oct: Soil sampling and second ryecorn spray. Eleven 7.5 cm deep soil cores were collected along a diagonal transect through each plot and bulked. The ryecorn plots were again sprayed with 450 ml of glyphosate (360 g/l) in 45 l of water. 26th Oct: Sowing and fertiliser treatment application for year 2 of the experiment. Block 1 was not sown due to it being intercepted by a newly built fence through the farmers' paddock. The original lucerne and Russell lupin plots were resown and one of each of the ryecorn plots were sown with more lucerne and Russell lupins per block. Fertiliser was hand spread on the plots at rates of 100 and 400 kg/ha of Ravensdown 'Sulphur super 30' (7% P, 30% S) according to the trial plan. Urea was spread on the plots at 40 kg/ha to aid in plant establishment. 16th Dec: Plant establishment counts and height measurements. One 0.2 m^2 quadrat used per plot. All plants counted and 8 height measurements taken per quadrat. Plant establishment had been much more successful after the second sowing. 21st March: Harvest 2 Only blocks 2-4 sampled due to the fence in block 1 and spray damage in block 5 from the neighbouring paddock. One 0.2 m^2 quadrat used per plot. All plants counted and 8 height measurements taken per quadrat. The lucerne was then harvested to 5 cm height and the lupins to 10 cm height. All grass and weeds were removed from the collected samples and dried and weighed separately. Once harvested all plots were mown to a height of 5-10 cm with a sicklebar mower. 23rd May: The experimental site was sprayed with haloxyfop-P (520 g/l, 37.5 ml with 150 ml Uptake spray oil in 45 l water) to remove grass weeds from the plots.
2017/18	30th Nov: Harvest 3. Only blocks 2-4 sampled. Lucerne: One 0.5 m^2 quadrat per plot. 8 height measurements taken per quadrat. Plants harvested to 5 cm height. Weeds were discarded from the collected sample. All stems were then counted, dried and weighed. Lupins: One 1 m^2 quadrat used per plot. 4 height measurements taken per quadrat. Plants were harvested to 10 cm height and the number of plants harvested was recorded. The whole

	collected sample was weighed fresh and a 500-600 g subsample was dried to determine % DM. Another 500-600 g subsample was used for morphological separation. After being sampled all plots were mown to 5-10 cm height with a sicklebar mower. 20th Apr: Harvest 4. Only blocks 2-4 sampled. All lupins: One 0.5 m^2 quadrat per plot. All plants in quadrat counted and measured for height. Harvested to 7 cm height. All lucerne: One 0.5 m^2 quadrat per plot. Eight height measurements per quadrat. Harvested to 3 cm height ensuring to leave some young leaves on plant.

F.3 Detailed experiment methods for The Dasher experimental site.

Year	Event and Procedure
2015/16	November: Two sites are selected at The Dasher for experimentation based on previous soil test results. The first of the two sites is the 'Packhorse site' located in the Packhorse Lane block which was selected due to its extreme pH and Exch Al content. The second site is the 'Paradise site' located in the Paradise paddock and selected out of interest of the farmer. Having selected the site each was extensively soil core and auger sampled to 45 cm depth. 17th Jan: Both sites were sprayed with Buster (glufosinate-ammonium, 200 g/l, 900 ml in 90 l water over the two sites). February: The Packhorse site was cultivated to 10-15 cm depth with offset discs in order to dislodge the tussocks from the soil so they could then be removed from the site to prevent clogging up the lime ripper and plot drill. The tussocks at the Paradise site were dug out by hand. 3rd Mar: Lime application. Optimise pelleted lime was applied in 20 m long rips at simultaneous depths of 5 and 25 cm at rates of 0, 500, 1000, and 2000 kg/ha. A 1000 kg/ha surface applied lime treatment was also applied. Each treatment was replicated three times at the Packhorse site and twice at the Paradise site. Following lime application each site was heavy rolled.
2016/17	28th Oct: The site was sown with one replicate of lucerne, Russell lupin, *Lotus pedunculatus*, and French serradella per block using the Flexiseeder plot drill. Following sowing urea was spread on all plots at 40 kg/ha to aid in plant establishment and the high and low fertiliser treatment was hand spread on the respective plots according to the experimental design in Appendix E. Flexinet fences were erected around each of the two sites. 13th Dec: Plant establishment counts and height measurements. All plants were counted in two 0.2 m^2 quadrats per plot at each of the two sites. The 0.2 m^2 quadrats were placed to cover four drill rows of plants (15 cm spaced rows) and were placed two drill rows in from the edge of the plots. Four plant height measurements were taken per quadrat. 8-9th Mar: First harvest of the Paradise site. One 0.2 m^2 quadrat used per plot. Eight height measurements were taken of the sown species before it was harvested. The harvested sample was sorted into sown species, broadleaf weeds and grass before each was dried to determine dry matter yield of each. 4-5th May: First harvest of the Packhorse site. As for first harvest of the Paradise site. The Paradise site was opened for grazing which occurred over the winter.
2017/18	13th Sept: Following grazing the Paradise site was again fenced off. 10th Nov: Second harvest of the Paradise site. One 0.2 m^2 quadrat used per plot. Eight height measurements were taken per quadrat of the sown species before it was harvested and all plants in the quadrats of the lucerne and Russell lupin plots were counted. Being an annual species the French serradella had failed to re-establish for a second season. The Packhorse site was found to have been opened to grazing and apart from the Lotus plots was completely stripped bare. The fences were put back up. 16th Nov: The original lucerne and Russell lupin plots at the Packhorse site were resown and the original French serradella plots were resown with additional Russell lupins. Urea was spread on all the plots at the Packhorse site at 40 kg/ha to aid in plant establishment. 12th Jan: Third harvest of the Paradise site. One 0.2 m^2 quadrat used per plot. Eight height measurements were taken of the sown species before it was harvested. The harvested sample was sorted into sown species, broadleaf weeds and grass before each was dried to determine dry matter yield of each. The Packhorse site was again found to have sheep in it and had failed to establish. Conclusion of experiment.

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