Investigation of the interactions between soil acidity, phosphorus biochemistry and dynamics and legumes in acid grassland soils

---

A thesis

submitted in partial fulfilment

of the requirements for the Degree of

Doctor of philosophy

at

Lincoln University

by

Moussa Bouray

Lincoln University

2021

Pre-Publication of Part of this thesis

Chapter 2 – a manuscript from this section has been published as follows:

Bouray, M., Moir, J., Condron, L., and Lehto, N. (2020). Impacts of phosphogypsum, soluble fertilizer and lime amendment of acid soils on the bioavailability of phosphorus and sulphur under lucerne (*Medicago sativa*). Plants 9, 1-17.

A poster has also been presented as follows:

Bouray, M., Moir, J., Condron, L., and Lehto, N. Phosphogypsum as an alterantive source of Phosphorus and Sulphur. Poster session presented at the 9th International Phosphorus Workshop. 2019, July 8–12. Zurich, Switzerland.

Chapter 3 – a manuscript from this section has been submitted as follows:

Bouray, M., Moir, J., Condron, L., Lehto, N., and Bayad, M. (2021). Effect of phosphogypsum on aluminium speciation in acid pasture soils. Journal of Soils and Sediments (Under Review).

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

Bouray, M., Moir, J., Condron, L., and Lehto, N. (2021). Lime-induced pH elevation influences phosphorus biochemical processes and dynamics in the rhizosphere of *Lupinus polyphyllus and Lupinus angustifolius.* Journal of Soil Science and Plant Nutrition, 1-15.

Chapter 5 – a manuscript from this section has been published as follows:

Bouray, M., Moir, J., Lehto, N., Condron, L., Touhami, D., and Hummel, C. (2021). Soil pH effects on phosphorus mobilization in the rhizosphere of *Lupinus angustifolius*. Plant and Soil, 1-21.

Chapter 6 – a manuscript from this section has been published as follows:

Bouray, M., Moir, J., Condron, L., and Dharini, P. (2021). Surface liming effects on soil P biochemistry and dynamics in extensive grassland. Nutrient Cycling in Agroecosystems, 1-15.

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

Investigation of the interactions between soil acidity, phosphorus biochemistry and dynamics and legumes in acid grassland soils

by

Moussa Bouray

Soil acidity and associated phosphorus (P) deficiency and aluminium (Al) toxicity are major constraints to agricultural production worldwide. For instance, in New Zealand steep lands, the so-called hill and high country, many commercial legume species fail to establish and persist in these acidic, low fertility environments. This PhD research project investigated (1) the impact of phosphogypsum (PG) on soil fertility and Al speciation in the soil solution and (2) the impact of lime-induced pH elevation on P biochemistry and dynamics in the rhizosphere of lupins and under open field grassland conditions. For the first part of the investigation, two experiments were conducted, each with a specific objective; in the first experiment (Chapter 2) the objective was to compare the effects of PG amendment, soluble fertilizer, and lime on short-term lucerne yield and P and sulphur (S) uptake in two different acidic soils under controlled environment conditions. The objective of the second experiment (Chapter 3) was to examine the impact of PG on Al speciation in the porewaters of both planted and incubated (unplanted) soils using the Visual Minteq Model. These two complementary experiments revealed that (1) PG has increased P and S bioavailability and therefore improved lucerne P and S uptake and yield. However, the application of PG to low pH soils necessitates its combination with lime because it has been found that pH was the most important factor controlling the nutrition and growth of lucerne as evidenced by the large difference in the yield and P and S uptakes between PG alone and PG combined with lime. The second key result (2) was that PG reduces soil exchangeable Al and monomeric Al^{3+}in the soil solution if applied at 1-3 t ha^{-1}. Higher application rates could acidify the soil and displace Al from the soil exchangeable sites into the soil solution. The mechanisms by which PG reduced Al^{3+} activity included the immobilization process through sulphate (SO_4^{2-}) and fluoride (F^-) binding and via precipitation reactions.

For the second part of the investigation, three experiments were conducted (Chapters 4, 5 and 6), each with a specific objective. Experiment 1 (Chapter 4) objective was to examine the effects of increasing

soil pH from 5.3 to 6.0 using lime on P-related processes and dynamics in the rhizosphere of two lupins (*Lupinus polyphyllus and Lupinus angustifolius*) after 11 weeks of plant growth in pots under glasshouse conditions. Experiment 2 (Chapter 5) was conducted to examine the impact of soil pH increase to near-neutral (pH 6.3) using lime on (1) acid phosphatase activity and labile P (DGT-P) distribution patterns in the rhizosphere of *Lupinus angustifolius* grown in two contrasting acid pasture soils, using innovative imaging techniques, (2) root morphological and physiological root traits. Experiment 3 (Chapter 6) was carried out in the field to study and quantify the effects of liming on P biochemistry and fractionation during 18 months on a long-term (+60 years) permanent fertilized grassland. All three experiments investigated the same soil (Mt Grand soil), collected from Central Otago, NZ—they proved unanimously that liming increases P availability and increases the mineralization of labile and moderately labile organic P (P_o) in this soil. For instance, in the field experiment, labile inorganic P (P_i) increased by 42% at pH 7.0 compared to pH 5.4, while labile and moderately labile P_o decreased by 33% and 25%, respectively. It was concluded from Chapters 4 and 5 that increasing soil pH above 6.0 negatively affects *Lupinus angustifolius* growth and P acquisition processes such as organic anions exudation and fine root length, while *Lupinus polyphyllus* was unresponsive to liming. Another conclusion drawn from the field trial (Chapter 6) is that liming enhances the mobilization of the historically applied P fertilizer and promotes P_o mineralization.

Keywords: soil pH, phosphorus dynamic, phosphorus acquisition, phosphorus fractionation, phosphorus biochemistry, aluminium toxicity, aluminium speciation, visual Minteq, lucerne, lupin, organic anion, phosphatase, lime, phosphogypsum, diffusive gradient in thin films (DGT), zymography, rhizosphere, legume, hill country, high country.

Acknowledgments

To my late father, who unfortunately didn't stay in this world long enough to see his son become a doctor.

First and foremost, I am extremely grateful to Mohammed 6 Polytechnic University (UM6P, Morocco) and Office Chérifien des Phosphates (OCP, Morocco) for their generous funding of this project and for giving me the opportunity to pursue a PhD in this exciting area of research under a collaborative research program with Lincoln University (Lincoln, New Zealand). I'm thankful to my supervisors, Assc. Prof. Jim Moir, Prof. Leo Condron, and Dr. Niklas Lehto for their invaluable advice, continuous support, and patience during my PhD study. I especially enjoyed the experience of driving down to central Otago with Jim Moir and working in the field together setting trials, applying lime and fertilizer, and sampling soils on the slippery hills of Mt Grand with plenty of fun– again I'm appreciative of all the time you've given, Jim, your encouragement and understanding. I would like also to thank Lincoln University's technical staff, in particular Roger Cresswell, Lynne Clucas, Leanne Hassall, Shiv Prasad Pokhrel, Joy Jiao, Jian Zhang, Neil Smith, and Brent Richards for technical assistance. Many thanks to Dharini Paramashivam for lab assistance and Dharshika Welikala, Christina Hummel, Raphael Tiziani and Jacob Santner for sharing their knowledge about the preparation of DGT gels and zymography. I thank Amal Torky for her patience and help with administration work. To my friends, fellow students, and the Moroccan community in Christchurch, especially Driss Jamradi, Badiaa Sabil and Noureddine Travis thank you all for being part of my journey. I also wish to thank my colleagues (Driss, Mohamed and Meryem) for their wonderful collaboration during the last four years.

I'm thankful to Ravensdown people, in particular: Mike Manning, Greg Campbell, and Chris Richards for their unconditioned and endless support and care, you have made my study and life in New Zealand a wonderful time. I also enjoyed the valuable discussions with the Ravensdown scientific team, in particular: Ants Robert, Alister Metherell, Richard Christie, and Hendrik Venter. To Scott Gower family and Andy and Trish MacFarlane, all from Ravensdown, it was so great of you to have me at your farms. A special thank you to Toni Laming, former director of Lincoln Hub for her support and hospitality. Another special thanks to Dougal Ferguson, former international commercial manager at Lincoln Hub for facilitating my arrival to New Zealand and for his hospitality.

Finally, I would like to express my gratitude to my mother Aarbia, my sisters, and my brothers for their prayers. Your unconditional love and support have meant the world to me, I hope that I have made you proud. To my wife Fatiha for her tremendous love, understanding, and encouragement in the past few years, it would be impossible for me to complete my study without you. You have made me stronger, better, and more fulfilled than I could have ever imagined. To my little boy Ziyad, I love you to the moon and back.

Table of Contents

List of Tables

List of Figures

List of Plates

List of Abbreviations

AcPME	Acid Phosphomonoesterase
Al	Aluminium
Al_{CaCl2}	$CaCl_2$-extracted aluminium
Al-DOM	Dissolved Organic Matter bound aluminium
Al-F	Fluoride bound aluminium
Al_{KCl}	KCl-extracted aluminium
Al-OH	Hydroxide bound aluminium
AlPME	Alkaline Phosphomonoesterase
Al-PO_4	Phosphate bound aluminium
Alsat.	Aluminium saturation
Al-SO_4	Sulphate bound aluminium
AMN	Anaerobic Mineralizable Nitrogen
ANOVA	Analysis of Variance
BS	Base Saturation
C	Carbon
CEC	Cation Exchange Capacity
DCL	Diamter Class Length
DGT	Diffusive Gradient in Thin Films
DOC	Dissolved Organic Carbon
DOM	Dissolved Organic Matter
Ex. H^+	Exchangeable hydrogen
FAO	Food and Agriculture Organization
ITPS	Intergovernmental Technical Panel on Soils
HPLC	High-Performance Liquid Chromatography
IS	Ionic Strength
masl	Meters above sea level
MBP	Microbial Biomass Phosphorus
MU	Methyllumbelliferone
MUB	Modified Universal Buffer
MUP	Methyllumbellieryl Phosphate
N	Nitrogen
NZ	New Zealand
OAs	Organic Anions

P	Phosphorus
PDE	Phosphodiesterase
PG	Phosphogypsum
pH_{CaCl2}	$CaCl_2$-extracted pH
pHsoln.	Soil Solution pH
pH_w	Water-exracted pH
P_i	Inoganic phosphorus
PLS	Partial Least Square
P_o	Organic phosphorus
SE	Standard Error
SM	Soil Moisture
SRL	Specific Root Length
SSP	Single Super Phosphatse
TC	Total Carbon
TDM	Total Dry Matter
TN	Total Nitrogen
TOA	Total Organic Anion
Tot.Al	Total dissolved aluminium
USDA	Unites State Department of Agriculture

Chapter 1

Introduction

1.1 Background

The uncultivable hill and high country below 1,000 m, with a slope > 20˚, comprises about 40% of New Zealand's land surface area (Tozer et al., 2021). These typical areas cover 6.6 million ha, of which 5.2 million ha are in pastoral agriculture (Mackay, 2008). The hill and high country play a critical role as the primary land resource for the red meat production industry in New Zealand. As such, these areas contribute a great proportion of livestock finishing, and are also the breeding platform for sheep and beef cattle and therefore a key support element for lowland intensified grassland systems (Moot et al., 2009; Morris and Kenyon, 2014). However, the livestock grazing systems in these areas generally rely on perennial pastures; the annual rate of pasture renewal of sheep and beef cattle hill country farms has been reported to be just 2.3% compared with 8% on dairy farms which are generally more fertile and on flatlands (Stewart et al., 2000). Although there has been an increase in livestock performance and sheep meat production since 1989/90 (Mackay et al., 2012), the intensification of less productive lands and the on-farm productivity in the hill and high country are still facing several strong limitations due to:

- Topography: inability to cultivate widely due to moderate and steep slopes. Moreover. topographical features such as slope and aspect induce variation in soil temperature and moisture by modulating precipitation and water flows, causing large variations in potential pasture production across landscapes (López et al., 2003; Radcliffe, 1982).

- Climate: continental-like with hot dry summers and long cold frosty winters with a short, moisture limited production season (Moir et al., 2000; Scott et al., 1985) which affects stocking rates.

- Low fertility soils: over 500,000 ha of NZ farmed high country soils are acidic (Moir and Moot, 2010), and most of these are deficient in terms of N, S, and P. The present research is mostly focusing on these soil-related constraints.

Nitrogen (N) is one of the major limiting nutrients in NZ hill and high country soils (Gillingham et al., 1998; Lambert et al., 2003). However, the application of inorganic (N) fertilizer is usually uneconomic and so does not occur. Therefore, legumes (clovers, lucerne, lupins, etc.) are the key for sustainable

farming in these environments due to biological N fixation through a symbiotic relationship with N-fixing rhizobia, thus improving soil N inputs (Suzaki et al., 2015) at low cost. For instance, white clover (*Trifolium repens*) and subterranean clover (*Trifolium subterraneum*) being among the most valuable crops in NZ pastures, contribute inputs of around 30 kg N ha^{-1} y^{-1} per ton of legume dry matter (DM) grown (Parfitt et al., 2006). Legumes are also a high-quality (high protein) feed for livestock compared to grass-based pastures (Kemp et al., 2010; Moot, 2013). However, legumes are often adapted to high soil fertility. Thus, their establishment and persistence in NZ hill and high country are limited by the native edaphic constraints, mainly low pH, Al toxicity, P and S deficiency and moisture stress (Maxwell et al., 2013; Maxwell et al., 2016; Moir et al., 2016; Whitley et al., 2019).

Acidification and associated Al toxicity have been identified as critical issues in New Zealand, particularly in high and hill country areas (Morton and Moir, 2018; Whitley et al., 2019). Berenji et al. (2017), in a field trial on a South Island brown acid soil (pH 5.2), demonstrated that the ability of rhizobia to inoculate lucerne (*Medicago sativa*) was strongly inhibited at a high exchangeable Al (0.02 M $CaCl_2$) concentration of 15.1 ppm. High free Al^{3+} concentration and low pH also negatively affect the growth and survival of rhizobia (Wigley et al., 2018). Commercial clovers (white, red, and subterranean), the most commonly sown legumes in NZ, are also affected by low pH and associated Al toxicity, but they are much more resistant than lucerne which is considered to be the most sensitive legume to Al toxicity (Moir et al., 2016), while subterranean clovers are the most resistant (Olykan et al., 2018; Shah et al., 2021). On the other hand, naturalized, adventive annual clovers (suckling, haresfoot, striated, and cluster) in NZ are adapted to the hill and high country environments even in dry summer environments (Maxwell et al., 2010). These are also tolerant to soils with Al levels higher than the reported toxicity threshold (Maxwell et al., 2012) of 3 mg kg^{-1} suggested by Moir et al. (2016) for legumes. This highlights the potential of this annual species; if successfully established and spread, for hill and high-country farming, especially where the aerial application of lime to hills is uneconomic. Russell lupin (*Lupinus polyphyllus*) has been identified as tolerant to low pH, high Al, and low P soils (Black et al., 2014; Hendrie et al., 2018; Martin-Hendrie, 2019; Moot and Pollock, 2014; Ryan-Salter et al., 2014; Scott, 2014). However, the persistence of these perennial species in dry and unfavourable hill and high-country areas is poorly understood, especially in terms of P mobilization, P requirements, and their Al detoxification strategies.

Agricultural lime ($CaCO_3$) application is a widely used farming practice in NZ for acidity correction: increasing soil pH and reducing Al bioavailability. However, the efficacity of lime in NZ hill and high country is limited to the near-surface layer and has a very limited effect on subsoil acidity, at least in the short term (Moir and Moot, 2010). This is mainly due to its low solubility and then slow movement

down the soil profile (Hendrie et al., 2018). The low rainfall and consequently low soil moisture content, particularly in hill and high-country areas of South Island (e.g., central Otago), contribute also to reducing lime reactivity in the soil. Additionally, due to topography, very few of these areas are sufficiently limed because of the economics of aerial application (Craighead, 2005; Gillingham et al., 1999). This is the main reason why lime has historically been sparingly applied to NZ hill and high country because the cost of aerial application of large quantities of lime is very expensive and so often not practical. The relationship between liming and pasture production is well established for some NZ hill and high-country soils (Edmeades et al., 1985; Edmeades et al., 1984; Kearney et al., 2010; Moir et al., 2016; Wheeler and O'Connor, 1998). Also, the role of liming in reducing soil extractable Al has been verified for several soil orders (Morton and Moir, 2018). However, studies of soil pH change with liming and associated changes in soil P chemistry, in South Island hill and high-country soils are scarce. The conventional use of surface-applied lime is often insufficient at correcting subsurface acidity. Plus, although has shown positive effects, the deep placement of pelletized lime to rectify subsoil acidity is not affordable for most of the farmers (very expensive) in the lowlands and is impractical for high-lands (Kalkhoran et al., 2020; Martin-Hendrie, 2019). Therefore, further research is required to investigate the use of novel materials of higher solubility such as phosphogypsum for hill and high-country soils. Also, the mixture phosphogypsum-lime could be one way to improve lime solubility (Crusciol et al., 2016; Lauricella et al., 2021).

After N, P is the second most limiting macronutrient that drives the productivity of legume-based pasture systems in NZ hill and high country (Bowatte et al., 2006; Maxwell et al., 2013). In a long-term grazing trial at Te Kuiti, North Island hill country, Roach et al. (1996) demonstrated that withholding P fertilizer inputs for 10 years resulted in 54-72% less legume production and 29-35% reduction in the annual pasture production. Also, soil N availability has been reduced. Similar impacts of P fertilizer withholding (15 years) were observed in Whatawhata, North Island hill country, where the abundance of productive and desirable species (white clover and ryegrass) decreased by 15-20%, while the abundance of undesirable low fertility grasses such as browntop increased (Dodd and Ledgard, 1999). A piece of more recent evidence to further stress the importance of P fertilizer for NZ hill and high-country farming performance is given in Figure 1.1. For instance, figure 1.1a showed that mean annual stocking rates at Ballantrae Hill Country Research Station, Southern Hawke's Bay, New Zealand over the period 1980–2014 were 6.9 (NF = no annual P applied), 9.8 (LF = 125 kg SSP ha^{-1}), and 15.9 (HF = 375 kg SSP ha^{-1}) SU ha^{-1}.

(Material removed due to copyright compliance)

Figure 1.1 Sheep stocking rates (SU ha^{-1}) (a) and soil Olsen P values (µg mL^{-1}) (b) at the Ballantrae Hill Country Research Station over time. Farmlets: NF = no annual P applied, LF = 125 kg single superphosphate (SSP) ha^{-1} , HF = 375 kg SSP ha^{-1} , applied on an annual basis since 1980. Each data point represents the mean value of 18 sampling sites. Adapted from Mackay et al. (2021).

Soil P fertility in NZ hill country was originally poor. However, in some area there was a build up with fertilizer application, but it declined due to the sharp decrease in fertilizer inputs simply because of curtailed farm income following the policy of farming subsidies removal in the mid-1980s and to the economics of the aerial application of fertilizers (MacLeod and Moller, 2006). However, most of the research on P addition has been limited to the North Island hill country. Also, soil P monitoring relied exclusively on plant-available P (Olsen P) measurement and less is known about other P pools such as organic P which could represent a considerable fraction (30% to 65%) of soil P (Condron et al., 2005; Turner et al., 2003): in some soils organic P could constitute 80% of total P due to its stabilizations through sorption and precipitation processes (Turner et al., 2002). Unfortunately, there have been insufficient studies, if there are any, which have quantified the importance of organic P in hill and high-country soils and hence there remains a lack of understanding of how this source of P could be

mobilized. Furthermore, the effects of soil properties, in particular, the interaction between pH and P availability is poorly understood in these soils. Further, Al and Fe oxides are known to drive P immobilization/fixation in acid soils and therefore control P availability in low pH soils (McDowell and Condron, 2001; McDowell et al., 2003). However, these mechanisms are also poorly investigated in hill and high-country soils. Besides, the ability of legumes to mobilize the sparingly available inorganic and organic P in these soils requires further investigation, especially from the rhizosphere perspective where P acquisition and mobilization are effectively occurring. This would help identifying which legumes species are potentially able to persist in low P soil and mobilize the legacy P. These findings support the view that liming has the potential to enhance the bioavailability and utilisation of inorganic and organic P resources in soil. For instance, Simonsson et al. (2018) found recently that liming had a positive effect on the solubility of P added as fertilizer during the decades following the lime application. Lime has also been reported to enhance organic P mineralization in some earlier studies (Condron and Goh, 1990; Condron et al., 1993; Halstead et al., 1963). However, to date, there is a paucity of information about how lime could affect P cycling in NZ hill and high country. Moreover, there is a notorious inconsistency within the international scientific community regarding the relationship between soil pH and P availability. As such it is among the aims of this PhD research to investigate liming effects on soil P biochemistry and dynamics in some soils of NZ South Island hill country from both soil and plant perspectives, so as to bring new insights for the improvement of acid pasture soils in these typical environments and therefore facilitate the integration of legumes to pastoral agricultural systems. This PhD research would contribute to elucidating the current controversy regarding liming-P relationship.

1.2 Literature review

1.2.1 Soil acidity

Soil acidity is one of the main constraints to crop production worldwide. It increases due to the accumulation of hydrogen (H^+) and free aluminium cation (Al^{3+}) or when bases such as calcium (Ca^{2+}), magnesium (Mg^{2+}), sodium (Na^+), and potassium (K^+) are leached and replaced by H^+ and Al^{3+} (Rengel, 2003). The base cations leaching occurs naturally because of long-term rainfall and soil weathering processes. The removal of cations in farm products such grain, wool and meat also contribute in soil acidification (Tang and Rengel, 2003). Soil acidification processes can also be induced anthropogenically in grassland through the excessive use of ammonium-based fertilizers and/or acid producing fertilizers (e.g., elemental S) (FAO and ITPS, 2015). Legumes themselves can contribute to the soil acidification process through excess cation uptake and exudation of acidic compounds by roots. Also, nitrogen fixation is well documented as an acidifying process (Bolan et al., 1991a; Tang et

al., 1999). Nonetheless, in fertilized soils, nitrification of NH^{4+} in historically applied ammonium suphate is the major source of acidification (Johnston et al., 1986). Moreover, organic matter (OM) decomposition by microorganisms produces carbon dioxide (CO_2) which transforms into carbonic acid (H_2CO_3) in the presence of water, then the H_2CO_3 dissociates releasing H^+ protons. For instance, forests as they are covered by a thick layer of litter, tend to be more acidic than grassland soils (Bolan and Hedley, 2003). About 50% of the world's arable lands are acidic (Von Uexküll and Mutert, 1995), which mostly occur in developing countries like Central African, South American, and Southeast Asian countries (FAO and ITPS, 2015). Figure 1.2. illustrates the topsoil acidy distribution worldwide. Topsoil acidity (pH < 5.5) affected 30% of the world's ice-free land area, and subsoil acidity affected 75% (Sumner and Noble, 2003) based on the data reported by Eswaran et al. (1997)

(Material removed due to copyright compliance)

Figure 1.2 A world map of the estimated topsoil pH (FAO and ITPS, 2015). FAO: Food and Agriculture Organization, ITPS: Intergovernmental Technical Panel on Soils

1.2.2 Aluminium toxicity

On acid soils (pH < 5.5), plant growth is generally limited. This is because acidified soil reduces the availability of several essential elements such as P and molybdenum (Mo) (particularly in legumes Hafner et al. (1992); Mitran et al. (2018)) while exacerbating the toxic levels of others such as Al (Kidd and Proctor, 2001). Aluminium is the most abundant metallic element in the earth's crust. However, it is not essential for plant metabolism (Sade et al., 2016). Aluminium toxicity is not harmful to plants only, it could also represent a serious threat to human health (Peters et al., 2020). In the soil, Al is generally found as Al oxide minerals and stable forms like aluminosilicates, which undergoes

hydrolysis at low pH (pH < 5; Figure 1.3) releasing monomeric Al^{3+} and mononuclear species such as $AlOH^{2+}$, $Al(OH)_2^+$, $Al(OH)_3$, and $Al(OH)_4^-$. However, among all species, Al^{3+} is considered the most rhizotoxic form (Kinraide, 1997; Schmitt et al., 2016; Singh et al., 2017). Although few studies have been performed, polynuclear Al species (Al_{13}) have also been supposed to be toxic (Kinraide, 1991; Vitorello et al., 2005).

(Material removed due to copyright compliance)

Figure 1.3 A diagram showing the formation of different Al species in the soil as a function of pH (Baes and Mesmer, 1986).

The symptoms of Al toxicity to plants are not easily identifiable. Root growth inhibition is the most used tool for the measurement of Al toxicity (Gupta et al., 2013; Kinraide, 1991). Although it is plant species-specific, Al toxicity can also manifest as leaves senescence, yellowing of leaf tip, leaf curling, and lateral roots thickening and discoloration (Rahman and Upadhyaya, 2020). Additional details on Al toxicity effects on plants are given in Figure 1.4. These deleterious effects restrict the uptake and translocation of water and nutrients (specifically P), altering plant metabolism, growth, and persistence on acid soils (Silva, 2012; Tamás et al., 2006). However, some plants have shown several mechanisms to cope with toxic Al concentrations such as (1) compartmentalization of Al in the vacuoles by cytosolic chelation with phenolic compounds and organic anions (internal detoxification), (2) external exclusion of Al by inducing the exudation of organic anions into the rhizosphere minimizing the entry of Al to the roots, and (3) reducing the production of reactive oxygen species (ROS) and augmenting ROS scavenging system (Chen and Liao, 2016; Delhaize and Ryan, 1995; Ma et al., 2001; Wang et al., 2020; Zhang et al., 2019b).

(Material removed due to copyright compliance)

Figure 1.4 Phytotoxicity of Al in Plants (Rahman and Upadhyaya, 2020), ROS: reactive oxygen species.

1.2.3 Phosphorus in acid soils

Researchers are becoming increasingly aware that adaptation to acid soil involves not just Al resistance but also enhanced ability to acquire P and other limiting nutrients. Phosphorus is an essential element required by living organisms (Elser and Haygarth, 2020). For example, inside the living cells, genetic information in the form of DNA and RNA molecules contains P as an integral structural component. In plants, P is involved in numerous functions such as photosynthesis (Marschner, 1995); one of the keys to ''life on earth''. Also, in legumes, P is a major component for energy transformation in nodules where biological-N-fixation occurs (Mitran et al., 2018). Phosphorus deficiency is considered to be one of the main problems in acid soils, predominantly Ultisols and Oxisols (Fairhust, 1999). Phosphorus use efficiency (PUE) in acid soil is 10 to 15% only, because of their high fixation capacity due to high concentrations of Al and Fe oxides (Scherer and Sharma, 2002; Thomas Sims and Pierzynski, 2005). However, the P adsorption characteristics of mineral oxides as well as clay minerals are controlled by soil pH (Asomaning, 2020; Gustafsson, 2001; Hiemstra and Van Riemsdijk, 1999). For instance, soil pH change affects two key factors that control P adsorption reactions: (1) the electrostatic potential of the adsorbing surfaces, which becomes more negative as pH increases and therefore less attractive to P, and (2) P speciation: as pH increases the concentration of the divalent phosphate ion (HPO_4^{2-})–the P species which is more susceptible to be adsorbed (Barrow, 1984; Bowden et al., 1980), increases. Nonetheless, reports on soil pH change effects concerning P availability to plants are very controversial. For instance, Penn and Camberato (2019) supported the classical view that P availability is generally maximized at near-neutral pH which coincides with the lowest degree of P fixation by Ca, Al, and Fe according to a diagram (Figure 1.5) redrawn from Price (2006).

(Material removed due to copyright compliance)

Figure 1.5 General representation of the relationship between phosphorus fixation and pH according to Price (2006).

However, this view was rejected by Barrow (2017) who contended that P uptake by plants and desorption by soil occur with a much lower pH optimum. This was also confirmed recently by Barrow et al. (2021); Barrow (2020); Barrow et al. (2020b). These two contrasting views in interpreting the relationship between soil pH and P availability were both based mostly on abiotic/chemical processes (adsorption/desorption and precipitation reactions) and fewer efforts were deployed to understand how pH could affect P availability via biological processes especially in the vicinity of the roots (e.g., rhizosphere) where P is effectively taken by plant roots. Moreover, up to half of soil P can be in the organic form which must undergo mineralization (biological/biochemical activity) before it can be accessed by plants (McLaren et al., 2020; Oehl et al., 2004). To advance science in these current knowledge gaps, the investigation of soil pH change effects on rhizosphere processes controlling P availability, its mobilization, and acquisition by plants is required. Progress in a more detailed understanding of rhizosphere mechanisms holds great promise for improving phosphorus use efficiency (PUE) and crop productivity. Consequently, promoting sustainable management of P resources in agroecosystems because there are limits to global rock phosphate reserves (Brownlie et al., 2021).

Many plant species have evolved in P-limited acid soils, and as a consequence, are known to have adaptive mechanisms that result in an increased acquisition of phosphorus from the soil (George et al., 2011; Ramaekers et al., 2010), including:

- Root morphological strategies such as increased length and frequency of root hairs, production of more adventitious roots, high specific root length, the formation of specialized root structures such as cluster (or proteoid) roots formed on white lupin (*Lupinus albus*). These strategies result in a larger exploration and foraging of soil volume (Haling et al., 2018; Lynch and Brown, 2008; Richardson et al., 2011).

- Association with mycorrhizae: The main benefit of this association for the uptake of P comes from the ability of mycorrhizal fungi to increase the surface area and effective length of roots, enabling the exploitation of a larger volume of soil. Mycorrhizas also enhance the utilization of soil organic P in some cases (Beltayef et al., 2021; Liyuan et al., 2021; Richardson et al., 2009). However, not all legumes are hosts of mycorrhizae, for example, lupins are known to be non or weakly-mycorrhizal (Lambers and Teste, 2013), whereas lucerne and clover associate with mycorrhizae (Peng et al., 2020).

- Root physiological strategies such as the release of extracellular organic anions and phosphatase enzymes:

Organic anions, as illustrated in Figure 1.6, have been shown to increase soil P availability by reducing inorganic and organic P sorption via competition for sorption sites and the alteration of surface characteristics of soil particles, and through the chelation of Al, Fe and Ca. They also stimulate the plant growth-promoting microorganisms in the rhizosphere such as bacteria and mycorrhizal fungi (Jones and Brassington, 1998; Lambers et al., 2015; Ryan et al., 2001; Wang and Lambers, 2020).

Organic P is abundant in soils and can contribute to the P nutrition of plants and microbes (Condron et al., 2005). The hydrolysis of organic P in the soil is mediated by phosphatase enzymes; several studies have demonstrated that higher rates of organic P mineralization were related to higher phosphatase activity either in natural ecosystems (Fox and Comerford, 1992; Polglase and Attiwill, 1992) or in pasture systems (Oberson et al., 2001; Richardson et al., 2009; Turner and Haygarth, 2005).

(Material removed due to copyright compliance)

Figure 1.6 Schematic diagram showing the effects of organic anions on soil P mobilization (Wang and Lambers, 2020), PGPR: Plant Growth-Promoting Rhizobacteria.

Soil phosphatases includes: (1) phosphomonoesterases which are involved in the degradation of orthophosphate monoesters including, sugar phosphates, polyphosphates, and mononucleotides, but the predominant monoesters in soils is inositol phosphate (Turner et al., 2002). Phosphomonoesterases consist of acid phosphomonoesterase and alkaline phosphomonoesterase, the former is released by both plants and microbes. However, the latter has not been detected in plants and is thought to be synthesized by soil microorganisms only (Nannipieri et al., 2011). (2) phosphodiesterase is involved in the hydrolysis of orthophosphate diesters like nucleic acids and phospholipids. Although occurring in a much smaller amounts compared to monoesters, diesters are soluble and rapidly mineralizable (Condron et al., 2005; Magid et al., 1996). This enzyme is reported to be secreted mainly by microorganisms. However, plants were also found to release phosphodiesterase under severe P limitation (Abel et al., 2000; Asmar and Gissel-Nielsen, 1997), but their contribution in total phosphodiesterase activity in the soil is thought to be negligible according to Turner and Haygarth (2005). Having said that, it is also worthwhile mentioning that phosphatase activity is not only mediated by the nature of the organic P substrate available in the soil. Soil pH is also commonly known to have a significant role in altering it since different phosphatases have distinct pH optima. For example, acid phosphatase activity exhibits maximum activity around pH 5.0-6.5 (Eivazi and Tabatabai, 1977; Hui et al., 2013), while phosphodiesterase has been found to have an

alkaline pH optimum (Browman and Tabatabai, 1978; Herbien and Neal, 1990). Moreover, the stability and conformation of phosphatases in soil are influenced by pH; the stabilized phosphatase due to soil sorption can be mobilized via soil pH increase (Allison, 2006; Skujiņš and Burns, 1976). Hence, the degree of stabilization on solid surfaces may change the pH optima of these enzymes. This suggests that the usually used enzyme assays, although they simplify and standardize the assay procedure, may confound interpretation of results if there are marked differences in optimum pH values in different soils. This is supported by the study of Turner (2010), who found that pH optima of each enzyme, especially phosphodiesterase, differed depending on soil type used. Some of the relevant results of this study are presented in Figure 1.7. Additionally, Margenot et al. (2018) found a strong linear decrease in acid phosphomonoesterase with an increase in pH across the pH 4.7 to 6.4 gradient. This result also did not reflect the generally accepted pH optima.

(Material removed due to copyright compliance)

Figure 1.7 pH optima of phosphodiesterase activity (right panel) and phosphomonoesterase activity (left panel) in six soils under lowland tropical rain forest in the Republic of Panama. Assays were conducted at 26°C using fluorogenic substrates in a modified universal buffer, MU: methyllumbelliferone. Error bars are the standard errors of the means for eight replicate wells per pH. Source: Turner (2010).

1.2.4 Liming

Liming is the most effective and long-established management practice for reducing soil acidity and maintaining an optimal pH for crop production (Li et al., 2019b). Although there are distinct differences in the response to lime, for most crops there is a positive yield response to liming, and the relationship between yield and soil pH has been quantified for several crops in the previous studies for both arable and grasslands (Holland et al., 2018; Holland et al., 2019; Moir et al., 2016). Because of the large differences in land use and the potential different management objectives for a given land or parcel, the management of lime is complex. This is due to several factors:

- Lime material type and quality: ground limestone ($CaCO_3$) is the most common liming material, then comes dolomite limestone ($CaMg(CO_3)_2$). However, calcitic limestone is known to have higher solubility compared to dolomite (Conyers et al., 1995). There is also some liming value in other products like compost, biochar, and rock phosphate (Basak and Biswas, 2016; Dai et al., 2020; Mokolobate and Haynes, 2002; Sikora, 2002). Moreover, the most important quality characteristics of a given liming material are : (1) the neutralizing value (e.g., the amount of acidity that a liming material can neutralize) and (2) particle size: the finest material shows better results (Álvarez et al., 2009).

- Application method: for example, in the no-tillage systems such as permanent pasture higher rates of lime should be applied compared to the system where lime is incorporated by tillage because surface liming is less effective than incorporated lime (Auler et al., 2019). Further, a single application is more effective than annual split doses (Álvarez et al., 2009).

- Soil properties: mineral soils with a high percentage of sand requires less lime compared to soils with high clay content (Sinclair et al., 2014). Also, other soil characteristics like organic matter content, cation exchange capacity, Al/Fe content, and initial/target pH influence lime requirements (Aitken, 1992; Curtin and Trolove, 2013; Lemire et al., 2006; Tunney et al., 2010).

Lime has numerous far-reaching impacts on plants: biomass production (Hayes et al., 2016), nodulation (Newbould and Rangeley, 1984), mineral content (Hamilton et al., 2012), herbage quality (Yu et al., 2011), and crop diseases (Lacey and Wilson, 2001). Soil processes and functions are also extensively impacted by liming, and these include increased nutrient availability for crops– more details on liming effects on soil chemical processes are listed in Table 1.1.

Table 1.1 Impacts of liming on soil chemical processes of selected macronutrients, heavy metals, and trace elements.

Nutrient/element	Process effect	Reference
Aluminium	Decreased soil exchangeable Al	Morton and Moir (2018)
	Decreased Al^{3+} activity in the soil solution	Brown et al. (2008); Miotto et al. (2020)
	Changes to Al speciation in the soil solution	
Phosphorus	Changes to plant-available P	Bouray et al. (2020); Moir et al. (2016)
	Increased organic P mineralization	Condron and Goh (1990); Condron et al. (1993)
	Increased solubility of added P	Simonsson et al. (2018)
	Affects P loss depending on soil type	Eslamian et al. (2021); Murphy and Sims (2012)
Sulphur (S)	Increased S mineralization	Bolan et al. (2003)
	Greater release of SO_4^{2-} and increased risk of SO_4^{2-} loss	Valeur and Nilsson (1993); Valeur et al. (2002)
	Increased SO_4^{2-} immobilization	
Calcium (Ca)	Increased Ca in the soil solution	Takamoto et al. (2021)
Heavy metals	Increased Cd immobilization	Cao et al. (2018); Wang et al. (2021)
	Decreased plant uptake of Ni, Cd, Pb and Mn	Cioccio et al. (2017); Kaninga et al. (2021)
	Decreased risk of heavy metal leaching	Fageria and Baligar (2008); Houben et al. (2012)
Potassium	Increased K availability	Han et al. (2019); Li et al. (2019b)
Trace elements	Increased Se availability	de la Luz Mora et al. (2008)
	Increased adsorption of Zn, Co, B and Cu	Hale et al. (2012); Lombi et al. (2003)

Besides, the application of lime has been found to have significant impacts on several soil biological processes such as mineralization, decomposition, mobilization and nitrification (Kemmitt et al., 2006; Kunhikrishnan et al., 2016; McCallum et al., 2015; Paradelo et al., 2015; Wachendorf, 2015). A simplified qualitative framework (Figure 1.8) has been presented recently by Holland et al. (2018) to illustrate and summarize the extensive and temporal impacts of liming on the processes and function of soils and crops. According to a systematic review done by Holland et al. (2018), despite the volume of research that exists on liming there remain knowledge gaps; for example, the liming impacts on greenhouse gas emissions (Kunhikrishnan et al., 2016), soil carbon stocks (Paradelo et al., 2015), and soil P uptake (Barrow, 2017, 2020; Barrow et al., 2020b, 2021). For instance, in terms of P, reports on liming effects on soil P availability are generally inconsistent, limited to the chemical effects (less work is done on the biological/biochemical side), and focused mainly on measuring P in the bulk soil (studies on rhizosphere soil and processes in response to liming are scarce). Hence, the importance of the present PhD research (see Chapters 4, 5 and 6).

(Material removed due to copyright compliance)

Figure 1.8 A qualitative framework of liming impacts for soils, crops, and biodiversity with a chronological scale for (a) properties and processes, and (b) function (ecosystem services) within an agricultural ecosystem. The solid circular/oval lines represent the time-span for the standard management practices; the dashed circular/oval lines represent a shorter time for improved management practices. Components in rectangular boxes represent different soil properties, soil processes, and related ecosystem services, components in hexagonal boxes represent crop or grass responses and related ecosystem services, components in diamond boxes represent regulation type ecosystem services and components in circles represent biodiversity and cultural services, adapted from Holland et al. (2018).

1.2.5 Phosphogypsum

Phosphogypsum (PG) is a solid by-product (waste) of the phosphate industry originating from the wet process of phosphoric acid production (Figure 1.9). The storage and recycling of PG are essential for many countries around the world and represent a serious concern for the phosphate industry (Chernysh et al., 2021; Saadaoui et al., 2017), as it contains some heavy metals and radioactive nuclides. The main areas where PG is produced and stacked are the USA, China, Africa, Middle East, and Russia (Tayibi et al., 2009). The annual production of PG worldwide is estimated to be 100-280 million tons, but the utilization rate is only 10–15% on the global scale including construction materials, agriculture, and others (Chernysh et al., 2021; El Zrelli et al., 2018). Therefore, PG management and utilization are important and adequate research must be undertaken. Phosphogypsum contains predominantly S and CaO and small amounts of P depending on the origin

of rock phosphate; the content of main elements and impurities in PG from different countries is presented in Table 1.2.

Table 1.2 Contents (% weight) of main elements and impurities in phosphogypsum from different countries

Main elements and impurities	Algeria[a]	Egypt[b]	Morocco[c]	Tunisia[d]	Turkey[e]	Brazil[f]	Canada[g]
CaO	31.18	32.13	38.14	30.7	32.04	37.05	30.2
P_2O_5	0.87	1.82	0.69	2.51	0.50	*nm*	1.3
SiO_2	0.88	8.78	0.86	1.38	3.44	1.39	6.38
SO_3	40.90	37.60	48.12	43.8	44.67	*nm*	43.1
Al_2O_3	0.10	0.29	0.19	0.1	0.88	0.14	0.24
Na_2O	1.32	*nm*	0.17	0.06	0.13	*nm*	0.05
Fe_2O_3	0.03	0.35	0.21	0.02	0.32	0.89	0.04
MgO	0.06	0.09	*nm*	0.01	*nm*	0.30	0.41
SrO	*nm*	*nm*	0.08	*nm*	*nm*	0.48	*nm*
K_2O	*nm*	*nm*	0.01	*nm*	*nm*	*nm*	0.06
F	1.20	0.80	*nm*	1.93	0.79	0.2	*nm*

nm: not measured

[a] Kacimi et al. (2006)

[b] Taher (2007)

[c] Rentería-Villalobos et al. (2010)

[d] Hentati et al. (2015)

[e] Degirmenci et al. (2007)

[f] Da Conceicao and Bonotto (2006)

[g] Luther et al. (1993)

(Material removed due to copyright compliance)

Figure 1.9 Flowchart A represents the production of phosphogypsum (PG) wastes, and B represents its marketing as an agriculture improver. TENORM: technologically enhanced naturally occurring radioactive materials. Source: Wang (2020).

The utilization of PG in agriculture as an amendment has become a topic of considerable interest. For instance, PG has been widely used to ameliorate the degraded soils, including saline-sodic soils (Nayak et al., 2013), acid soils (Caires et al., 2008; Caires and Guimarães, 2018; Crusciol et al., 2016), eroded soils (Cochrane et al., 2005; Mamedov et al., 2010) and contaminated soils (Mahmoud and Abd El-Kader, 2015). Details about the PG effects and mechanisms involved in the remediation process of the degraded soils are summarized and presented in Figure 1.10. Besides, the beneficial and adverse effects of PG utilization in agriculture were comprehensively synthetized in many recent reviews (Chernysh et al., 2021; Saadaoui et al., 2017; Wang, 2020).

(Material removed due to copyright compliance)

Figure 1.10 Degraded soil amelioration using phosphogypsum: mechanisms and effects. Source: Wang (2020).

1.3 Research objectives and thesis structure

Given the knowledge gaps highlighted and discussed in this literature review, this present PhD research aimed to push the current science boundaries and contribute to investigating and understanding the edaphic conditions in New Zealand hill and high-country soils in a way that serves not only NZ context but also the wider science community. The role of the hill and high-country areas in promoting the agriculture sector and subsequently the whole economy is indisputable. However, from a soil science point of view, farming development and sustainability in these typical environments necessitate a holistic understanding where four pieces are interconnected, excluding soil moisture (Figure 1.11).

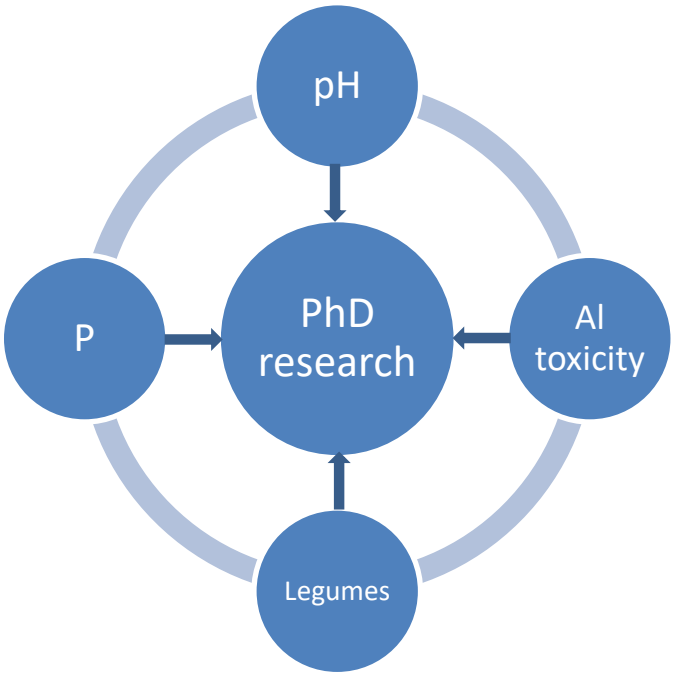


Figure 1.11 The main research components of the present PhD project.

Specifically, the aim was to investigate the four research components above through five experiments (five Chapters) as illustrated and summarized above, in Figure 1.12. One part of this research (three Chapters 4, 5 and 6, each with a separate objective) was dedicated to understanding how lime-induced pH elevation could influence soil P biochemistry and dynamics in the rhizosphere of lupins and under open field grassland conditions. The second part was composed of two Chapters (2 and 3, each with a separate objective) devoted to investigating the possibility of using phosphogypsum for NZ acid soils to improve soil fertility and reduce Al toxicity. Three legumes were used: lucerne (*Medicago sativa*, known for its sensitivity to Al toxicity and has high P requirements) and two lupin species: Russell lupin (*Lupinus polyphyllus*) and blue lupin (*Lupinus angustifolius*), Lupins were selected because of their active rhizosphere and their potential as an alternative crop for NZ hill and high-country environments.

The objectives of this thesis are:

Objective 1: Compare the effects of PG amendment, soluble fertilizer, and lime on short-term lucerne yield and P and S uptake in two acidic soils under controlled environment conditions (Chapter 2).

Objective 2: Examine the effect of phosphogypsum application on the distribution of Al species in the porewaters of both planted and incubated (unplanted) acid soils using the Visual Minteq Model (Chapter 3).

Objective 3: Examine the effect of soil pH change through liming from 5.3 to 6.0 on rhizosphere properties involved in P mobilization and acquisition by *Lupinus polyphyllus and Lupinus angustifolius* (Chapter 4).

Objective 4: Examine the impact of soil pH increase to near-neutral (pH 6.3) using lime on (1) acid phosphatase activity and labile P (DGT-P) distribution patterns in the rhizosphere of *Lupinus angustifolius* grown in two contrasting acid pasture soils, using colorimetric DGT with zymography, (2) root morphological and physiological root traits (Chapter 5).

Objective 5: Evaluate and quantify the effects of liming on P biochemistry and dynamics by conducting an 18-month field experiment on a long-term (+60 years) permanent fertilized grassland (Chapter 6).

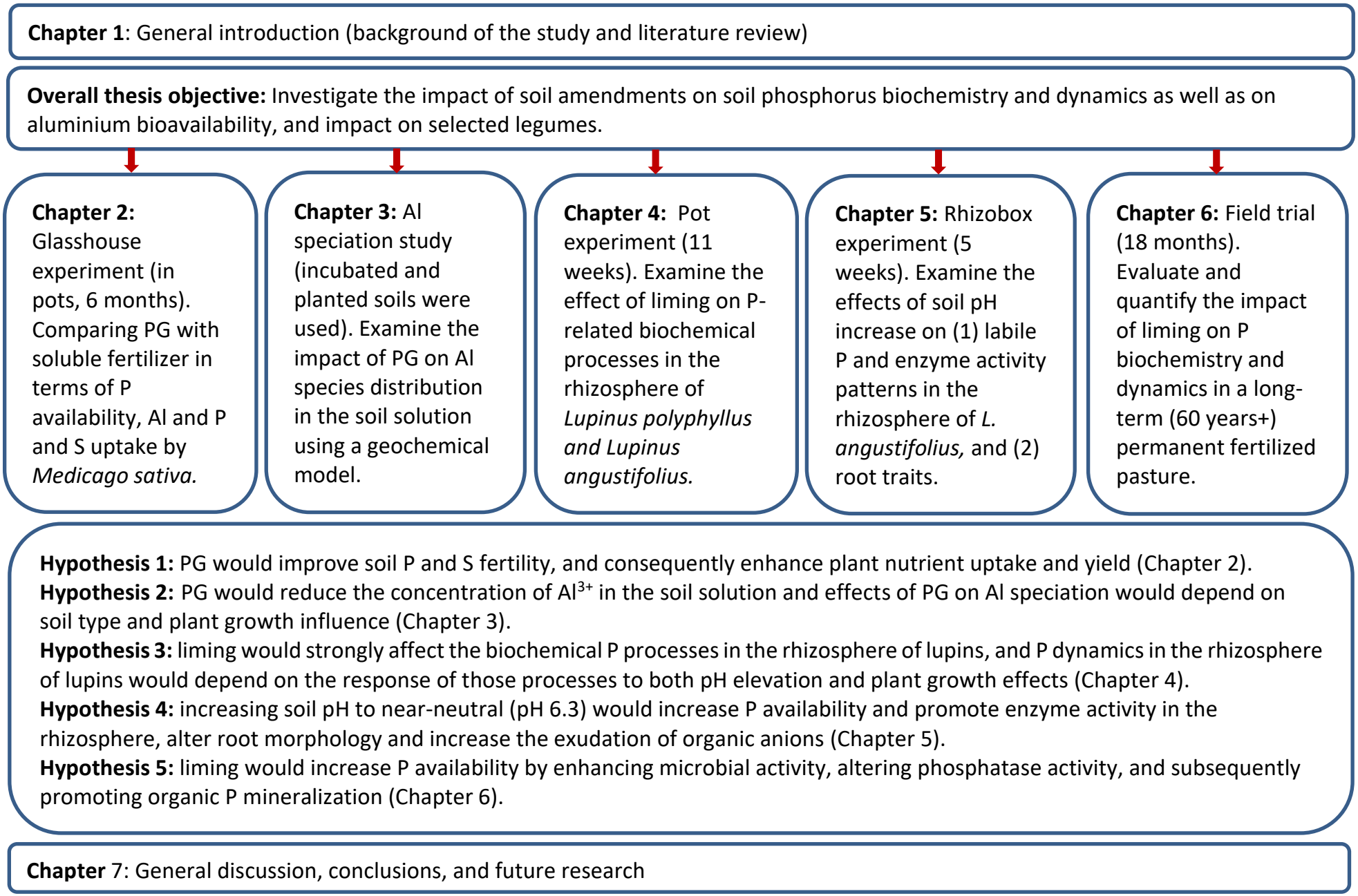


Figure 1.12 Overview of thesis structure.

Chapter 2

Impacts of Phosphogypsum, Soluble Fertilizer and Lime Amendment of Acid Soils on the Bioavailability of Phosphorus and Sulphur Under Lucerne (*Medicago sativa*)

(Published paper)

2.1 Introduction

Soil acidity coupled with phytotoxic concentrations of soil exchangeable aluminium (Al) and low P availability and very low sulphur (S) availability are among the major limitations to legume establishment and growth in New Zealand hill and high country farms (Haynes and Williams, 1993; Maxwell et al., 2013; Moir et al., 2016). Lucerne (*Medicago sativa*) is one of the most valuable forage legumes in New Zealand but is very sensitive to Al and manganese toxicities that prevail in low pH (Rechcigl et al., 1988; Su and Evans, 1996) and P availability (Moir et al., 2016). Hence the lime application is often an essential prerequisite to counteract soil acidity and enhance legume establishment and persistence in grasslands (Edmeades et al., 1983). However, liming effectiveness is limited to the shallow top-soil layer and has a very limited effect on subsoil acidity in the short term (Moir and Moot, 2010) due to its low solubility and passive movement down the soil profile (Hendrie et al., 2018; Kirchhof et al., 1995).

Most productive pasture legumes are adapted to highly fertile soils (Haynes and Williams, 1993). In contrast, soil fertility levels (plant-available P and S) are often low in many hill and high country soils in New Zealand (Maxwell et al., 2013) due to low fertilizer inputs (Moir et al., 2000) which are driven by the economics of fertilizer and lime application in these complicated topographical areas requiring aerial application (Gillingham et al., 1999). As such, the sustainability of legume production in New Zealand hill and high-country pastures may depend on new alternative affordable sources of nutrients for the farmers.

Phosphogypsum (PG), a by-product of the phosphoric fertilizer industry, is produced when rock phosphate (fluorapatite) is digested with concentrated sulphuric acid according to the following chemical formula:

$$Ca_{10}(PO_4)_6F_2 + 10\ H_2SO_4 + 20\ H_2O \leftrightarrow 6\ H_3PO_4 + 10\ CaSO_4.2\ H_2O + 2\ HF. \quad (2.1)$$

About 160 million tons of phosphogypsum are produced annually worldwide and it is mainly disposed of in large stockpiles or discharged in water bodies (IAEA, 2013). It contains predominantly sulphur and calcium oxide and small amounts of phosphorus (Saadaoui et al., 2017). It may contain small amounts of heavy metals and radioactive element impurities, whose concentrations depend on the composition of raw materials (Arnold and Hurst, 1980; Mazzilli et al., 2000) and the processing method used (Rutherford et al., 1995). Because of these impurities, the use of PG has been restricted in some markets, although these restrictions did not always have a proper scientific justification (IAEA, 2013).

Phosphogypsum is used in agriculture all over the world, for example in Brazil, Spain, Australia, India, Pakistan, USA and Egypt (Abril et al., 2008; Alcordo and Rechcigl, 1993; Vyshpolsky et al., 2008), either as soil amendment under the category ''calcium sulphate'' or as fertilizer (Mesić et al., 2016). Several benefits of PG application in agriculture have been reported worldwide for saline / sodic soils (AĞAR, 2011; Armstrong and Tanton, 1992; Nayak et al., 2011) or acidic soils (Caires and Guimarães, 2018; Crusciol et al., 2016; Degirmenci et al., 2007; Masud et al., 2015). However, research on PG application on acid soils has mostly focused on its effects in alleviating the toxic effects of high Al bioavailability or used in providing calcium for crops (Crusciol et al., 2016). Studies examining PG effects on soil fertility in general and on P and S availability in acid soils (pH ≤ 5) for legumes are limited; to date, there has been no research on PG use in NZ grasslands.

The main objective of this study was to compare the effects of PG amendment, soluble fertilizer and lime on short-term lucerne yield and P and S uptake in two acid soils under controlled environment conditions. We hypothesized that PG would improve soil P and S fertility, and consequently enhance plant nutrient uptake and yield.

2.2 Material and methods

2.2.1 Soil characteristics

Two acid soils with different chemical and physical properties were used (Table 2.1). They were collected from two sites and are known to be phosphorus-deficient and have high bioavailable Al concentrations (Martin-Hendrie, 2019; Whitley, 2018). The ''GM'' soil was sampled from Glenmore station, located on the southern banks of Lake Tekapo, central Canterbury, while the ''MO'' soil was collected from Molesworth station, in the Marlborough region. The two soils are classified as Dystrudepts (USDA, 2014) or Brown soils (NZ soil classification after (Hewitt, 2010)). Upon collection (0–15 cm), plant material and stones were removed. The soils were air-dried and sieved (4 mm mesh).

Table 2.1 Results of soil chemical and particle-size distribution before the establishment of the experiment.

Soil Analysis	Molesworth	Glenmore	By method of
pH (H_2O)	4.7	5.0	Blackmore et al. (1987)
Olsen P (mg kg^{-1})	13	18	Olsen et al. (1954)
Resin P (mg kg^{-1})	24	31	Saggar et al. (1990)
P retention (%)	59	42	Blackmore et al. (1987)
Inorganic P (mg kg^{-1})	160	196	Bowman and Moir (1993); Dick and Tabatabai (1977b); Turner et al. (2005)
Organic P (mg kg^{-1})	440	696	
P organic/P inorganic ratio	2.75	3.55	
Sulphate sulphur (µg g^{-1})	9	15	Watkinson and Kear (1994)
Reserve K (me $100g^{-1}$)	6.45	2.10	Carey and Metherell (2003)
Anaerobic Min N (kg ha^{-1})	102	169	Keeney and Bremner (1966a)
Organic matter (% w w^{-1})	8.5	10.6	Blackmore et al. (1987)
Exchangeable Al (mg kg^{-1})	21	8	Hoyt and Nyborg (1972)
Total N (% w w^{-1})	0.38	0.53	(Dumas combustion method using an Elementar Vario Max Cube Analyzer)
Total C (% w w^{-1})	4.91	6.18	
Carbon/Nitrogen	12.9	11.7	
CEC (meq $100g^{-1}$)	14	17	Brown (1943)
Ca (meq $100g^{-1}$)	0.9	4.7	Rayment and Higginson (1992)
Mg (meq $100g^{-1}$)	0.43	0.79	
K (meq $100g^{-1}$)	0.40	0.36	
Na (meq $100g^{-1}$)	0.06	0.07	
Base saturation (%)	12.9	34.1	
Particle-Size distribution %			
Clay (0.05–2µm)	17	13	ISSS Classification
Sand (20–2000 µm)	51	48	
Silt (2–20 µm)	32	40	

ISSS International Society of Soil Science.

2.2.2 Experimental design and treatments

The sieved soils were subjected to one of four treatments. In PG treatment, four rates of phosphogypsum: 0, 1, 3 and 9 t ha^{-1} (5.4, 16.2 and 48.6 kg P ha^{-1} and, 113, 339 and 1017 kg of S ha^{-1} respectively) were applied. In the soluble fertilizer (PS) treatment, P and S were applied at four rates to match the amount of the nutrients in the PG treatment: P was supplied as 0, 22, 66 and 198 kg of monocalcium phosphate ($CaHPO_4$) ha^{-1}, while S was supplied as 0, 0.5, 1.5 and 4.5 t ha^{-1} of sodium sulphate (Na_2SO_4). The rates of PG and PS were gradually increased to achieve an optimum Olsen P range of 25–30 mg kg^{-1} for lucerne (Pang et al., 2010b; Roberts et al., 1994; Sandral et al., 2019) at the highest rate. The chemical composition of PG and soluble fertilizers used in this study is presented in Table 2.2.

The four rates of PG and PS are reported in the text, tables and figures as R0, R1, R2 and R3 respectively and each rate supplies the same amount of P and S for both PG and PS. The control treatment corresponds to rate 0 (R0) where no P and S inputs were supplied.

Table 2.2 Chemical composition of the fertilizers used in the experiment.

		pHw	P	K	S	Ca	Mg	Na	Al	As	Cd
PG	% (wt wt^{-1})	3.5	0.54	0.08	11.30	16.11	0.03	0.19	0.12	4.10^{-4}	2.10^{-4}
Na_2SO_4	% (wt wt^{-1})	-	-	0.01	22.6	0.01	-	-	-	0	0
$CaHPO_4$	% (wt wt^{-1})	-	24.6	-	0.1	15.9	-	-	-	-	-

wt wt^{-1} = weight/weight.

Both PG and soluble fertilizer (PS) treatments were applied alone (without lime: 0 t ha^{-1}) but were also applied in combination with lime (2 t $CaCO_3$ ha^{-1}, lab-grade lime). This experiment was a 4×2×2 factorial design with 4 rates of PG or PS separately, two soils and two lime rates (0 and 2 t ha^{-1}). Four replicates were used for each treatment level, giving a total of 112 pots. The lime treatment was included to increase soil pH and thus facilitate seedling emergence and plant establishment and to test for possible interactions with PG and PS. The lime rate of 2 t ha^{-1} has been used based on the findings of a pot experiment (Whitley, 2018). It has been reported to be an optimum lime rate for lucerne yielding under the same soil types investigated in this study.

Lime, PG and soluble fertilizer treatments were thoroughly mixed with 200 g of air-dried soil. Basal potassium (K) was also mixed with the soil (300 kg of K ha^{-1} as KCl). The treated soils were deployed

in 250 mL plastic plant pots (66 mm diameter × 75 mm height) with holes at the bottom and distributed in a complete randomized block design on a table at Lincoln University (Lincoln, NZ) glasshouse facilities (Plate 2.1a). The daily average temperature for the experiment period was 18 °C. A small pot size was used to create a rhizosphere environment where nutrient uptake could be enhanced and to speed up soil nutrient cycling enabling to see treatment effect in short period of time. However, this could negatively impact plant growth (Poorter et al., 2012).

Lucerne (*M. sativa*, cv. Grasslands Kaituna) seeds were directly sown into the pots. After germination, the plants in each pot were thinned to 3 seedlings per pot and grown for six months, between March 16th to September 23rd, 2018. The pots were inoculated with a commercial (diluted peat culture) rhizobia strain, Group AL (New-Edge Microbials Pty. Ltd, Albury, Australia) 30 days post-germination to insure that an active rhizobia population is present in the soil. Throughout the growth period, soil moisture was monitored using high-frequency capacitance volumetric water content sensors (Decagon 5TM, Decagon Devices LTD, Pullman, Washington, USA) installed within the soil and maintained at 22–25% (v/v) by an automated dripper irrigation system (Plate 2.1b).

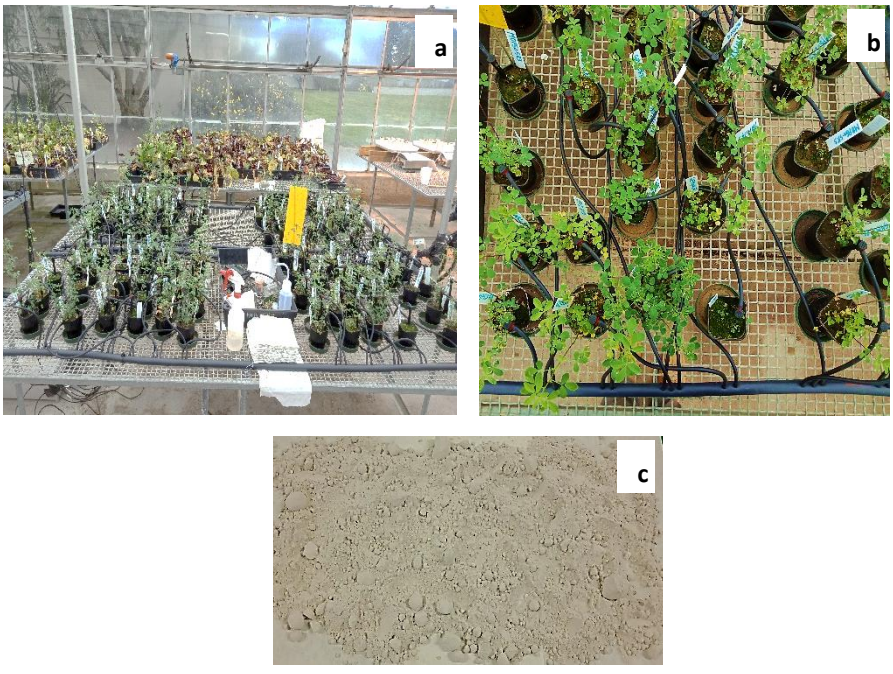


Plate 2.1 Pots distributed to four blocks on a table in the glasshouse (a), automated irrigation system (b) and phosphogypsum product used in this experiment (c).

2.2.3 Plant and soil sampling and analysis

Lucerne shoots were harvested four times during the experiment period by cutting 2 cm above the crown of each plant, oven-dried at 70 °C for 48 h, weighed, finely ground and bulked on an individual pot basis. Therefore, shoot nutrient uptake data shown in this study represents the average of the four harvests and shoot DM yields data represents the sum of the four harvests per pot. At the end of the experiment, the roots were harvested, whereupon they were carefully cleaned using deionized water, dried at 70 °C for 48 h and then weighed. The soils were then collected and air-dried at 30 °C for 7 days. After drying the soil was sieved (2 mm) and stored at room temperature in polyethylene bags to await for analysis.

The chemical characteristics of the soils were determined using standard methods (Table 2.1). Soil pH (1:2.5 soil: water ratio) was measured using both deionized water and 0.01 M $CaCl_2$. Bioavailable soil P (Olsen P) was extracted using 0.5 *M* sodium bicarbonate and was analyzed in a discrete wet chemistry analyzer (Smartchem TM 200, AMS Alliance, Paris, France). Exchangeable aluminium was extracted using 0.02 *M* $CaCl_2$ (1:4 soil: water ratio) and analyzed using Inductively Coupled Plasma Optical Emission Spectrophotometry (ICP-OES: Varian 720-ES ICP-OES, Varian, Melbourne, Australia).

Herbage samples underwent acid digestion (Nitric acid (HNO_3 69%)-Hydrogen Peroxide (H_2O_2 30%), 1:1 v/v) using a microwave digester (CEM MARS XpressTM, CEM Corp., Matthews, North Carolina, USA) (NIST, 1995). The digest solution was analyzed for total P and S using Inductively Coupled Plasma Optical Emission Spectrophotometer (ICP-OES: Varian 720-ES ICP-OES, Varian, Melbourne, Australia).

2.2.4 Statistical analysis

The data were analyzed at the end of the experiment and were subjected to analysis of variance (ANOVA) using Minitab® statistical software version 18 (Minitab, Inc., State College, Pennsylvania, USA). The normality of data distribution was verified as well as the homogeneity of variances to meet the ANOVA assumptions. The outliers were removed from the data set using Grubbs' test at the significance level of 0.05. The phosphogypsum or soluble fertilizer treatments, lime treatments

and soil types were considered as fixed factors. Three-way ANOVA was carried out to test the significance of the main effect of each factor and to identify any significant interactions between them, it was carried out for (PG, lime and soil types) and (PS, lime and soil types) separately. One-way-ANOVA was used to test the effect of treatment levels on soil parameters (Olsen P, pH and exchangeable Al) and plant parameters (yield and uptakes). Where differences between the means were significant ($p < 0.05$), the Dunnett test ($\alpha = 5\%$) was used to compare treatment levels to the control level. The comparison between PS and PG effect per rate on TDM yield was performed using a two-sample t-test at 5%. The effects were considered to be significant when $p \leq 0.05$. A correlation matrix was developed using the Pearson method to establish the relationships between P and S concentrations in the shoots and shoot DM yields. A simple linear regression was used to study the relationship between PG and PS application rates and soil Olsen P. A multiple linear regression (backward elimination) was used to determine the most important soil variable (pH, Olsen P or exchangeable Al) in impacting TDM yield. The variables were standardized by subtracting the mean then divide by standard deviation.

2.3 Results

2.3.1 Phosphogypsum effects on yield and nutrient uptake

Plant response to soil inputs varied depending on the soil type and treatment type and rate (Figure 2.1 and 2.2). Total dry matter (TDM) yields were different ($p < 0.001$) between the two soils, an average of 1.2 g and 3.2 g TDM yield per pot were recorded for Molesworth (MO) and Glenmore (GM) soils respectively, across all treatments. Likewise, the main effect of liming and phosphogypsum (PG) was significant on TDM yield per pot. However, the soluble fertilizer's (PS) main effect was not significant (Table 2.3). Moreover, the effect of the interaction on the TDM yield was only significant for soil × PG. As such, the significance of the simple effect of PG was presented for GM and MO separately under the two lime rates (Figure 2.2).

Table 2.3 Summary of the analyses of variance (3 way ANOVA) to evaluate the effect of soil type, lime and phosphogypsum (PG) or soluble fertilizer (PG) and their interactions on total dry matter yield of lucerne (TDM, g pot^{-1}).

Factors	Phosphogypsum (PG)	Soluble fertilizer (PS)
Soil (S)	***	***
Lime (L)	***	***
Rate (R)	***	n.s.
S*L	n.s.	n.s.
S*R	**	n.s
L*R	n.s.	n.s
S*L*R	n.s.	n.s

Asterisks indicate significant effect levels (*p < 0.05, **p < 0.01, ***p < 0.001), n.s. not significant.

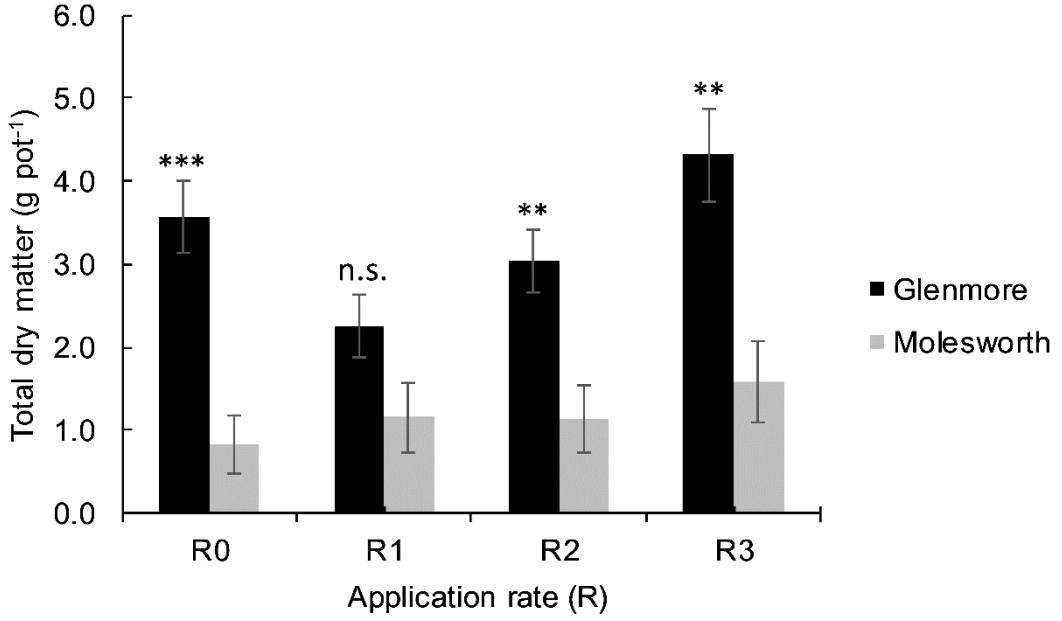


Figure 2.1 Total dry matter yield (g pot^{-1}, n = 8) of lucerne (*Medicago sativa*) after six-month growth period under two different soils: Molesworth and Glenmore , as affected by four rates of phosphogypsum (PG) across two lime treatments (0 and 2 t ha^{-1}). Error bars indicate standard errors (± SE, n = 8). Means of TDM for GM and MO per rate (R0 to R3) were separated using a two-sample t-test at 5%. Asterisks above bars indicate the level of significance in the difference between the two soils within each rate of PG (p < 0.01, ***p < 0.001, n.s. not significant).**

At the highest tested rate, R3 = 9 t ha^{-1}, the PG increased (p < 0.01) the TDM yield compared to the control (R0) in the unlimed MO soils (Figure 2.2a), whilst at the rates of (R1 = 1 t ha^{-1}) and (R2 = 3 t ha^{-1}), the yields were lower and no significant effects were recorded compared to the control. Whereas, in unlimed GM soil, the PG (9 t ha^{-1}) effect on the yield was not significant compared to the control unless combined with lime. The yields were greater in the presence of lime for both PG

and PS application. Phosphogypsum at R3 (9 t ha^{-1}) combined with lime (2 t ha^{-1}) increased TDM yield in the order of 46% and 77% compared with PG (9 t ha^{-1}) alone for GM and MO respectively. Similarly, soluble fertilizer applied at the same rates of S and P and combined with lime (R3 + Lime), increased the yield by 20% and 91% compared to PS (R3) alone for GM and MO respectively.

The comparison between PG and PS in terms of TDM yields generated per rate under the two investigated soils and lime rates are shown in Figure 2.2. Trends in dry matter yields were relatively similar for PG and soluble fertilizer (PS) treatments in MO soil (Figure 2.2a); the yields increased proportionally to the application rate. However, where lime was added, an opposite trend was observed between PG and PS for GM soil (Figure 2.2b). In most cases, the average TDM yield produced per rate were not different ($p > 0.05$) between PG and PS regardless of soil and lime effects, except for R3 under unlimed MO and R3 under limed GM where PG effect was significantly ($p < 0.01$) higher compared to PS.

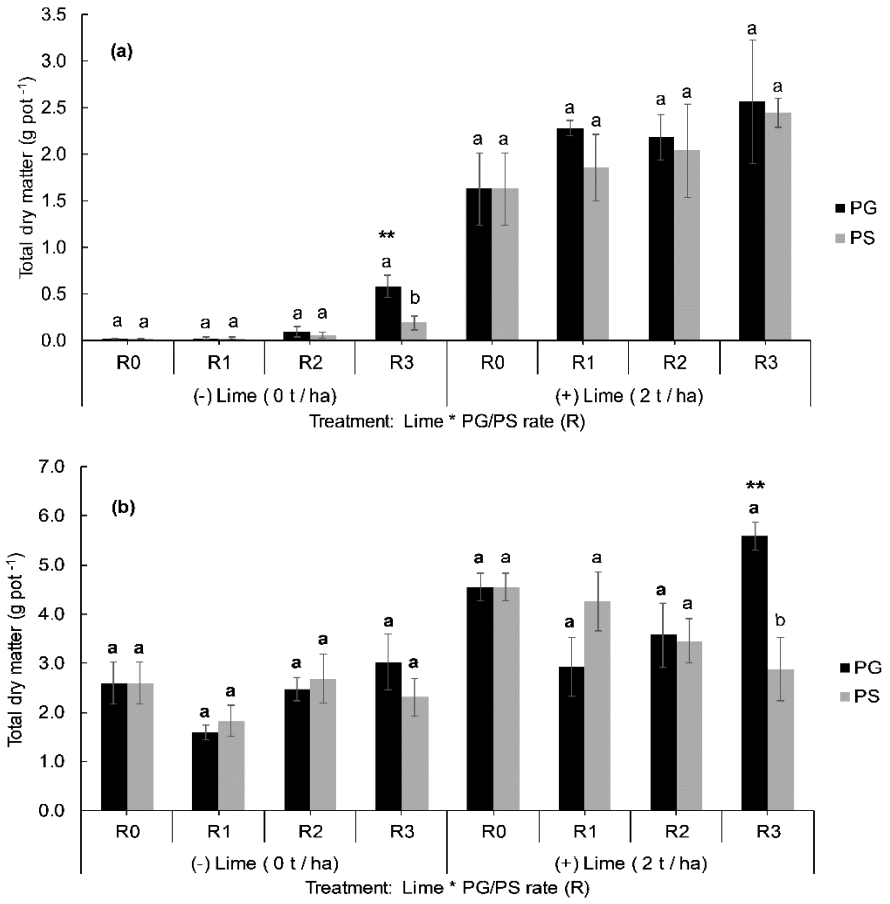


Figure 2.2 Comparison between the effects of phosphogypsum (PG) and soluble fertilizer (PS) on total dry matter yield (g pot^{-1}, n = 4) of lucerne (*Medicago sativa*) after six month growth period under two different soils: Molesworth (a) and Glenmore (b) treated (+) or not (–) with lime. Error bars indicate standard errors (± SE, n = 4). Means of TDM for PG and PS per rate (R0 to R3) were separated using a two-sample t-test at 5%, means indicated by the same lower-case letter per rate are not significantly different. Asterisks above bars indicate the application rate where TDM was significantly higher compared to the corresponding control under PG and PS separately, according to the Dunnett test at 5%. ** indicates a one-way ANOVA significance level (1%).

For the MO soil, most of the treatments without lime did not result in enough shoot dry matter for herbage analysis; the quantities harvested were less than 0.2 g which is the minimum required shoot weight for nutrients analysis through digestion solution, thus data of P and S uptakes were not determined. Shoots of the plants grown on limed GM soil showed higher P and S uptakes than those grown on limed MO soil. Significant treatment effects were recorded for the two soils regarding S uptake (Table 2.4). The highest S uptakes were found under PS (R3) and PG (R3) both combined with lime irrespective of soil type, though PG (R3) either combined with lime or not significantly enhanced S uptake compared with the control under GM soil. For P, the highest uptakes were recorded under

PG (R3) combined with lime regardless of soil type. For example, under limed GM soil, PG (R3) increased ($p < 0.05$) P uptake by 35% compared to PS (R3) and by 21% compared with the control (R0). However, without lime addition, PG's effect on nutrient uptake, particularly P, was similar to PS.

Table 2.4 The effects of phosphogypsum (PG), soluble fertilizer (PS) and lime on lucerne (*Medicago sativa*) shoot P and S uptakes from Glenmore and Molesworth soils after a six-month plant growth period. Within rows, means followed by the same lower-case letter are not significantly different (Dunnett test at 5%, R0 = control). Within columns, means were compared using a two-sample t-test at 5%.

		P uptake (mg pot^{-1})					S uptake (mg pot^{-1})				
		R0	R1	R2	R3	p value‡	R0	R1	R2	R3	p value‡
Glenmore											
(−)	Phosphogypsum	3.76	2.31	3.11	3.87	n.s.	4.55a	4.31a	5.96b	8.32b	< 0.001***
Lime	Soluble fertilizer	3.76	2.79	3.78	2.87	n.s.	4.55	4.57	5.71	7.69	n.s.
	P value†	n.s.	n.s.	n.s.	n.s.		n.s.	n.s.	n.s.	n.s.	
(+)	Phosphogypsum	4.44	3.73	4.61	5.65	n.s.	5.92a	5.79a	7.72a	9.77b	< 0.001***
Lime	Soluble fertilizer	4.44	3.89	4.15	3.65	n.s.	5.92	6.77	7.13	8.36	n.s.
	P value†	n.s.	n.s.	n.s.	0.043*		n.s.	n.s.	n.s.	n.s	
Molesworth (MO)											
(−)	Phosphogypsum	n.d.	n.d.	n.d.	0.50	N.A.	n.d	n.d.	n.d.	1.33	N.A.
Lime	Soluble fertilizer	n.d.	n.d.	n.d.	n.d.	N.A.	n.d	n.d.	n.d.	n.d.	N.A.
	P value†	N.A.	N.A.	N.A.	N.A.		N.A.	N.A.	N.A.	N.A.	
(+)	Phosphogypsum	1.43	2.08	2.26	2.52	0.053n.s.	1.97a	3.46a	3.86b	5.36b	0.001**
Lime	Soluble fertilizer	1.43	1.68	1.81	2.20	n.s.	1.97a	3.02a	3.63a	5.67b	0.002**
	P value†	n.s.	n.s	n.s.	n.s.		n.s.	n.s.	n.s.	n.s.	

Asterisks indicate significant effect levels (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$). N.A. not applicable, n.d. not determined, n.s. not significant, ‡ One-way ANOVA at 5%, † two-sample t-test at 5%.

The multiple linear regression analysis results are presented in Table 2.5. The data of PG and PS treatments were compiled to conduct this analysis (n = 128). The comparison of standardized coefficients for the three considered variables (pH, exchangeable Al and Olsen P) revealed that pH was the most important factor in impacting the TDM yield followed by Olsen P, then exchangeable aluminium. The interaction between these three predictors was not significant, therefore excluded from the model. The regression equation is presented below:

$$\text{TDM yield} = -13.20 + 2.89\ \text{pH}_{\text{CaCl2}} + 0.042\ \text{Exchangeable Al} + 0.082\ \text{Olsen P} \qquad (2.2)$$

(n = 128, $p < 0.001$, Adjusted R^2 = 25%)

Table 2.5 Multiple linear analysis regression results (coded coefficients).

	Standardized coefficients	SE coefficients	t value	*p* value	VIF
Constant	2.15	0.13	16.63	< 0.001	
pH ($CaCl_2$)	0.70	0.14	5.08	< 0.001	1.14
Exchangeable Al	0.31	0.16	2.01	0.047	1.45
Olsen P	0.40	0.15	2.55	0.012	1.43

VIF variance inflation factor, SE standard error.

2.3.2 Phosphogypsum effects on soil pH, Olsen P and exchangeable aluminium

Soil pH and exchangeable Al concentrations in both soils changed during the six-month growth period (Tables 2.6 and 2.7). There were decreases in both pH (H_2O) and pH ($CaCl_2$) between the highest and lowest PG application rates in the GM soil and for the pH (H_2O) in the MO soil, while the pH ($CaCl_2$) in the MO soil was relatively stable. Adding lime increased the pH of both soils and while the PG treatments mostly negated this effect at the highest rate, the lime generally reduced the acidifying effect of increasing PG application rate. When lime was applied, a significant decrease in pH was seen in the pH (H_2O) of both soils between the highest and lowest PG application rates. Another notable exception was the pH ($CaCl_2$) in the MO soil, where the effect of the lime was comparatively similar across the four levels of PG. The PS treatments did not significantly change the pH (H_2O) of the soils. Further, an increase ($p < 0.05$) in pH ($CaCl_2$) was observed at the highest rate (R3) compared with the corresponding controls for limed GM and unlimed MO soils.

Exchangeable aluminium concentrations under different treatments in both soils exceeded the toxicity threshold of 3 mg kg^{-1} (Moir et al., 2016) for most grassland legume species. However, a substantial decrease of exchangeable Al content was observed for some treatments. For example, 5.9 and 7.5 mg kg^{-1} decrease of exchangeable Al concentration in GM and MO respectively were found under limed control (R0) compared with the unlimed control. A decrease of 5.3 and 2.4 mg kg^{-1} of exchangeable Al was recorded in unlimed GM and MO soils respectively at R1 = 1 t ha^{-1} of PG compared with the corresponding controls, also a reduction of 5.7 mg of exchangeable aluminium per kg of unlimed MO soil at R2 (3 t PG ha^{-1}) compared with the corresponding control was observed. Whereas, at R3 (9 t PG ha^{-1}) the soil exchangeable aluminium increased in both soils. The same effect has been observed for soluble fertilizer PS (R3) in both soils, but PS was less effective in reducing soil exchangeable Al at low rates (R1 and R2) compared to PG; a decrease of 2.9 mg kg^{-1}

was observed for PS (R1) in the unlimed GM soil only. The exchangeable Al concentrations were relatively higher across all PS treatments compared to PG treatment in the absence of lime.

Table 2.6 Effects of phosphogypsum and soluble fertilizer on soil pH (water and $CaCl_2$) and exchangeable aluminium in both Glenmore and Molesworth soils under no lime application, after six months plant growth period. Within rows, means followed by the same lower-case letter are not significantly different (Dunnett test at 5%, R0 = control). Within columns, means were compared using a two-sample t-test at 5%.

	Phosphogypsum (PG)					Soluble fertilizer (PS)				
	R0	R1	R2	R3	p value‡	R0	R1	R2	R3	p value‡
pH water										
Glenmore (GM)	4.84a	4.82a	4.63a	4.42b	0.037*	4.84	5.03	5.00	4.86	n.s.
Molesworth (MO)	4.83	4.66	4.59	4.49	n.s	4.83	4.81	4.80	4.82	n.s.
P value†	n.s.	n.s	n.s.	n.s.		n.s.	0.041*	n.s.	n.s.	
pH $CaCl_2$										
Glenmore (GM)	4.47	4.39	4.31	4.23	n.s.	4.47	4.38	4.41	4.46	n.s.
Molesworth (MO)	4.16	4.24	4.30	4.31	n.s.	4.16a	4.33b	4.30a	4.43b	0.006**
P value†	n.s.	0.040*	n.s.	n.s.		n.s.	n.s.	n.s.	n.s.	
Exchangeable Al (mg kg^{-1})										
Glenmore (GM)	22.6	17.3	26.6	31.9	n.s.	22.6a	19.7a	29.7b	31.8b	0.037*
Molesworth(MO)	20.9	18.5	15.2	21.0	n.s.	20.9	21.5	20.1	20.7	n.s.
P value†	n.s.	n.s.	n.s..	n.s.		n.s.	n.s.	n.s.	n.s.	

Asterisks indicate significant effect levels (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, n.s. not significant). R0 to R3 indicates the four rates of PG (0, 1, 3 and 9 t ha^{-1}) and their equivalence for PS. ‡ One-way ANOVA at 5%; † two-sample t-test at 5%.

Table 2.7 Effects of phosphogypsum and soluble fertilizer on pH (water and $CaCl_2$) and exchangeable aluminium in Glenmore and Molesworth soils under liming (2 t ha^{-1}) conditions. Within rows, means followed by the same lower-case letter are not significantly different (Dunnett test at 5%, R0 = control). Within columns, means were compared using a two-sample t-test at 5%.

	Phosphogypsum (PG)					Soluble fertilizer (PG)				
	R0	R1	R2	R3	p value‡	R0	R1	R2	R3	p value
pH water										
Glenmore (GM)	5.26a	5.16a	5.00b	4.66b	<0.001***	5.26	5.27	5.27	5.20	n.s.
Molesworth(MO)	5.35a	5.23a	5.03b	4.82b	<0.001***	5.35	5.33	5.33	5.32	n.s.
P value†	n.s	n.s.	n.s.	0.042*		n.s.	n.s.	0.018*	n.s.	
pH $CaCl_2$										
Glenmore (GM)	4.64a	4.74a	4.73a	4.52b	0.011*	4.64a	4.75a	4.80a	4.86b	0.043*
Molesworth(MO)	4.73	4.74	4.74	4.65	n.s.	4.73	4.76	4.82	4.88	n.s.
P value†	n.s.	n.s.	n.s.	0.045*		n.s.	n.s.	n.s.	n.s.	
Exchangeable Al (mg kg^{-1})										
Glenmore (GM)	16.7a	16.8a	20.6a	30.4b	0.004**	16.7	16.3	19.5	22.0	n.s.
Molesworth(MO)	13.4	10.9	12.9	20.2	n.s.	13.4	15.7	13.2	14.3	n.s.
P value†	n.s.	0.026*	n.s.	n.s.		n.s.	n.s.	n.s.	n.s.	

Asterisks indicate significant effect levels (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, n.s. not significant). R0 to R3 indicates the four rates of PG (0, 1, 3 and 9 t ha^{-1}) and their equivalence for PS. ‡ One-way ANOVA at 5%, † two-sample t-test at 5%.

The relationships between PG and PS treatments and soil Olsen P are presented in Figure 2.3. The Olsen P measured in both soils increased ($p < 0.05$) in the presence of PG with a maximum recorded at 9 t PG ha^{-1} where Olsen P increase by 8 and 7 mg kg^{-1} compared with the control (0 t ha^{-1}) for MO and GM soils respectively. Similarly, PS increased ($p < 0.05$) Olsen P by 6 and 11 mg kg^{-1} at the highest rate (198 kg MCP ha^{-1}) compared with the control (0 kg MCP ha^{-1}) for MO and GM soils respectively. The average Olsen P across all treatments of PG or PS was higher ($p < 0.05$) in GM soil compared to MO soil for both 0 and 2 t lime ha^{-1} (Table 2.8). The average Olsen P across all PG rates decreased under liming in both soils but to a lesser extent compared to PS. For example, in limed GM soil, the average Olsen P for PG decreased by 0.9 mg kg^{-1} compared to the unlimed GM. However, for PS the decrease was 5 times higher than that of PG. A similar trend was observed in the limed MO soil compared to unlimed MO, though the difference between PG and PS is not as large as in GM soil.

Table 2.8 Average soil Olsen P (mg kg^{-1} ± SE) measured after 6 months of phosphogypsum (PG), soluble fertilizer (PS) and lime application to two different soils (Glenmore and Molesworth). Within columns, means followed by the same lower-case letter are not significantly different (Dunnett test at 5%). Within rows, means were compared using a two-sample t-test at 5%.

	(–) Lime (0 t ha^{-1})			**(+) lime (2 t ha^{-1})**		
	Glenmore	Molesworth	*p* value‡	Glenmore	Molesworth	*p* value
Control	17.1 ± 1.51a	14.5 ± 0.67a	n.s.	17.3 ± 0.80a	11.0 ± 0.73a	0.002**
Phosphogypsum	20.0 ± 1.10a	16.9 ± 1.06a	0.051n.s.	19.1 ± 0.86a	13.2 ± 0.70b	<0.001***
Soluble fertilizer	21.8 ± 1.40b	16.9 ± 0.85a	0.008**	17.5 ± 1.1a	12.5 ± 0.41a	<0.001***
p value†	0.025*	n.s.		n.s.	0.044*	

Asterisks indicate significant effect levels (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$), n.s. not significant.
† One-way ANOVA at 5%, ‡ two-sample t-test at 5%.

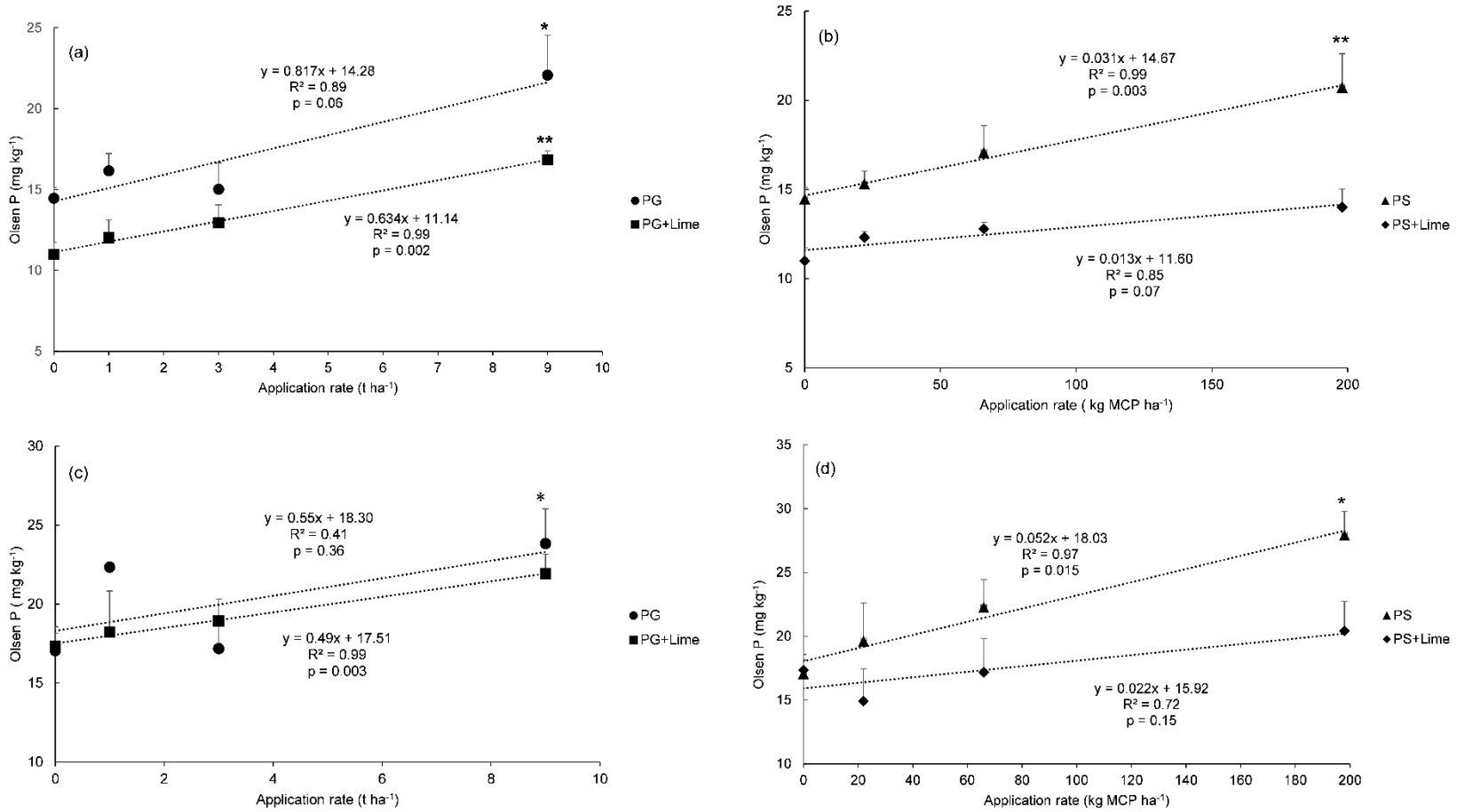


Figure 2.3 Relationship between mean Olsen P (mg kg^{-1}, n = 4) and application rate of phosphogypsum (PG, t ha^{-1})) and soluble fertilizer (PS, kg of MCP ha^{-1}) combined or not with lime under Molesworth ((a) and (b)) and Glenmore ((c) and (d)) soils, separately. Error bars indicate upper standard errors (SE, n = 4). MCP: mono-calcium phosphate ($CaHPO_4$). Asterisks indicate the application rates which are significantly different from the control (0 tons or 0 kg ha^{-1}), according to the Dunnett test at 5%. * and ** indicate a one-way ANOVA significance level of 5 and 1%.

2.4 Discussion

2.4.1 Phosphogypsum effects on yield and nutrient uptake

The difference in TDM yields produced under MO and GM soils regardless of treatment type can be attributed to the initial soil fertility. Low soil pH coupled with high exchangeable Al in the MO soil prevented seedlings from germinating and establishing (TDM = 0 g pot^{-1} for control), thus depressing the overall average TDM in that soil compared with GM. Despite showing a decrease in exchangeable Al at 1 and 3 t ha^{-1}, PG effects on TDM yields were less efficient than lime alone even at high application rates independently of soil type. These findings support the evidence that reducing exchangeable Al without improving soil pH will not be sufficient for legumes to persist. Therefore, lime was more efficient as it significantly increased soil pH while reducing exchangeable aluminium, which was not the case for PG. However, the mixture PG + Lime enhanced dry matter yield better than lime alone due to the supply of P and S through PG application. These two elements are considered the most limiting edaphic requirement to legumes in NZ hill and high country farms (Haynes and Williams, 1993; Moir et al., 1997) which is consistent with the highest DM yields being observed when PG + Lime or PS + Lime were applied to MO soil. However, under GM soil TDM yield response to PS and PG did not support this hypothesis, except for PG at the highest rate where a significant increase in the yield was observed compared to the control under liming conditions.

The response to PG + Lime or PS + Lime was more pronounced for the MO soil compared to the GM soil, presumably because of the low initial P and S content of MO soil. The SO_4^{2-}-S level of the GM soil was above 10–12 mg S Kg^{-1}, which is the range for near-maximum pasture on hill and high country farms in NZ (Morton and Roberts, 1999). For the MO soil, SO_4^{2-}-S level was below that range and its initial Olsen P was 5 units lower than GM. Additionally, the poor response to PS and PG in the unlimed GM soil compared to unlimed MO soil in terms of TDM yield could also be due to the exhibited higher Al bioavailability. For example, the drop in TDM yield observed at PS (R3) under unlimed GM soil was coincided with a significantly higher exchangeable Al concentration compared to the rest of the PS treatments.

The improvement of TDM yield under limed GM at PG (R3) against PS (R3) is supported by the significantly higher P uptake and higher S uptake for PG (R3) compared to PS (R3). This was in agreement with the difference in Olsen P concentration between the two treatments (ΔOlsen P = 1.5 mg kg^{-1}) even though PS (R3) has greatly increased Olsen P compared to PG (R3) in the absence of lime (ΔOlsen P = 4 mg kg^{-1}) for the same soil. These findings suggest that the depressive effect of lime on P availability is higher for PS than PG. Moreover, at R3 (9 t ha^{-1}), PG supplied large amounts of Ca which were found to decrease Al activity in soil solution when Ca^{2+}/Al^{3+} ratio is high even if exchangeable Al

is high (Cunha et al., 2018), therefore alleviating its deleterious effects on roots (Bakker et al., 2000; Brady et al., 1993; Manoharan et al., 1996).

The fact that P uptakes were not significantly affected by P supply for PG and PS treatments in the absence of lime compared with the control (R0), gives insight that this was likely related to other factors, probably soil pH. This explanation is strongly supported by higher P uptakes measured under Lime + PG and Lime + PS where pH is significantly higher. Similar findings were recently found by Otieno et al. (2018) in acid soils of western Kenya. Additionally, the combination PG + L has been reported to stimulate soil microbial activity (Inagaki et al., 2016; Lee et al., 2009) and this could have improved phosphorus bioavailability.

The continuous increase in shoot uptaken S per pot with PG and PS rate increase indicates that lucerne was still S-uptake responsive to S supply even at high rates and that PG can be an alternative source for S fertilization as it performed almost identically to soluble fertilizer in both soils. The sulphur concentration of shoots under different PG and PS rates exceeded the optimum range of 0.18–0.22% S, suggested by Craighead and Metherell (2006) for NZ high and hill country farms.

Lime addition increased S uptake under both PG and PS treatments. This was likely due to the mobilization of the adsorbed SO_4^{2-} at low pHs (Martini and Mutters, 1984; Mehlich, 1964). The effect of lime is usually attributed to the competition between OH^- and SO_4^{2-} on adsorption sites on Fe and Al hydrous oxides and P compounds may also compete for adsorption sites as they become more soluble at higher pHs (Korentajer et al., 1983). Furthermore, the enhancement of root growth following lime application could also explain the greater uptakes of P and S with increased soil pH (Appendix A: Figure A.1).

We can conclude from the negative linear relationships exhibited between shoot dry matter yields and the shoot concentration of P and S (Table 2.9), that P and S supply may not be limiting the yield in GM soil. This explanation is supported by the observed mean nutrient concentrations in the plant shoots, which are in ''adequate'' range according to Craighead and Metherell (2006); Morton et al. (1999); Venter et al. (2004). This hypothesis is also in line with the TDM yield data in GM soil where no significant differences were found between the control and treated soils with P and S either through PS or PG, except PG (R3 + Lime). Conversely, the decrease of P and S content of shoots with increased yield could be due to the '' dilution effect'' associated with the extra dry matter production (Jarrell and Beverly, 1981).

Table 2.9 Correlation matrices of the nutrient concentrations (g kg^{-1} of shoot DM) and shoot DM yield (g) produced in GM and MO soils

	Glenmore (GM) soil		Molesworth (MO) soil	
	Shoot DM	S content	Shoot DM	S content
S content	−0.65***		−0.19 n.s.	
P content	−0.53***	−0.40**	−0.11 n.s.	0.41*

Asterisks indicate significance levels of the relations (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$), n.s. not significant.

The correlation matrix showed weak relationships between nutrients concentrations and shoot yield under MO soil. However, the mean P concentrations across all treatments were below deficiency values. This indicates that the uptake of P is not solely dependent on its availability in this soil but influenced by other factors, most likely soil pH. Although no strong correlation was found between shoot yield and shoot S concentrations, an increase in S concentration was recorded when sulphur was supplied through PG and PS compared to the control. This could be explained by the luxury consumption of S by lucerne (Venter et al., 2004), which means that not all the S removed by the crop is essential for plant growth. Moreover, the absence of the relationship between shoot yield and shoot P and S concentrations could to some extent be associated with the small pot-size used in this experiment because plants could have been pot bound for a relatively long period, which likely affected shoot yield due to restricted growth.

2.4.2 Phosphogypsum effects on soil pH, Olsen P and exchangeable aluminium

The lower soil pH (H_2O) resulting from phosphogypsum addition was consistent with the findings of Jarak et al. (2003); Lee et al. (2009); Nayak et al. (2011). The PG used in this study had a high calcium content which likely displaced Al^{3+} and H^+ on cation exchange sites into the soil solution resulting in low pH, this view is supported by the measured pH (H_2O) under PS treatments, where Ca supply was largely lower compared to PG, which resulted in a relatively stable pH (H_2O). Besides, the low pH (H_2O, 3.5) of this PG product likely contributed to acidifying the soil. On the other hand, Smith et al. (1994) found that the pH of surface amended soil with 2.5 t ha^{-1} of PG was unchanged.

The pH ($CaCl_2$) results in this study showed an opposite trend to pH (H_2O), especially for unlimed MO soil as its pH ($CaCl_2$) increased slightly with PG. This confirmed what was reported recently about the increase of soil pH at depths of 0 to 5 cm by Crusciol et al. (2016), after the surface application of phosphogypsum. Further, this result corroborates early reports by Toma et al. (1999), who found that gypsum application decreased pH (H_2O) while pH ($CaCl_2$) did not. This could be explained by pH (H_2O) overshadowing the liming effect of phosphogypsum due to the salt effect, whereas measuring pH in 0.01 M $CaCl_2$ kept constant the effect of salt on the hydrolysis of Al forms releasing H^+ protons. Moreover, in a pot experiment conducted by Edmeades et al. (1983) where they recorded the same behavior between pH (KCl) and pH (H_2O), this was claimed to be related to the decrease of ionic

strength of the solution after dilution with H_2O. Therefore, in glasshouse studies where soil volume is limited promoting high ionic strength conditions, the interpretation of pH (H_2O) must be done carefully. Similarly, $CaCl_2$ extracted pH increased significantly under PS for unlimed MO and limed GM. The observed rise in pH ($CaCl_2$) following PS and PG application to the soil can also be ascribed to ligand exchange, whereby the supplied SO_4^{2-} replaces OH^- (Hue et al., 1985; Turner and Kramer, 1991). So, considering these factors, we can state that the PG and PS effect on soil pH likely depends on the balance between Ca^{2+} and SO_4^{2-} reactions.

The observed reduction in soil Al concentrations under soils treated with 1 t ha^{-1} of PG agrees with Crusciol et al. (2016). This effect can be explained by the association of the Al^{3+} ions with SO_4^{2-} and F^- forming ionic pairs $AlSO^{4+}$, AlF_2^+, AlF^{2+} and AlF_3^0 (Carvalho and van Raij, 1997). The absence of a reduction in exchangeable Al concentration at PG 9 t ha^{-1} for both MO and GM soils was probably due to the higher ionic strength in the soil solution. This would favor ionic exchange reactions to the detriment of adsorption and precipitation reactions. Hence, the ionic exchange of Ca^{2+} would have increased Al^{3+} in the solution and dominated the ligand exchange reactions. Additionally, at PG (9 t ha^{-1}) the pH (H_2O) has dropped by 0.4 and 0.34 units compared to the control in unlimed GM and MO soils respectively. This triggered an increase of exchangeable Al in GM and MO soils. This inverse relationship between pH and exchangeable Al in New Zealand soils has been confirmed by several researchers (During et al., 1984; McIntosh and Backholm, 1981; Moir and Moot, 2014; Moir and Moot, 2010; Morton et al., 2005; Venter, 2017; Wheeler and O'Connor, 1998; Whitley et al., 2016). However, the observed increase in exchangeable Al under PS treatments was not related to soil pH which was unchanged. This agrees with the findings of Horsnell (1985), in a glasshouse experiment, where those workers found that neutral salts (K_2SO_4 or $CaSO_4$) in the presence of calcium phosphate increased aluminium concentrations in soil solution.

The difference between the influence of PG (R1 = 1 t ha^{-1}) and PG (R3 = 9 t ha^{-1}) in the absence of lime on Olsen P was larger (6 units) for MO soil, while under GM soil it was small (only 1 unit). This has probably resulted from a sharp decrease in GM soil pH when 9 t ha^{-1} of PG was applied, which would have consequently increased P adsorption (Barrow, 2017) on oxide surfaces. This explanation is also supported by the high exchangeable Al content measured in unlimed GM under PG (9 t ha^{-1}), which exceeded that of PG (1 t ha^{-1}) by 14.6 mg kg^{-1} of soil.

The effect of PG on soil P availability is comparatively similar to that of soluble fertilizer used in this study as a standard source of P and S. This indicates the high solubility of total P contained in PG materials and its ability to be easily released into the soil and therefore be available to the plants in the same manner as soluble fertilizers. Phosphogypsum amendment could also have improved the

microbial activity and population in the soil (Al-Enazy et al., 2018; Inagaki et al., 2016; Lee et al., 2009; Nayak et al., 2011) resulting in a higher P solubility.

Lime application decreased Olsen P for both soils regardless of treatment type. Our results are in line with studies done by other workers on acid soils (Curtin and Syers, 2001; Sorn-Srivichai et al., 1984). This decline in soil P under lime application can be due to the formation of Ca-P precipitates (Penn and Camberato, 2019). Moreover, when pH is increased, the proportion of absorbable P species increases such as the divalent phosphate (HPO_4^{2-}) (Barrow, 1984). The formation of insoluble hydroxyl-Al species following lime can also be highly active adsorption surfaces for phosphate (McLean, 1976). Haynes and Ludecke (1981) reported an increase in Al-bound P fraction under liming. Moreover, the stability of hydroxyl-Al-P complexes has been reported to be high around pH 5 (White and Taylor, 1977). However, the decrease in P availability following liming is not supported by plant shoot P uptake in this experiment, which showed a significant increase when lime was applied. Alternative soil P tests under liming conditions are recommended as the decline of Olsen P could be due to an artifact in the Olsen procedure which uses high pH extractant (pH 8) favoring the Ca-P precipitation (Curtin and Syers, 2001; Sorn-Srivichai et al., 1984). On the other hand, the resulting decline in Olsen P under liming could to some extent be explained by the higher removed of P by plants as reflected by the higher P uptake by lucerne in the presence of lime.

2.5 Conclusions

Phosphogypsum application on acid soils showed positive effects on soil P availability, P and S uptakes and consequently on lucerne biomass production. However, the effects of PG on soil acidity depends on soil properties. Its magnitude of pH-neutralizing effects does not support PG to be used as a lime substitute but rather as a fertilizer supplement. The responses to PG would not be maximized without being necessarily combined with a pH ameliorant such as lime in our case. The strategy of blending materials might be feasible for acid soils and can be a real solution for the improvement of lime solubility and therefore its reaction time and movement down to the sub-soil layers. However, there is a lack of evidence about it, hence further studies are required in this aspect. Phosphogypsum has decreased exchangeable Al at low rates, this warrants further investigations are necessary to evaluate the effects of PG on Al species. Further studies are also required to identify Al toxicity thresholds for legumes. The Ca effect on Al activity and phytotoxicity could also support the use of Ca-rich materials such as PG on acid soils. Moreover, being a pot experiment, this study may not reflect what could happen under open field conditions. Therefore, the general applicability of the results of this experiment requires a confirmation using field trials.

The sustainable utilization of PG in agriculture necessitates long-term experiments focusing not only on fertilizing effects of PG but paralleled with an environmental impact assessment. Also, the assessment of PG effects on nutritional imbalances especially at high application rates is needed.

Soluble fertilizer used in this study (MCP + $NaSO_4$), if used without lime, may not be the most effective for the establishment and growth of lucerne on acid soils with high native exchangeable aluminium levels. The increased Al concentrations following the combination of MCP and $NaSO_4$ requires further investigation.

Chapter 3

Effect of Phosphogypsum on Aluminium Speciation in New Zealand Acid Pasture Soils

(Submitted paper)

3.1 Introduction

Soil acidity is one of the major issues in agriculture worldwide. About 50% of the world's arable lands are acidic (Von Uexküll and Mutert, 1995), which mostly occur in developing countries like Central Africa, South America and Southeast Asia (FAO and ITPS, 2015). Although the poor fertility of acid soils is due to low pH and nutrient deficiencies (e.g. P and Mo), Al toxicity is the most important factor, being a major constraint for crop production on 67% of the total acid soil area (Eswaran et al., 1997). Aluminium phytotoxicity to plants is often seen when soil pH decreases below 5.5 (Delhaize and Ryan, 1995; Rout et al., 2001). The Al effect on plant growth depends on the species of Al in the soil solution, of which Al^{3+}, $AlOH^{2+}$ and $AlOH_2^+$ are considered the most toxic (Kinraide, 1991; Singh et al., 2017). Root growth inhibition is the most easily recognized symptom of Al toxicity, and affects uptake and translocation of water and nutrients altering plant metabolism, growth and persistence (Rahman and Upadhyaya, 2020; Schmitt et al., 2016).

Aluminium toxicity represents a serious impediment to legume establishment and survival in New Zealand (NZ) grasslands (Moir et al., 2016), particularly in the steep-lands, popularly called the "hill and high country'' (Morton and Moir, 2018; Whitley et al., 2019). However, Al chemistry in NZ soils is poorly investigated and understood (Taylor et al., 2012; Wang et al., 1999). Methods of the analysis of Al in NZ soils have been mainly based on 0.02 M $CaCl_2$ and 1 M KCl extractions (Whitley et al., 2020), which provide only information on the soil exchangeable Al fraction. Also, they do not allow the identification of Al forms with specific inorganic and organic ligands. Moreover, the extractable-Al methods are known to solubilize Al from plant-unavailable fractions and provide a misleading indication of potential Al toxicity status (Marques et al., 2002; Percival et al., 1996). For instance, Schroth et al. (2000) found that the lack of toxicity symptoms in plants was attributed to very low Al content in soil solution (0.01 $cmol_c\ kg^{-1}$), even when exchangeable Al in the soil was 3 $cmol_c\ Kg^{-1}$. Several studies showed that the better measure to relate to plant growth is the activity of Al^{3+} in the soil solution (Menzies et al., 1994; Percival et al., 1996; Shuman, 1990). Therefore, examining Al species in the soil solution should provide a better estimate of Al phytotoxicity.

To understand the Al behaviour in soil environments it is necessary to know the distribution of different Al species in the soil solution and the potential for Al complexation with organic and inorganic ligands (Brautigan et al., 2012; Ščančar and Milačič, 2006). Numerous analytical procedures have been developed for fractionation of Al compounds and the determination of individual species, but many of them have limited selectivity, are not harmonized and the data comparison is scarcely reported (Bi et al., 2001; Ščančar and Milačič, 2006). In addition to experimental analytical techniques, computer modelling is a widely used speciation approach in soil science. Several computer models have been used in studies of Al speciation in soil solutions such as Visual MINTEQ (Alleoni et al., 2010; Chamier et al., 2015; Martins et al., 2020; Miotto et al., 2020) and WHAM (Tipping, 2005).

Agricultural lime ($CaCO_3$) is the most common amendment used in NZ to counteract the effects of soil acidity (Morton and Moir, 2018). However, its effectiveness is limited to the shallow top-soil layer and has a very limited effect on subsoil acidity in the short term (Caires et al., 2008; Caires et al., 2005), due to its low solubility and passive movement down the soil profile (Hendrie et al., 2018). As such, an alternative material with a comparatively higher solubility is necessary. Phosphogypsum, a by-product of the phosphoric acid industry, mainly contains calcium sulphate and small amounts of P and F. This product has been proven to be mobile in the soil and has been widely used when subsoil acidity is an important yield-limiting factor (Caires et al., 2011; Caires and Guimarães, 2018; Illera et al., 2004). Moreover, a mixture of lime and phosphogypsum is a strongly recommended strategy to improve lime reactivity and movement in acid soils (Carmeis Filho et al., 2017; Crusciol et al., 2016). However, studies examining phosphogypsum effects on Al speciation in acid soils, particularly with pH ≤ 5 are scarce. Recently, Bouray et al. (2020) attempted to investigate the effect of four rates of phosphogypsum on soil exchangeable Al (using 0.02 *M* $CaCl_2$ method) in two different New Zealand acid soils. They found that phosphogypsum decreased soil exchangeable Al, if applied at low rates. However, analysing soil exchangeable Al alone is not enough to reveal the mechanisms involved. Also, the fact that those soils were grown with lucerne for six months would make it difficult to distinguish phosphogypsum effect from the plant growth effect on soil Al chemistry. Therefore, further investigations are necessary to elucidate the mechanisms and to understand the phosphogypsum rate effect.

In this context, we aimed to examine the effect of phosphogypsum application on the distribution of aluminium species in the porewaters of both planted and incubated (unplanted) acid soils. We hypothesized that (1) phosphogypsum would reduce the concentration of Al^{3+} in the soil solution (2) the effect of phosphogypsum on Al speciation would depend on soil type and plant growth effects.

3.2 Materials and methods

3.2.1 Soil characteristics

Three acid soils with different physical and chemical properties were used. They were collected (0-15 cm) from three sites and are known to have different exchangeable Al concentrations. The "Glenmore" soil was sampled from Glenmore station (43 ° 44' 24''S, 170° 28' 42''E), located on the southern banks of Lake Takapo, Central Canterbury. The "Molesworth" soil was collected from Molesworth station (42° 06' 17''S, 173° 07' 33''E), in the Marlborough region, while the "Lindis Peaks" soil was sampled from Lindis Peaks station (44° 46' 26''S, 169° 27' 21''E), in the Tarras region. Plant material and stones were removed, and then the soil was thoroughly mixed, air-dried and sieved (2 mm). The "Glenmore" and "Molesworth" are classified as Brown soils according to NZ soil classification (Hewitt, 2010), while ''Lindis Peaks'' is classified as Pallic. All soils are classified as Inceptisols in the USDA classification (Schoeneberger et al., 2012). The soil's physical and chemical characteristics are given in Table 3.1.

Table 3.1 Results of soil chemical and particle-size distribution before the establishment of the experiments.

Soil Analysis	Glenmore (GM)	Molesworth (MO)	Lindis Peaks (LP)	By method of
pH (H_2O)	5.0	4.7	5.3	Blakemore et al. (1987)
Olsen P (mg kg^{-1})	18	13	13	Olsen et al. (1954)
P retention (ASC, %)	42	59	21	Blakemore et al. (1987)
Sulphate sulphur (µg g^{-1})	15	9	11	Watkinson and Kear (1994)
Organic matter (% w w^{-1})	10.6	8.5	4.7	Blakemore et al. (1987)
$Al_{KC}l$ ($cmol_c\, kg^{-1}$)	1.7	2.3	0.07	Rayment and Lyons (2011)
Al_{CaCl2} ($cmol_c\, kg^{-1}$)	0.2	0.5	0.02	Hoyt and Nyborg (1972)
Total N (% w w^{-1})	0.53	0.38	0.24	(Dumas combustion method
Total C (% w w^{-1})	6.19	4.91	2.74	using an Elementar Vario
Carbon/Nitrogen	11.7	12.9	11.4	Max Cube Analyser)
CEC (meq $100g^{-1}$)	17	14	13	Brown (1943)
Ca (meq $100g^{-1}$)	4.7	0.9	5.7	Rayment and Higginson
Mg (meq $100g^{-1}$)	0.79	0.43	0.82	(1992)
K (meq $100g^{-1}$)	0.36	0.40	0.29	
Na (meq $100g^{-1}$)	0.07	0.06	0.07	
Base saturation (%)	34.1	12.9	53.6	
Particle-Size distribution				ISSS Classification
Clay (0.05- 2µm)	13 (%)	17	5.8	
Sand (20-2000 µm)	48 (%)	51	62	
Silt (2-20 µm)	40 (%)	32	32.3	

ISSS International Society of Soil Science.

3.2.2 Experimental design and treatments

A 60-day incubation experiment was carried out in a laboratory (Lincoln University, NZ) in 2019, using the Molesworth and Lindis Peaks soils, amended with a 2 mm-sieved phosphogypsum. These soils were chosen for their high (Molesworth) and low (Lindis Peaks) exchangeable Al concentrations (Table 3.1). The chemical composition of the phosphogypsum used in this study is presented in Chapter 2, Table 2.2. The phosphogypsum was added to a soil sample of 100 g at four rates: 0, 1, 3 and 9 t ha^{-1} matching the rates used previously in the pot experiment (Bouray et al., 2020) and mixed thoroughly. All treatments were performed with four replicates. The treated soils were placed in 200 mL glass jars with screw top lids left partially open to allow aeration, while minimizing water loss through evaporation. The soils were incubated at 25 °C in randomized blocks and their order was randomized weekly. Water was added to the soil during incubation to maintain moisture content at 20-24% (v/v). At the end of the incubation period, the lids were removed, and the incubation temperature was raised to 30 °C for 5 days to dry the soils. The soils were then sieved (2 mm mesh) for analysis (Plate 3.1).

Plate 3.1 Glass jars with treated soils (a), incubator (b) and soil grinding and sieving after drying at the end of the incubation (c).

The details of pot experiment are described by Bouray et al. (2020). Briefly, Molesworth and Glenmore soils were treated with four rates of phosphogypsum (0, 1, 3 and 9 t ha^{-1}). The soils were grown with lucerne (*Medicago sativa*, 3 seedlings per pot) for six months, between March 16th and September 23rd, 2018 in a temperature-controlled glasshouse with natural light conditions at Lincoln University (Lincoln, NZ); there were four replicates pots for each treatment. The pots were watered using an automated dripper irrigation system that maintained the soil moisture at 22-25% (v/v), the average

temperature in the glasshouse was 18 °C. At the end of the experiment, the soils were air-dried, sieved (2 mm mesh) and stored at room temperature in polyethylene bags to await analysis.

The Incubation experiment, as a complement to the pot experiment, allows the comparison of phosphogypsum application effects on Al distribution in the soil solution between the absence and presence of plants. However, this comparison is only possible for Molesworth soil, which was used in both experiments because of its very high Al content. The Lindis Peaks soil was included in the incubation experiment as a low Al soil, in contrast to the two other investigated soils.

3.2.3 Soil solution analyses

The soil solutions were extracted after the end of the incubation and plant growth experiments according to Alleoni et al. (2010) and Cunha et al. (2018). Briefly, 20 g of air-dried soil was mixed with 20 mL deionized water, after which the samples were shaken horizontally at 150 rpm for 15 min. After a 60 min equilibration period, the samples were shaken for another 5 min and then centrifuged at 3500 rpm for 15 min. The pH and electrical conductivity (EC) were measured immediately in 5 mL of extract from each sample. The soil solution used for cation and dissolved organic carbon (DOC) analyses was passed through 0.45 µm cellulose acetate syringe filters, while the solution for anion analyses was passed through 0.20 µm cellulose acetate syringe filters. Cation (Ca^{2+}, Mg^{2+}, K^{+}, Na^{+}, Al^{3+}, Zn^{2+}) concentrations were determined using Inductively Coupled Plasma Optical Emission Spectrophotometer (ICP-OES: Varian 720-ES ICP-OES, Varian, Melbourne, Australia), anion concentrations (F^{-}, Cl^{-}, PO_4^{3-}, NO_3^{-}, SO_4^{2-}) were determined using ion chromatography (Dionex Corp., Sunnyvale, USA), and DOC was determined in a TOC analyzer (Elementar GmbH, Hanau, Germany). The ionic strength (IS, expressed as mM) was calculated from the EC of the soil solution, according to equation 3.1, proposed by Sposito (2008) who adopted the results of Marion and Babcock (1976).

$$\log_{10}(\mathrm{IS}) = 1.159 + 1.009 \times \log_{10}(EC) \qquad (3.1)$$

Where *EC* (dS m^{-1}) represents electrical conductivity at 25 °C.

3.2.4 Soil solid phase analyses

The soil pH (1:2.5 soil:water ratio) was measured using both deionized water and 0.01 *M* $CaCl_2$. The plant-available soil P fraction (Olsen P) was estimated using 0.5 *M* sodium bicarbonate extraction (Olsen et al., 1954) and was analyzed in a discrete wet chemistry analyzer (Smartchem TM 200, AMS Alliance, Paris, France). Exchangeable Al was extracted using 0.02 *M* $CaCl_2$ (1:4 soil:extractant ratio) and 1 M KCl (1:10 soil:extractant ratio), then analyzed using Inductively Coupled Plasma Optical Emission Spectrophotometry (ICP-OES: Varian 720-ES ICP-OES, Varian, Melbourne, Australia). Cation exchange capacity (CEC), base saturation (BS) and Al saturation were determined by extracting the soil

cations (Ca^{2+}, K^{+}, Mg^{2+}, Na^{+} and Al^{3+}) using 1 *M* ammonium acetate buffered at pH 7 followed by analysis with ICP-OES. The exchangeable H^{+} was calculated from the pH value of the ammonium acetate extracts according to Brown (1943). The Total C and N were determined by combustion using Vario-Max CN Elemental analyzer (Elementar GmbH, Hanau, Germany).

3.2.5 Aluminium speciation in the soil solutions

The Al speciation in soil solutions was modelled using a geochemical software Visual Minteq version 3.1 (Gustafsson, 2020). The model inputs were: pH, ionic strength, temperature (25 °C and 18 °C for incubation and plant growth experiments, respectively), and the concentrations of Ca^{2+}, Mg^{2+}, K^{+}, Na^{+}, Al^{3+}, Zn^{2+}, F^{-}, Cl^{-}, PO_4^{3-}, NO_3^{-}, SO_4^{2-} and DOC. To quantify the metal binding to DOM (dissolved organic matter), the NICA Donnan model (Kinniburgh et al., 1999) was used. It was assumed that ratio of active DOM to DOC was 1.65 (Sjöstedt et al., 2010) and that all the active DOM was fulvic acids (Adams et al., 2000; Hawke et al., 1996; Sjöstedt et al., 2010). The partial CO_2 pressure (pCO_2) in the soil solution was assumed to be 10-100 times higher than atmospheric CO_2 pressure (Ma et al., 2013). However, its effect on Al speciation in our soils was negligible within that range. The charge differences between cations and anions were balanced by changing Na^{2+} and/ or Cl^{-} concentrations, where needed, to achieve a charge difference of < 1%.

The formation of soluble inorganic complexes was evaluated using the equilibrium constants of the model database (Smith et al., 2004) and the model was run for each soil-treatment replicate independently. The sum of modelled Al species bound to the same inorganic ligands (OH^{-}, SO_4^{2-}, PO_4^{3-} or F^{-}) are reported as totals for that Al-ligand combination (e.g., Al-F includes AlF^{2+}, AlF_2^{+}, AlF_3 and AlF_4; Appendix B: Table B.1). The sum of Al bound by DOM species (Al-DOM) included weakly-bound Al^{3+}, and Al bound to carboxylic and phenolic functional groups.

3.2.6 Equilibria with possible mineral phases

Modelled saturation index (SI) values were used to assess the potential for an equilibrium of aluminium species in the soil solution with mineral phases. A solubility diagram was generated by plotting the modelled Al^{3+} activity across all soil-phosphogypsum treatment combinations (n = 62), against measured soil solution pH. The values were then compared to the predicted Al^{3+} activities for the following Al minerals: soil amorphous $Al(OH)_3$, crystalline gibbsite ($Al(OH)_3$), diaspore (AlOOH) and alunite ($KAl_3(SO_4)_2(OH)_6$). These mineral phases were selected based on the saturation indexes (Appendix B: Table B.2) which indicated that those minerals were most likely to form. The basaluminite ($Al_4(SO_4)_{10}5H_2O$) mineral was also included in the prediction, though it is not on the list of the minerals specified by the model. Equilibrium with basaluminite has been suggested as a possible mechanism controlling Al solubility in soils with a high SO_4^{2-} content (Adams and Rawajfih, 1977; Jones et al., 2011).

To predict the Al^{3+} activities (pAl^{3+} = - log {Al^{3+}}) of these minerals the following formulas were used:

Amorphous $Al(OH)_3$: $\mathrm{pAl}^{3+} = 33.71 - 3\,pOH$ (3.2)

Crystalline gibbsite: $\mathrm{pAl}^{3+} = 34.26 - 3\,pOH$ (3.3)

Diaspore: $\mathrm{pAl}^{3+} = 35.12 - 3\,pOH$ (3.4)

Basaluminite: $\mathrm{pAl}^{3+} = 29 - 2/5\,pOH\ -\ 1/4\,log_{10}(SO_4^{2-})$ (3.5)

Alunite: $\mathrm{pAl}^{3+} = 28.47 - 2\,pOH\ - 2/3\,log_{10}(SO_4^{2-})\ - 1/3\,log_{10}(K^{+})$ (3.6)

Equations 3.2–3.6 were generated by substituting (14-pOH) for pH and re-arranging the solubility product of each mineral phase (Jones et al., 2011). The following solubility products were used: log K_{sp}= - 116 for basaluminite (Adams and Rawajfih, 1977; Nordstrom, 1982), - 85.4 for alunite (Adams and Rawajfih, 1977; Nordstrom, 1982), - 8.29 for soil amorphous $Al(OH)_3$ (Gustafsson, 2020), - 7.74 for crystalline gibbsite (Palmer and Wesolowski, 1992) and - 6.88 for diaspore (Gustafsson, 2020; Peryea and Kittrick, 1988). The solubility line of alunite mineral is not included in the solubility diagram. The theoretical Al^{3+} activities (pAl^{3+}) of this mineral phase were too high, ranged 11.7–14.8 within 4.3–5.4 soil solution pH interval.

3.2.7 Statistical analysis

Analysis of variance (ANOVA) was used to identify significant differences between means and in cases of significant differences ($p < 0.05$), subsequent comparison using Tukey's post-hoc test ($p < 0.05$) using Minitab® statistical software version 18 (Minitab, Inc., State College, Pennsylvania, USA). The soil types and phosphogypsum treatments we considered as fixed factors. The ANOVA assumptions: normality (using Anderson-darling test) and homogeneity of variances (using Levene's test) were considered during the data analysis. The data of each soil were separately subjected to one-way ANOVA followed by the post-hoc test to distinguish between the effect of phosphogypsum treatment levels on the concentrations of Al species. Pearson correlation was used to evaluate relationships between the variables. Multiple linear regression (backward elimination, $\alpha < 0.05$) was used to determine the most important factor (Al^{3+}, Al-SO_4, Al-F, Al-PO_4, Al-DOM, Al_{KCl} and Al_{CaCl2}) impacting the TDM (total dry matter = shoot+ root DM yields) yield across the two soils and four phosphogypsum treatments. Here the variables were standardized by subtracting the mean then dividing by the standard deviation (see standardized coefficients in Appendix B: Table B.3 and Equation 3.7).

The partial least square (PLS) regression was used to build a statistical model to describes the relationship between one or more x-variables (soil and soil solution attributes, Table 3.2) and the y-variable(s) (Al^{3+} only in our case) and, thus revealing the relative importance of the different variables

in constructing the pattern of co-variation. Additionally, each object (soil-treatment combination) will have a score showing if it is high or low with respect to the x-variables creating the pattern. A number of PLS components of different loading and scores are then calculated with an increasing explained variance of both x and y variables. The optimum number of PLS components corresponds to the first minimum for the prediction error from the full cross-validation (leaves out only one sample at the time). All variables used in the PLS analysis were centred and scaled to unit variance. The data set of each soil were run separately with 16 objects (4 phosphogypsum rates x 4 replicates), 30 x-variables and 1 y-variable, then all soils data were compiled together and included in the general PLS analysis (62 objects, 30 x-variables and 1 y-variable).

Table 3.2 Summary of the partial least square regressions.

	Optimal components numbers	Cumulative explained variability in X (%)	Cumulative explained variability in Y (%)	RMSE ($\mu m\ L^{-1}$)	r^2
MO (incubated)	5	87	100	58.0	0.91
MO (planted)	2	72	90	11.3	0.82
GM (planted)	6	83	100	4.9	0.94
LP (incubated)	6	91	100	0.02	0.98
All soils	7	88	94	24.9	0.86

x-variables of PLS regressions

Soil	C, N, BS, CEC, Alsat. Olsen P, Ex.H^+, pH_w, pH_{CaCl2}, Al_{KCl}, Al_{CaCl2}
Soil solution	IS, DOC, Ca, K, Mg, Na, Zn, F, Cl, NO_3, SO_4, PO_4, Al-OH, Al-SO_4, Al-F, Al-DOM, Al-PO_4, Tot.Al, pHsoln

MO– Molesworth, GM– Glenmore, LP– Lindis Peaks
IS – Ionic Strength, Tot.Al – Total dissolved Al, pHsoln. – pH of soil solution, Alsat. – Al saturation
BS – Base saturation, Al-SO_4– sulphate bound Al, Al-PO_4– phosphate bound Al, Al-F – fluoride bound Al, Al-DOM – dissolved organic matter bound Al, Ex. H^+– Soil exchangeable H^+

The average soil and soil solution chemical attributes (x-variables), as affected by phosphogypsum application, used for PLS regression analysis are presented in Tables 3.3 and 3.4.

Table 3.3 Average measured soil solution attributes (n = 4) as affected by phosphogypsum application.

Soil	Rate ($t\ ha^{-1}$)	pH	IS (µM)	DOC ($mg\ L^{-1}$)	Ca ($mg\ L^{-1}$)	Mg ($mg\ L^{-1}$)	K ($mg\ L^{-1}$)	Na ($mg\ L^{-1}$)	Zn ($mg\ L^{-1}$)	Al ($mg\ L^{-1}$)	Cl^- ($mg\ L^{-1}$)	NO_3^- ($mg\ L^{-1}$)	PO_4^{3-} ($mg\ L^{-1}$)	SO_4^{2-} ($mg\ L^{-1}$)	F^- ($mg\ L^{-1}$)
MO	0	4.4	20.6 d	77.7	100.5c	39.3 a	39.1 b	15.9 b	0.6	8.5 b	7.7	742.9 a	0.5	22.1 d	1.8 b
(incuba	1	4.6	23.3 c	78.3	172.1b	32.1 b	43.5 ab	14.4 b	0.4	4.5 c	8.2	696.9 b	0.7	199.3 c	1.6 b
ted)	3	4.5	38.8 b	102.3	476.8ab	38.0 ab	40.1 ab	20.5 ab	0.4	9.3 b	8.3	644.6 c	0.9	989.9 b	2.1 ab
	9	4.4	48.7a	114.9	675.1a	41.8 a	42.7 a	25.3 a	0.4	13.3 a	8.3	645.7 c	1.1	1490 a	3.0 a
MO	0	4.5 b	24.3	188.4	141.2b	49.4	105.3	74.9	0.9	4.7 b	214.0	510.4 a	1.5 b	12.7 c	2.4 c
(plante	1	4.8 a	20.4	193.6	140.7b	34.8	77.6	61.7	0.5	3.1 b	175.6	302.4 ab	1.5 b	129.8 b	2.8 bc
d)	3	4.8 a	25.4	195.1	274.3b	34.2	72.1	61.3	0.6	5.1 b	145.3	115.3 b	2.3 a	549.0 a	3.1 ab
	9	4.7 a	38.2	189.9	599.3a	36.0	70.9	68.7	0.7	9.0 a	123.6	18.7 b	2.5 a	1334 a	3.5 a
GM	0	5.1 a	8.6 c	408.5 a	59.2d	12.2 b	10.2	53.4 b	0.4 b	8.8	136	1.9	4.3 a	33.1 c	3.7 b
(plante	1	4.9ab	16.9 b	421.9 a	163.5c	26.3 a	18.6	70.1 ab	0.5 b	5.5	184.5	1.8	3.1 ab	253.4 b	3.9 b
d)	3	4.7bc	23.3 b	314.5 ab	295.2b	26.5 a	11.8	75.4 a	0.5 b	5.7	154.3	1.5	2.8 b	605.8 b	4.1 ab
	9	4.5 c	40.8 a	256.5 b	690.4a	36.2 a	16.5	89.4 a	0.8 a	9.7	153.1	1.6	2.7 b	1485 a	5.3 a
LP	0	5.3 a	24.5 c	82.6	276.1d	35.1 c	20.4 c	20.3 d	0.19 c	0.6 c	4.7	878.2	0.9 c	54.5 d	1.4 b
(incuba	1	5.3 a	34.1 b	82.8	467.9c	46.9 b	23.1 bc	24.1 c	0.20 bc	0.7 c	4.9	884.6	1.0 bc	531.3 c	1.6 b
ted)	3	5.3 a	48.8 a	87.6	803.5b	68.6 b	34.1 ab	33.0 b	0.24 ab	0.9 b	4.8	879.9	1.2 b	1324 b	2.2 a
	9	5.0 b	50.1 a	90.9	836.3a	79.7 a	40.3 a	34.9 a	0.26 a	1.4 a	4.9	839.5	1.5 a	1365 a	2.5 a

IS = ionic strength, DOC = dissolved organic carbon. MO = Molesworth, GM = Glenmore, LP = Lindis Peaks

Lower-case letters indicate the significant differences between phosphogypsum rates per soil for each attribute ($p < 0.05$ after Tukey's test).

Table 3.4 Average measured soil chemical attributes (n = 4) as affected by phosphogypsum application.

Soil	Rate ($t\ ha^{-1}$)	pH_w	pH_{CaCl2}	Al_{KCl} ($cmol_c\ kg^{-1}$)	Al_{CaCl2} ($cmol_c\ kg^{-1}$)	Total N ($g\ kg^{-1}$)	Total C ($g\ kg^{-1}$)	Ex. H+ ($cmol_c\ kg^{-1}$)	CEC ($cmol_c\ kg^{-1}$)	BS (%)	$Al_{sat.}$ (%)	Olsen P ($mg\ kg^{-1}$)
MO	0	4.1 b	3.9 c	2.3 a	0.7 a	3.7	43.5	9.7 a	12.4 b	17.8 d	6.6	18.9 d
(incubated)	1	4.4 a	4.0 b	2.0 b	0.4 c	3.7	41.5	8.1 b	12.8 b	29.0 c	9.2	20.3 c
	3	4.3 a	4.1 a	1.8 c	0.4 c	3.6	41.8	8.3 b	15.0 b	41.2 b	5.2	22.4 b
	9	4.3 a	4.1 a	1.8 c	0.5 b	3.5	41.7	8.1 b	23.9 a	60.9 a	3.7	28.5 a
MO	0	4.7	4.1 b	2.3 a	0.5	3.5	42.0	8.9 b	12.7 b	24.4 c	7.7 a	14.0 b
(planted)	1	4.6	4.2 ab	2.1 b	0.4	3.4	43.0	8.9 b	12.3 b	27.6bc	1.9 b	15.7 ab
	3	4.6	4.3 a	2.0 b	0.2	3.4	39.0	8.6 b	13.9 b	33.8 b	5.9 a	15.1 ab
	9	4.5	4.3 a	2.0 b	0.3	3.5	43.2	9.9 a	17.0 a	41.2 a	2.1 b	22.1 a
GM	0	4.9 a	4.5	1.8	0.5	4.7	53.4	10.8	17.4 c	38.3 c	1.5	17.1
(planted)	1	4.8ab	4.4	1.5	0.4	4.7	52.8	11.3	19.0 b	41.2bc	1.1	22.9
	3	4.6ab	4.3	1.6	0.6	4.6	53.0	10.8	19.3 b	44.3 b	1.1	17.2
	9	4.4 b	4.2	1.9	0.9	4.4	54.6	10.7	22.8 a	54.0 a	1.0	23.9
LP	0	4.9 a	4.7 a	0.08 b	0.08	2.5	24.6	3.5	14.7 d	68.8 c	8.0 a	17.5 d
(incubated)	1	4.8 a	4.6 ab	0.07 b	0.10	2.5	24.2	4.3	16.4 b	71.1 c	3.4ab	19.5 c
	3	4.8 a	4.6 ab	0.08 b	0.13	2.6	24.8	4.0	18.9 b	77.6 b	1.9 b	25.4 b
	9	4.7 b	4.5 b	0.12 a	0.13	2.6	24.2	3.6	26.8 a	86.3 a	0.6 b	42.7 a

Ex. H^+ = exchangeable H^+, Alsat.. = Al saturation, MO = Molesworth, GM = Glenmore, LP = Lindis Peaks

Lower-case letters indicate the significant differences between phosphogypsum rates per soil for each attribute ($p< 0.05$ after Tukey's test).

3.3 Results

3.3.1 Aluminium species distribution as affected by phosphogypsum application

According to the modelled outputs, the total dissolved Al concentration in the solution of the incubated Molesworth soils was higher compared to the planted soils regardless of the phosphogypsum rate (Table 3.5). In the untreated Molesworth soils, free Al^{3+} was the predominant form and amounted to ~ 34% of the total dissolved Al. This was higher than the corresponding planted soil where the free Al^{3+} accounted for only 9% of the total dissolved Al. The free Al^{3+} ion concentrations in both the incubated and planted phosphogypsum-treated Molesworth soils were lower than the untreated soil ($p < 0.05$). However, higher concentrations of Al^{3+} were found under 9 t ha^{-1} relative to 1 and 3 t ha^{-1}. A similar trend was observed for hydroxylated Al forms (Al-OH). The concentration of Al species bound to inorganic ligands Al-SO_4 and Al-F increased linearly ($p < 0.05$) with phosphogypsum rate in the planted and incubated Molesworth soils. The Al-SO_4 fraction was more prevalent in the incubated Molesworth soils compared to the planted ones irrespective of phosphogypsum rate ($p < 0.05$), while the concentrations of Al-F fraction were similar in both. There were no significant differences in the concentrations of the Al-PO_4 fraction in the incubated soils; however, the concentrations of Al-PO_4 fraction were higher at 9 t ha^{-1} compared to the rest of the treatments in the planted soils. The concentration of Al in organic species (Al-DOM) decreased with increasing phosphogypsum rate in the incubated soils ($p < 0.05$), while there were no significant differences among the planted soils.

Table 3.5 Modelled concentrations of dissolved aluminium species (µM) in Molesworth soil solution after 60 days of laboratory incubation and after being planted for 6 months with lucerne (*Medicago sativa*) in pots under glasshouse conditions, treated with four rates of phosphogypsum.

Rate (t ha^{-1})		Al^{3+}	Al-OH	Al-SO_4	Al-F	Al-PO_4	Al-DOM	Total dissolved Al
0	Incubated	106.4 aA	14.7 aA	21.9 aD	87.1 aB	3.9 aA	79.6 aA	313.5 aB
	planted	15.6 bAB	2.2 bAB	1.5 bB	87.9 aAB	3.2 aAB	62.9aA	173.3 bB
1	Incubated	18.1 aC	4.3 aAB	34.7aC	70.0 aB	2.3 aA	35.3 aB	164.8 aC
	planted	0.9 bB	0.3 bB	1.2 bB	75.6aB	0.6 aB	36.2 a A	114.9 aB
3	Incubated	28.3aBC	3.1 aAB	178.8 aB	92.9 aAB	3.0 aA	37.7aB	343.8 aAB
	Planted	4.8 bAB	1.4 aAB	23.1 bAB	100.9 aAB	3.1aAB	54.3 aA	187.6. bB
9	Incubated	36.3 aB	3.4a B	284.1 aA	133.5 aA	3.4 aA	38.4 aB	499.0 aA
	Planted	12.9 bA	3.3aA	110.5 bA	132.0 aA	6.7 bA	66.1 bA	331.5 bA

Lower-case letters indicate the difference between the incubated and planted soils for each Al fraction under each phosphogypsum rate separately. Upper-case letters indicate the difference between the effect of phosphogypsum rates on each Al fraction for both the incubated and planted soils separately ($p< 0.05$ after Tukey's test).

After six months of being planted with lucerne, the total dissolved Al concentration in Glenmore soil solution decreased by 36% and 33% at 1 and 3 t ha^{-1} respectively, compared to 0 t ha^{-1}. Conversely, an increase of 8% was observed at 9 t ha^{-1} compared to 0 t ha^{-1} (Table 3.6). In the untreated Glenmore soils, 74% of total dissolved Al was bound by organic ligands. However, under phosphogypsum application, the size of that fraction decreased with increasing rates 1 (61%), 3 (70%) and 9 (74%) t ha^{-1} when compared to 0 t ha^{-1}. Sulphate and fluoride bound Al increased with increasing phosphogypsum. However, phosphate and hydroxyl Al fractions were not affected by phosphogypsum application in this soil. The free Al^{3+} concentration increased ($p < 0.01$) at the highest phosphogypsum rate (9 t ha^{-1}) compared to the other treatments.

The total Al concentration in Lindis Peaks soil solution was low ($p < 0.001$) compared to other soils regardless of treatment. After incubation, phosphogypsum application increased ($p < 0.001$) the total dissolved Al concentration in Lindis Peaks soil solution (Table 3.6), which was mainly in the Al-F fraction. The concentrations of the other inorganic Al species were very low (< 1% of total dissolved Al) irrespective of phosphogypsum rate; however, an increase in all these Al fractions was observed at 9 t ha^{-1} compared to the rest of the treatments ($p < 0.05$).

Table 3.6 Modelled concentrations of dissolved aluminium species (µ*M*) in the Glenmore soil solution after being cultivated with lucerne (*Medicago sativa*) for 6 months in pots under glasshouse conditions, and in Lindis Peaks (LP) soil solution after a laboratory incubation of 60 days, both soils were treated with four rates of phosphogypsum.

Rate (t ha^{-1})	Al^{3+}	Al-OH	Al-SO_4	Al-F	Al-PO_4	Al-DOM	Total dissolved Al
Glenmore							
0	1.3 b	1.1	2.8 c	76.4 c	5.1	241.5 a	328.2
1	0.9 b	0.5	3.7 bc	107.6 bc	1.8	94.4 ab	209.2
3	2.6 b	0.6	15.1 ab	124.5 ab	2.1	73.5 b	218.5
9	10.3 a	1.3	94.0 a	185.5 a	3.7	63.0 b	357.7
p-value	**	n.s.	***	**	n.s.	**	n.s.
Lindis Peaks							
0	0.01 b	0.01 b	0.0 c	13.4 c	0.01 b	7.2 ab	20.6 c
1	0.01 b	0.01 b	0.03 b	17.5 c	0.01 b	6.7 b	24.3 c
3	0.02 b	0.01 b	0.1 b	26.2 b	0.01 b	7.4 ab	33.8 b
9	0.06 a	0.03 a	0.3 a	44.5 a	0.03 a	8.2 a	53.2 a
p-value	***	**	***	***	**	*	***

n.s. not significant. The lower-case letters indicate the difference between the effect of phosphogypsum rate on each Al fraction separately ($p < 0.05$ after Tukey's test). Asterisks indicate the level of statistical significance (*p< 0.05, **p< 0.01, ***p< 0.001 after one-way ANOVA).

3.3.2 Equilibria with possible mineral phases

The relationship between pH and pAl^{3+} (- log $\{Al^{3+}\}$) in the incubated Molesworth soil was weaker (r^2= 0.17; Figure 3.1a) than in the planted Molesworth soil (r^2 = 0.49; Figure 3.1b). This relationship was stronger in the planted Glenmore soil (r^2= 0.69; Figure 3.1c), and in the incubated Lindis Peaks soil (r^2= 0.91; Figure 3.1d). Across all soils and phosphogypsum treatments, there was a strong positive correlation between pH and Al activity in the soil solution (r^2 = 0.84; Figure 3.2).

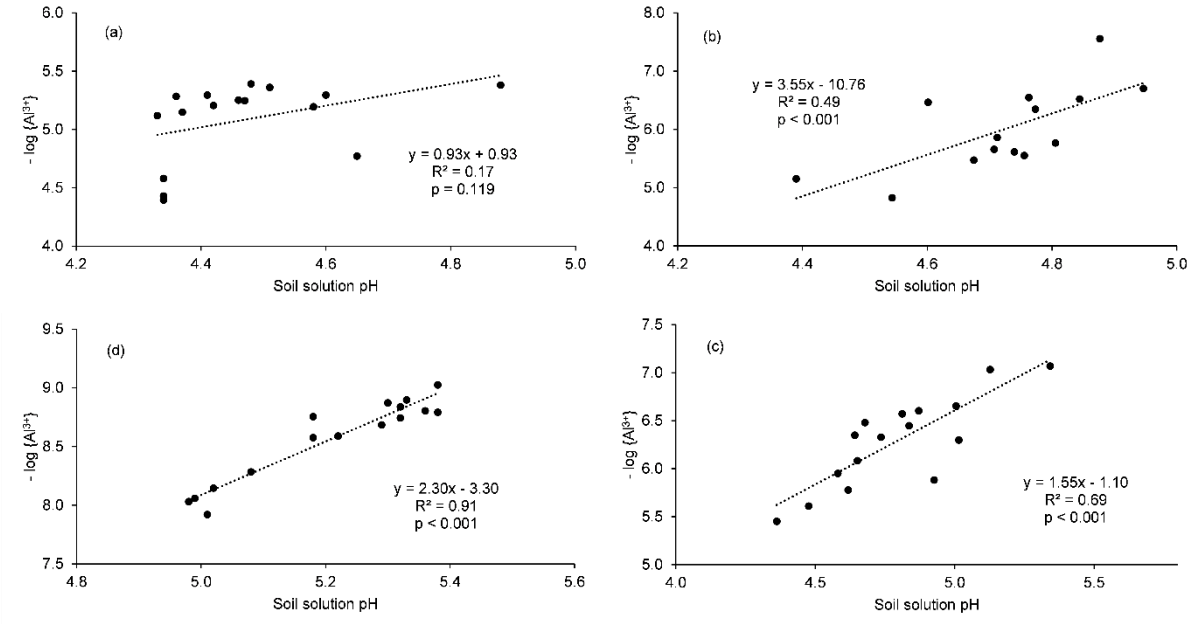


Figure 3.1 Relationship between pH and Al^{3+} activity in the soil solution of four different acid soils across four rates of phosphogypsum application: (a) incubated Molesworth soil; (b) planted Molesworth soil; (c) planted Glenmore soil and (d) incubated Lindis Peaks soil. The broken line indicates the linear regression of - log $\{Al^{3+}\}$ on pH (equations are presented in the plots).

Below pH 5, the Al solubility was likely controlled by four mineral phases (Figure 3.2): Al in most soil solution samples with pH < 5.0 was oversaturated relative to crystalline gibbsite, diaspore and basaluminite, while only 14 samples were oversaturated relative soil amorphous $Al(OH)_3$. However, above pH 5.0 the Al in soil solution appeared to be oversaturated relative to diaspore mineral only, while under-saturated relative to the rest of the predicted mineral formations. The predicted Al activity for alunite showed an oversaturation for all soil solution samples independently of soil pH (not shown). However, the average saturation indexes indicated an oversaturation relative to alunite only for soil solution samples below pH 5.

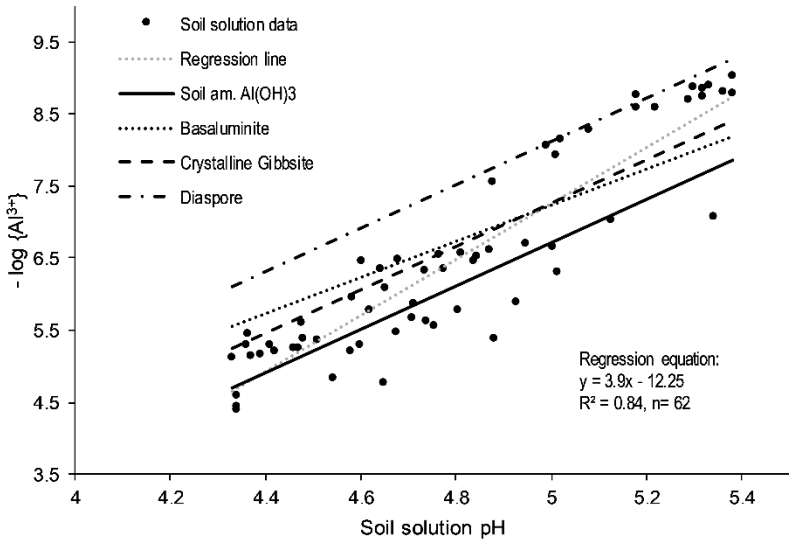


Figure 3.2 Solubility diagram of Al^{3+} activity vs. pH across all soil solutions in relation to four minerals phases.

3.3.3 Multivariate analysis

In the incubated Molesworth soil (Figure 3.3a), most x- variables showed negative loadings within the first PLS component, while y-variable (Al^{3+}) had high positive loading (0.26). Hydroxylated Al (Al-OH), Al-DOM, Al_{CaCl2}, Al_{KCl} and Ex. H^+ (exchangeable H^+) showed a high degree of co-variance with Al^{3+} (closely grouped loadings). A very close relationship between NO_3^- and Al_{KCl} was observed. The ionic strength (IS) had a negative loading of - 0.24 on the first PLS component and correlated strongly with SO_4^{2-} (r = 0.99, $p < 0.001$), Ca^{2+} (r = 0.98, $p < 0.001$), Al_{KCl} (r = -0.84, $p < 0.001$) and BS (r = 0.97, $p < 0.001$). A clear negative correlation was also obtained between pH_{CaCl2} and both Al^{3+} and Ex. H^+. Furthermore, Al-SO_4 and Al-F were closely correlated with SO_4^{2-} and F^- respectively and had negative loading of - 0.22 and - 0.11 on PLS component 1, respectively.

In contrast to the incubated soils, most x-variables in the planted Molesworth soils had positive loadings on the first PLS component (Figure 3.3b) which explained 82% of y-variability. All Al fractions including Al^{3+} had positive loading on PLS component 1, they were clustered together and co-varied with ionic strength and total dissolved Al, while negatively correlated with soil pH_w. As in the incubated soils, a clear co-variance was also found in the planted Molesworth soils between SO_4^{2-}, Olsen P, BS, CEC, Ca^{2+} and IS. A close relationship was also revealed between Al_{KCl} and NO_3^- in the planted Molesworth soil. However, Al_{KCl} had a small loading on PLS component 1 in this soil but had a high positive loading on the second PLS component (+ 0.32). In the planted Molesworth, soil solution pH (pHsoln.) seemed to co-vary with soil total C and N (lying along a thought line from pHsoln. to the origin).

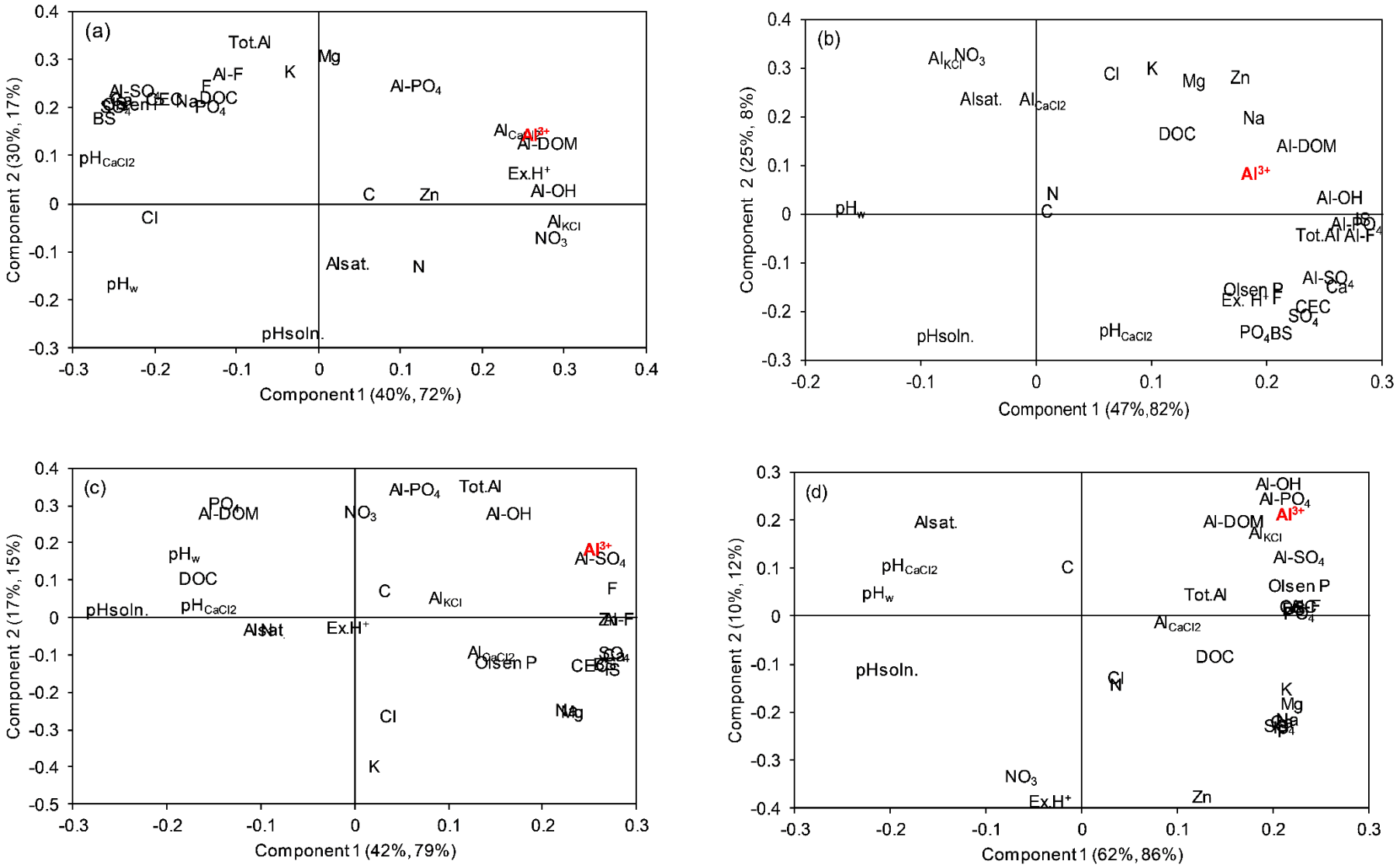


Figure 3.3 Loading plots of PLS regression for (a) Molesworth (incubated), (b) Molesworth (planted), (c) Glenmore (planted) and (d) Lindis Peaks (Incubated).

As in the planted Molesworth soil, in the planted Glenmore soil (Figure 3.3c), the Al^{3+} co-varied with IS, SO_4^{2-} and soil BS and CEC. Furthermore, Al^{3+} was positively correlated with other Al fractions in the following order: Al-SO_4 # Al-F # Al-OH # Total dissolved Al # Al-PO_4. Conversely, Al-DOM seemed to have a negative effect on Al^{3+} in this soil and showed high positive loading on PLS component 2. Contrarily to the planted Molesworth soil, in the planted Glenmore soil, the Ex. H^+ contribution in Al^{3+} concentration was negligible (very close to the origin). Additionally, a close relationship between DOC and soil pH_w was revealed in the planted Glenmore soil which is not the case for Molesworth soil.

In the incubated Lindis Peaks soil, most x-variables were loaded positively on PLS component 1 and showed a strong correlation with Al^{3+} (Figure 3.3d). Aluminium fractions in soil solution were clustered together and vary closely with soil exchangeable Al (Al_{KCl}). Moreover, they had higher positive loading on PLS component 2. Soil Al saturation ($Al_{sat.}$) had high positive loading on PLS component 2 showing a negative co-variance with Al^{3+} and other Al fractions. A clear negative correlation was revealed between soil/soil solution pHs, and Al fractions, total dissolved Al, ionic strength, exchangeable Al and SO_4^{2-}.

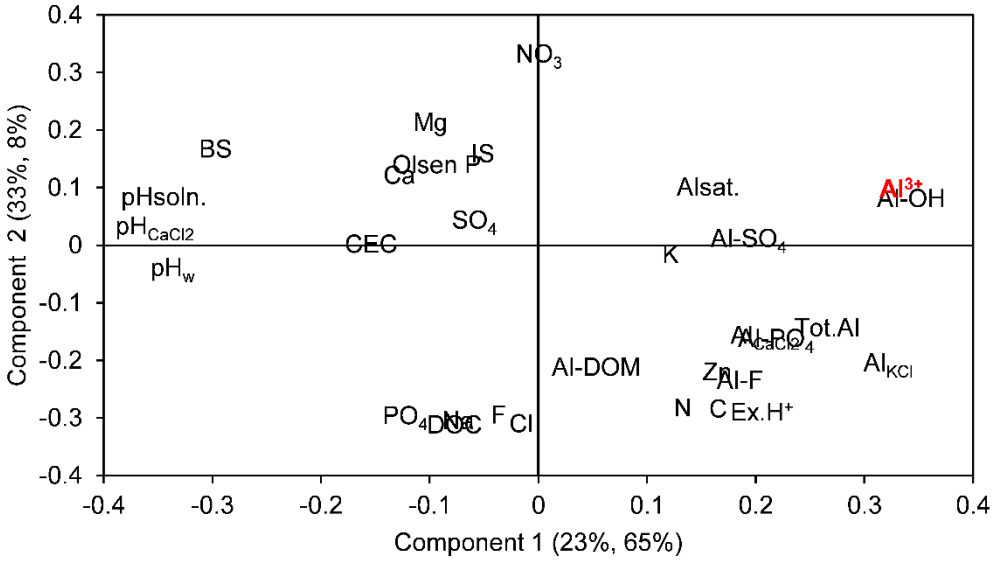


Figure 3.4 Loading plots of PLS regression for the whole soil solution data set (n = 62) across all soils and phosphogypsum treatments.

The PLS analysis of all soils together revealed that Al^{3+} co-varied positively with the rest of Al fraction, in particular Al-OH, and with total dissolved Al and exchangeable Al, while negatively correlated with soil and soil solution pH (Figure 3.4). Soil base saturation had a high negative load on Al^{3+} concentration in soil solution regardless of soil type. Also, ionic strength, SO_4^{2-} and soil CEC showed negative co-variance with Al^{3+}. Though, their loadings were small compared to soil BS. Exchangeable H^+ showed a high degree of positive co-variance with soil total C and N. The loadings of soil pH (water and $CaCl_2$) and soil solution pH were closely grouped and showed a higher correlation with soil BS.

3.3.4 Relationship between soil exchangeable Al, Al fractions and TDM yield

There was a positive linear correlation between both Al_{KCl} and Al_{CaCl2} and most of the modelled/estimated Al fractions in the soil solution (Table 3.5). However, these correlations seem to be stronger for Al_{KCl} compared to Al_{CaCl2}. Moreover, Al-F and total dissolved Al showed the highest r coefficient for both Al_{KCl} and Al_{CaCl2}, while Al-PO_4 showed a strong correlation with Al_{KCl} only.

Table 3.7 Correlation coefficients (Pearson's R) between soil exchangeable Al (KCl and $CaCl_2$) and Al fractions in the soil solution.

Modelled Al fractions ($\mu M\ L^{-1}$)	Al_{KCL}($cmol_c\ L^{-1}$)	Al_{CaCl2} ($cmol_c\ L^{-1}$)
Al^{3+}	0.43***	0.34**
Al-OH	0.48***	0.36**
Al-SO_4	0.30*	0.22 n.s.
Al-F	0.67***	0.63***
Al-PO_4	0.61***	0.39**
Al-DOM	0.43***	0.33**
Total dissolved Al	0.67***	0.55***

n.s. not significant
Asterisks indicate the level of statistical significance (*p <0.05, **p <0.01, ***p <0.001).

The comparison of the standardized multiple regression coefficients (Appendix B: Table B.3) revealed that Al-F was the most important fraction impacting positively the yield, followed by Al^{3+} with a negative impact, then Al-DOM with a positive impact. The regression equation is presented below:

$$TDM(g\ pot^{-1}) = 1.43 - 0.97\ [Al^{3+}] + 1.38\ [Al\text{–}F] + 0.86\ [Al\text{–}DOM] \qquad (3.7)$$

(n= 30, r^2_{adj} = 73%, p <0.001)

A strong positive relationship was found between TDM yield and Al_{KCl} in Glenmore soil (r^2 = 0.87; Figure 3.5) across phosphogypsum treatments, while in Molesworth soil this relationship was weak (r^2 = 0.2). On the other hand, no clear relationship was found between TDM and Al_{CaCl2} in both soils.

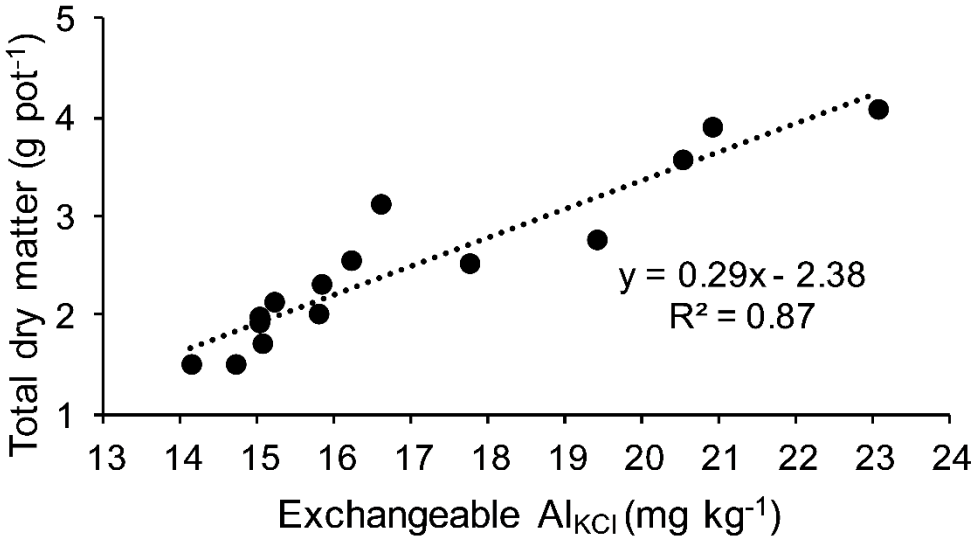


Figure 3.5 Relationship between TDM yield and Al_{KCl} in Glenmore soil.

3.4 Discussion

The high free Al^{3+} concentration in the untreated Molesworth soil solutions agrees with previous research that suggested that at low pH, the dissolution of inorganic Al pools by H^+ ions drives Al release (Guo et al., 2007). This view is also supported by the strong positive co-variance shown in PLS regression between exchangeable H^+ and Al^{3+} (r = 0.78, p < 0.005). However, the large differences observed in Al^{3+} and total dissolved Al concentrations between 1, 3 and 9 t ha^{-1} in the incubated Molesworth soil indicate that other factors beyond pH controlled the Al solubility in the presence of phosphogypsum, providing that soil/soil solution pH values were identical for these three rates. The poor relationship between Al activity and soil solution pH indicates that the decrease in the size of Al^{3+} fraction in the phosphogypsum-treated soils is due to the association of Al^{3+} ions with SO_4^{2-} and F^- (Carvalho and van Raij, 1997), this is also shown by the negative loadings of Al-SO_4 and Al-F fractions on Al^{3+}. The observed increase of Al^{3+} and total dissolved Al concentrations in the soil solution at 3 and 9 t ha^{-1} compared to 1 t ha^{-1} could be due to the higher ionic strength (Giesler et al., 1996), where the increased Ca^{2+} concentration resulted in Al^{3+} being displaced from soil cation exchange sites into the soil solution. This is in line with PLS regression loadings where soil exchangeable Al (Al_{KCl}) co-varied negatively with soil base saturation and ionic strength, which were strongly and positively correlated with Ca and SO_2^{4-} concentrations in soil solution. These results agree with the findings of Guo et al. (2004); Matschonat and Vogt (1998).

In the planted Molesworth soil, the PLS regression results showed that the amount of KCl-extractable Al did not seem to be that important for Al^{3+} concentration nor did Al saturation, despite the positive loading of base saturation, CEC and ionic strength on Al^{3+}. This implies that cation exchange reactions may not be the only process controlling Al^{3+} concentration in this soil, but pH also seemed to

contribute. This view is supported by two findings. First, the linear relationship between Al activity and soil solution pH. Second, the positive loading of soil exchangeable H^+ and the negative loading of soil pH_w on Al^{3+}. For instance, the higher concentration of Al^{3+} at 9 t h^{-1} compared to 1 and 3 t ha^{-1} was accompanied by a significant increase in soil exchangeable H^+ and a decrease in soil/soil solution pH_w. The soil protonation at 9 t ha^{-1} can be explained by soil acidification via plant roots exudations because this was not the case when the same soil was incubated without plants. The root density in the pots was higher enough to assume that the entire soil volume was influenced by the roots and can therefore be considered as rhizosphere soil. Thus, the higher ionic strength and Ca^{2+} supply could have enhanced the release of H^+ to counter-balance the excess uptake of cations. Hence, the positive loading of ionic strength and Ca^{2+} on Al^{3+}. This mechanism is often considered the major source of root-induced changes in rhizosphere pH (Cheng et al., 2004; Hinsinger et al., 2003). Additionally, the nitrogen fixation by legumes as an acidifying process is well documented (Bolan et al., 1991b; Tang et al., 1999). These mechanisms likely contributed together to increasing Al^{3+} and total dissolved Al, specifically at 9 t ha^{-1}, where average root dry matter produced per pot was 74% and 92% higher than 3 and 1 t ha^{-1} respectively, with an average number of nodules of 4.75 per pot against 1.5 and 0.75 nodules at 3 and 1 ha^{-1} (data not shown). Therefore, we hypothesize that rhizosphere acidification at 9 t of phosphogypsum ha^{-1}, had caused the dissolution of SO_4^{2-} and OH^- bound Al minerals. This view is supported by (1) the very high loadings of inorganic Al (e.g. Al-SO_4, Al-OH) fractions on Al^{3+} and (2) the decrease of saturation index (SI) relative to amorphous $Al(OH)_3$, gibbsite, and hydroxy-sulfate minerals (alunite) with the phosphogypsum rate increase. The implication of these mineral phases in controlling Al solubility in acidic sulphate rich soil environment has been confirmed by several studies (Adams and Rawajfih, 1977; Jones et al., 2011). On the other hand, the resulting decrease in Al^{3+} at 1 and 3 t ha^{-1} compared to 0 t ha^{-1} can to some extend be attributed to ion-pairing of Al^{3+} with SO_4^{2-} and F^-, also to a less pronounced plant growth effect on soil acidity referring to (1) the soil exchangeable H^+ which remained unchanged and to (2) the total dry matter data (Bouray et al., 2020) where no significant difference was revealed between 0, 1 and 3 t ha^{-1}. The lower concentration of inorganic Al fractions in the planted Molesworth soil solution compared to the incubated Molesworth soil could be due to plant Al uptake, and to the leaching of mobile Al forms (e.g. Al-SO_4) out of the pots, because they were irrigated throughout the growth period, and that drainage could have occurred, in contrast to the closed incubation system.

In the planted Glenmore soil, the relationship between pAl^{3+} and pH was linear with a slope of 1.55, which implies that ion-exchange or complexation reaction with soil organic substances are important for the control of Al^{3+} activity according to van Hees et al. (2001); Van Hees et al. (2000). Indeed, the dissolved organic carbon (DOC) seemed to be important for Al^{3+} in this soil because of the dominance of organically bound Al fraction (Al-DOM) in the untreated soils. However, the DOC decreased

significantly under phosphogypsum application, which was translated into a significant decrease in Al-DOM fraction. This was likely due to the resulting decrease in soil solution pH from 5.1 at 0 t ha^{-1} to 4.5 at 9 t ha^{-1}. Consistent with the present result, Gerke (1994) investigated humic-Al complexes at pH 4, 4.5, 5, and 6 in the soil solution and they found that organically bound Al decreased strongly as pH decreased below 5. Moreover, previous studies have shown that increasing the ionic strength of soil solution reduces the DOC flux into the soil solution (Evans Jr et al., 1988; Tipping and Hurley, 1988). The decrease in soil/soil solution pH_w following phosphogypsum application can partly be explained by plant growth effect as previously mentioned for planted Molesworth soil. Although soil/soil solution pH decreased at 1 and 3 t ha^{-1} compared to 0 t ha^{-1}, the free Al^{3+} concentration and soil exchangeable Al did not change, and a decrease in total dissolved Al concentration was observed. These findings suggest that phosphogypsum application at 1 to 3 t ha^{-1} could have induced Al precipitation reactions. This mechanism would appear likely, in view of the reported role of SO_4^{2-} in Al precipitation (Adams and Rawajfih, 1977; Alva et al., 1990; Pavan et al., 1984). Whereas, at 9 t ha^{-1} both free Al^{3+} and total dissolved Al increased significantly in the soil solution compared to the rest of the treatments. This was probably due to the sharp decline in soil/soil solution pH. Alternatively, the higher ionic strength in the soil solution, could have favoured ionic exchange reactions to the detriment of adsorption and precipitation reactions (Carvalho and van Raij, 1997). Hence, the ionic exchange of Ca^{2+} would have increased Al^{3+} in the solution and dominated the ligand exchange reactions between SO_4^{2-} and Al^{3+}

The pH in the incubated Lindis Peaks soil/soil solution decreased significantly at 9 t of phosphogypsum ha^{-1}, while no change was observed at 1 and 3 t ha^{-1} compared to 0 t ha^{-1}. This can be explained by the large supply of Ca^{2+} had displaced H^+ and Al^{3+} (which liberates H^+ after hydrolysis) into the soil solution (Alva et al., 1990), leading to a decrease in soil solution pH. These results are consistent with the findings of Alva et al. (1988); Black and Cameron (1984). Our hypothesis is supported by the decrease in soil Al saturation from 8% at 0 t ha^{-1} to only 0.6% at 9 t ha^{-1}. Also, the Ca^{2+} concentrations in soil solution correlated strongly with Al^{3+}(r = 0.69, p < 0.01), soil solution pH (r = - 0.68, p < 0.01) and soil pH_w (r = - 0.77, p < 0.001). Nevertheless, the Al^{3+} concentration in this soil regardless of the phosphogypsum rate was below the critical limit of 1 μM L^{-1} for negatively impacting plant growth (Kinraide, 1997; Shann and Bertsch, 1993). A significant decrease in soil Al saturation was also recorded at 1 and 3 t ha^{-1} compared to 0 t ha^{-1}. However, no change was observed for Al^{3+} and soil/soil solution pH. This reveals the role of SO_4^{2-} and F^- in binding free Al^{3+} in the soil solution, hindering its fixation into soil exchangeable sites as evidenced by soil exchangeable Al (KCl and $CaCl_2$) concentrations which remained unchanged at 1 and 3 t ha^{-1} compared to 0 t ha^{-1}. Further, the average modelled saturation index (SI) relative to alunite increased with the phosphogypsum rate in this soil. This stresses the role of precipitation reactions in immobilizing Al in the soil solution.

The general PLS regression showed a very strong co-variance between Al^{3+} and Al-OH species (r = 0.92, p < 0.001, n = 62) in the soil solution. This indicates that, in the investigated soils, Al hydroxide forms and minerals (amorphous Al $(OH)_3$ and gibbsite) are likely responsible for supplying soil solution with free Al^{3+}. Moreover, according to the Al speciation results and saturation indexes, the Al hydroxide forms and minerals were found to decrease under phosphogypsum application, which implies that this is another mechanism by which phosphogypsum could have reduced Al^{3+} activity in the soil solution. Furthermore, the correlation between both Al^{3+} and Al-OH, and soil base saturation (Al^{3+}: r = - 0.44, p < 0.001; Al-OH: r = - 0.53, p < 0.001, n = 62) confirms the role of phosphogypsum in decreasing the acidic cations (Al^{3+} and H^+) occupying the exchange sites. The role of soil base saturation in mitigating exchangeable Al (Al_{KCl}) has been confirmed recently by Whitley et al. (2019) for a range of New Zealand soils.

The Al-F and Al-DOM are supposed to be non-toxic (MacLean et al., 1992; Martins et al., 2020; Yerima et al., 2020). Therefore, the fact that Al_{KCl} and Al_{CaCl2} were strongly associated with these two fractions confirms that these two methods extract not only the Al^{3+} but also the non-phytotoxic forms. This is supported by the positive impact that Al-F and Al-DOM had on TDM yield according to the multiple regression analysis. This disagrees with the finding of Manoharan et al. (2007), in a glasshouse experiment, who found that high concentrations of Al-F complexes had restricted barley root growth at pH < 5.

Our findings suggest that the 1 *M* KCl or 0.02 *M* $CaCl_2$ method may not be a sufficient indicator to evaluate Al toxicity in acid soils. Further, $CaCl_2$ extraction being the standard method currently used in New Zealand commercial labs (Whitley et al., 2020), showed a relatively less relevant relationship with Al^{3+} (r = 0.34, p <0.01) relative to the KCl method (r = 0.43, p <0.001). Thus, we recommend using the KCl extraction method instead of $CaCl_2$ for exchangeable Al extraction. Yet, this recommendation requires further investigation using a range of soils across different orders excluding treatment effects.

3.5 Conclusion

This study shows that soil solutions, although from different soils treated with different rates of phosphogypsum, have a strong pH-pAl^{3+} relationship. This confirms the major role that pH plays in controlling Al solubility in acid soils (pH ≤ 5.3). The differences in plant growth in Glenmore and Molesworth soils was mostly explained by the variation in Al^{3+}, Al-F and Al-DOM concentrations, rather than exchangeable Al. Fluoride and DOM likely complexed Al and showed a positive impact on the total dry matter (shoot + root) yield of lucerne. The phosphogypsum, when applied at feasible on-farm rates (1 to 3 t ha^{-1}), can significantly reduce the activity of Al^{3+} in acid soil solution through the mechanism of Al displacement on soil exchange sites via Ca^{2+}, followed by Al^{3+} complexation with SO_4^{2-} and F^-, while the phosphate role in Al immobilization appeared to be less important. However, the

high rates of phosphogypsum (9 t ha^{-1} in our case) should be avoided on acid soil as this would further acidify the soil causing a release of Al and its accumulation unless combined with a pH neutralizing material such as lime. The lower rates of phosphogypsum did not affect soil pH and Al^{3+} concentration in the soil solution of Lindis Peaks soil (characterized with a very low exchangeable Al) suggesting that phosphogypsum can be used as fertilizer on this type of soils. Our study confirms that examining Al species in the soil solution would better assess Al phytotoxicity. Hence, the necessity of examining legumes growth versus soil Al species rather than exchangeable Al which seems to be an insufficient indicator. Also, an appropriate pot size is crucial in glasshouse experiments to avoid any eventual negative effects that small-size pots could have on plant growth.

Chapter 4

Lime-induced pH Elevation Influences Phosphorus Biochemical Processes and Dynamics in the Rhizosphere of *Lupinus polyphyllus and Lupinus angustifolius*

(Published paper)

4.1 Introduction

Phosphorus (P) is an essential element for plant nutrition, and soil P deficiency is one of the most limiting factors affecting crop production worldwide (Hou et al., 2020), especially in acid soils which represent over 50% of potentially arable lands in the world (Von Uexküll and Mutert, 1995). Phosphorus availability in acid soils is mainly limited by adsorption reactions between orthophosphate anions (e.g., $H_2PO_4^-$), amorphous metal oxides (e.g., Al and/or Fe oxides) or clay minerals (Sims and Pierzynski, 2005) due to low pH. Liming is a commonly used agricultural practice to maintain an appropriate pH for plant growth and decrease the solubility of phytotoxic elements, particularly exchangeable Al (Bouray et al., 2020; Morton and Moir, 2018; Whitley et al., 2019). However, there are inconsistencies in the literature regarding the impact of pH adjustment on plant P availability and uptake (Barrow et al., 2020b; Moir et al., 2016; Penn and Camberato, 2019). Phosphorus availability has been reported to increase (Griffin, 1971; Ryan and Smillie, 1975), decrease (Curtin and Syers, 2001; Haynes and Ludecke, 1981) or not be affected (Haynes, 1982) by soil pH change through liming. However, most of these studies are focused on how pH affects chemical processes that control P solubility, and little is known about the liming effect on biological P cycling.

Liming has been reported to decrease soil P_o (Condron and Goh, 1989; Condron et al., 1993), and this has been proposed to reflect the stimulation of microbial mineralization process which is known to be sensitive to changes in soil pH (Harrison, 1982; Trasar-Cepeda et al., 1991). Similarly, the activity of extracellular hydrolytic enzymes (e.g., phosphatases) which are actively secreted into the soil by microbes, and many plants, in response to the demand for P (Abel et al., 2000; Quiquampoix, 2005), is directly affected by soil pH (Acosta-Martinez and Tabatabai, 2000; Margenot et al., 2018). However, since different phosphatases have different pH optima, the effect of liming on the hydrolysis of P_o will likely be conditioned by soil pH target. Phosphodiesterase enzyme is the rate-limiting step in P_o mineralization (Turner and Haygarth, 2005) but it is relatively far less investigated compared to phosphomonoesterases under liming conditions.

On the other hand, liming can stimulate microbial P immobilization (Condron and Goh, 1989, 1990) by maintaining favourable pH conditions for higher microbial activity (Pietri and Brookes, 2008; Robson and Abbot, 2012) and by chemically increasing available P for microbial uptake. Microbial P turnover is also a potential source of available P for plants (Achat et al., 2010). To fully understand the effect that soil pH change through liming could have on P availability, a biochemical examination of all these processes is necessary. Most studies evaluate the liming effect on soil P availability by analyzing P in the bulk soil and/or plant P uptake. However, plant P nutrition and acquisition are mainly determined by many processes in the vicinity of the roots; that is, the rhizosphere (Clarkson, 1985) and soil P bioavailability differs significantly among plant species according to their ability to mobilize P_i in the rhizosphere (Hinsinger et al., 2011). Therefore, examining the effect of pH change via liming on rhizosphere P related biological and biochemical processes could improve our understanding of the relationship between liming and soil P availability, and clarify to some extent the controversial views regarding this relationship.

Soil P exists in potential P_o and P_i pools with varying stability in the soil. This has generated attention in quantifying their contribution to plant P nutrition and understanding their transformation into labile P forms. Sequential extraction procedures have been widely used to characterize soil P fractions and to investigate P dynamics (Condron and Newman, 2011; Tiessen and Moir, 2008). Thus, a soil P fractionation approach may provide important insights into the role of processes either directly mediated by plant roots or by microorganisms such as exudation of low molecular weight organic acids (Jones, 1998), secretion of phosphatases (Nannipieri et al., 2011) and rhizosphere acidification (Hinsinger et al., 2018), in the dynamic and bioavailability of P in the rhizosphere in response to soil pH change.

Previous rhizosphere studies have focused on mostly white lupin (*Lupinus albus*) as a model P-mobilizing plant (Dissanayaka et al., 2017; Lambers et al., 2013). *L. albus* modifies the rhizosphere through rhizodeposition (Nuruzzaman et al., 2006), especially under P-deficiency (Wasaki et al., 2003), and the formation of cluster roots (or proteoid) which efficiently increase the accessible soil volumes (Cheng et al., 2011; Ma et al., 2019). However, the potentials of other agriculturally important lupin species to mobilize P, such as Russell lupin (*Lupinus polyphyllus*) are poorly investigated. The *Lupinus polyphyllus* is recognized as a globally invasive plant (McDougall et al., 2005; Valtonen et al., 2006), and often invades soils with low pH and low P availability (Lambers et al., 2013). We hypothesize that this may be due to its ability to mobilize P. Contrary to Russell lupin, blue lupin (*Lupinus angustifolius*) is widely cultivated (Reinhard et al., 2006) and is known to release large amounts of organic acids (Robles-Aguilar et al., 2019). Here, we compare these two non-cluster root species to see if they differ in their P acquisition strategies.

This study aimed to examine the effect of soil pH change through liming from 5.3 to 6.0 on rhizosphere properties involved in P mobilization and its acquisition by plants. We hypothesized, first, that liming would affect the biochemical P processes in the rhizosphere of lupins; and second, P dynamics in the rhizosphere of lupins would depend on the response of those processes to both pH elevation and plant growth effects.

4.2 Material and methods

4.2.1 Soil sampling and characteristics

The soil was collected (0–15 cm) in late-November 2018, from Mt Grand (MG) station (44°40'19.49''S, 169°19'5.66''E), a commercial sheep and beef high-country farm operated by Lincoln University, located in central Otago district, New Zealand. The sampling site was on a moderately steep (20-25°) south-facing hillside within an altitude of 600 masl. Plant material and stones were removed and then the soil was thoroughly mixed, air-dried and sieved (2 mm mesh). The soil is classified as a Brown soil (NZ classification after Hewitt (2010)); the United States Department of Agriculture classification: Dystrudepts (USDA, 2014). Soil fertility status was characterized using the analyses listed in Table 4.1.

Table 4.1 Initial fertility status of Mt Grand (MG) soil (0-15 cm), before the establishment of the experiment.

Soil Analysis	Value	By method of
pH	5.3 (H_2O)	Blackmore et al. (1987)
Olsen P	7 (mg kg^{-1})	Olsen et al. (1954)
Resin P	14 (mg kg^{-1})	Saggar et al. (1990)
P retention (ASC)	20 (%)	Blackmore et al. (1987)
Inorganic P	243 (mg kg^{-1})	Bowman and Moir (1993);
Organic P	401 (mg kg^{-1})	Dick and Tabatabai (1977b);
P organic/P inorganic	1.65 (ratio)	Turner et al. (2005)
Sulphate sulphur	3 (µg g^{-1})	Watkinson and Kear (1994)
Organic matter	5.1 (% w w^{-1})	Blackmore et al. (1987)
Exchangeable Al_{KCl}	1.20 (meq $100g^{-1}$)	Rayment and Lyons (2011)
Exchangeable Al_{CaCl2}	5.80 (mg kg^{-1})	Hoyt and Nyborg (1972)
Total N	0.27 (% w w^{-1})	(Dumas combustion method
Total C	2.96 (% w w^{-1})	using an Elementar Vario
Carbon/Nitrogen	11 (ratio)	Max Cube Analyser)
CEC	11 (meq $100g^{-1}$)	Brown (1943)
Ca	3.4 (meq $100g^{-1}$)	Rayment and Higginson
Mg	0.65 (meq $100g^{-1}$)	(1992)
K	0.30 (meq $100g^{-}1$)	
Na	<0.02 (meq $100g^{-1}$)	
Base saturation	38.3 (%)	
Particle-Size distribution		
Clay (0.05–2µm)	3 (%)	
Sand (20–2000 µm)	50 (%)	ISSS Classification
Silt (2–20 µm)	47 (%)	

ISSS International Society of Soil Science

4.2.2 Experimental design and treatments

The experiment was conducted over an 11-week autumn period, from February to May 2019 in a temperature-controlled glasshouse with natural light conditions at Lincoln University (Lincoln, NZ). Average daytime and night-time temperatures were 22 °C and 18 °C, respectively.

Lime ($CaCO_3$, lab-grade) was applied to a subset of moist soil at a rate of 2.7 t ha^{-1} to raise soil pH_{water} from 5.3 (original) to 6.0. The latter pH was chosen as above this value nodulation has been found to be impaired in *Lupinus* species (Tang and Robson, 1993). Sulphur (S) was added as Na_2SO_4 at 100 kg of S ha^{-1}. The amended and unamended soils (200 g per pot) were placed in 250 mL plastic plant pots (ø 66×75 mm) with holes at the bottom that were then distributed in a completely randomized block design on the glasshouse table. The small-size pots were used to speed up the rhizosphere processes. However, this could have some negative effects on plant growth (Poorter et al., 2012). Four seedlings of blue lupin or Russell lupin were transplanted into each pot after seed had germinated on a moist tissue paper for 48 hours. Each plant-lime treatment combination was replicated four times. Soil moisture was maintained at 70% of field capacity for the duration of the experiment by watering pots to a specific weight with tap water every two days.

4.2.3 Soil sampling, organic anions collection, and determination

At the end of the experiment, the plants were carefully removed from the pot and gently shaken to remove the loosely adhering soil around the root (considered to be bulk soil). The soil that remained tightly adhered to the roots was partially sampled using tweezers and brush (Plate 4.1); this was defined as "rhizosphere soil" (Li et al., 2007; Wang et al., 2017). The bulk soil was air-dried immediately and stored at room temperature for further analyses.

The root system and remaining rhizosphere soil were immediately immersed in 40 mL 0.2 *mM* $CaCl_2$ solution, and gently shaken for 2 min (Pearse et al., 2007) in order to extract the rhizosphere. Despite careful handling of the root during the extraction, it cannot be excluded that some organic anions originated from damaged roots (Oburger and Jones, 2018). One drop of Micropur (0.01g L^{-1}, Katadyn Products, Kemptthal, Switzerland) was added to the $CaCl_2$ solution after the extraction to inhibit the activity of microorganisms. The extracts were then centrifuged at 3500 rpm for 5 min, the supernatant was transferred into a separate vial. The supernatant was filtered using a cellulose acetate syringe filter (pore size 0.45 µm, filter diameter: 28 mm). A subsample of the supernatant was analyzed using High-Performance Liquid Chromatography (Shimadzu Corporation, Kyoto, Japan) as described by (Shi et al., 2011). Briefly, the organic anions were separated on a Prevail TM organic acid column (250 x 4.6 mm, 5 µm particle size; Alltech, USA) using Waters 490 E programmable multi-wavelength detector (Waters Pty Ltd, USA). The mobile phase was 25 *mM* KH_2PO_4 (pH 2.35, adjusted with H_3PO_4). The samples (30

µL) were injected into the system and separated in the column at 50˚C. The organic anions were identified according to their retention time and absorbance at 210 nm. The analytical standards were prepared by dissolving L-malic acid, malonic acid, citric acid, shikimic acid, succinic acid, Pyruvic acid, DL-lactic acid, acetic acid, and fumaric acid (all from Sigma-Aldrich, UK) in 0.2 *mM* $CaCl_2$ matrix solution. The HPLC data was processed using Lab solutions LCMS software (Shimadzu Corporation, Japan). The organic anions concentrations were normalized by root dry matter (μM g^{-1} root DM). Total organic anions concentrations can also be normalized by the weight of rhizosphere soil left in the centrifuge tube after oven-drying (µM g^{-1} rhizosphere soil dry weight). The latter is only used once in this chapter to find out the relationship between total organic anions and root DM (discussion section: 4.4.1)

Plate 4.1 Pots distributed to four block on a table in the glasshouse (a) and rhizosphere soil sampling using a soft brush (b).

4.2.4 Phosphatases activity and microbial biomass phosphorus

Acid phosphomonoesterase (AcPME), alkaline phosphomonoesterase (AlPME), and phosphodiesterase (PDE) were assayed as described by Tabatabai (1994). Three replicates were performed for each assay using 1 g of moist-rhizosphere soil incubated at 37 °C for 1 hour in 4 mL of modified universal buffer (MUB) at pH 6.5 for acid phosphomonoesterase, pH 11 for alkaline phosphomonoesterase and pH 8 for phosphodiesterase using tris(hydroxymethyl)aminomethane (THAM) buffer instead of modified universal buffer, 1 mL of para-nitrophenyl phosphate (0.05 *M*) was added to acid and alkaline phosphomonoesterase assays and 1 mL of bis-para-nitrophenyl phosphate (0.05 *M*) to phosphodiesterase assays, all assays received 0.25 mL toluene.

The assays were stopped by adding 4 mL of 0.5 *M* NaOH and 1 mL of 0.5 *M* $CaCl_2$. After being rested for 5 mins, the assays were centrifuged at 3500 rpm for 10 min. The para-nitrophenol concentration in the supernatant was quantified at 410 nm using a UV-Vis spectrophotometer (UVmini-1240, Shimadzu, Japan). The phosphatase activity for each enzyme was expressed as (μM pNP g^{-1} dry soil h^{-1}).

Microbial P was determined using chloroform fumigation (Brookes et al., 1982). Briefly, three sets (fumigated, unfumigated, and P-spiked) of moist rhizosphere soil (2 g) per replicate were processed. The fumigated samples were treated with chloroform gas for 24 hours, the other two sets were

incubated for the same period. The fumigated and unfumigated sets were extracted with 30 mL of 0.5 *M* $NaHCO_3$ (pH 8.5, 30 mins), while the third set was extracted with 0.5 *M* $NaHCO_3$ spiked with 25 mg P L^{-1} to estimate the P_i recovery from the fumigated samples during the extraction. The extracts were centrifuged at 3500 rpm for 10 mins, then an aliquot from the clear supernatant was used for P_i analysis using the colorimetry method (Brookes et al., 1982; Murphy and Riley, 1962). Microbial P (P_{mic}) was calculated using equation 4.1.

$$\mathrm{P_{mic}} = \frac{[\mathrm{P_i(fumigated)} - \mathrm{P_i(unfumigated)}] \times 25}{[\mathrm{P_i(spiked)} - \mathrm{Pi(unfumigated)}] \times \mathrm{K_{pi}}} \qquad (4.1)$$

The K_{pi} coefficient was used to correct for P_i fixation during the fumigation period, a value of 0.4 was used for the calculation of P_{mic} in the present study (Brookes et al., 1982; Hedley and Stewart, 1982).

4.2.5 Phosphorus fractionation

The P fractionation was carried out for both rhizosphere and bulk soils using the method described by Condron et al. (1996) and modified by including the residual P fraction (recalcitrant P); the residual soil (0.1 g) was extracted with H_2SO_4 (1 *M*, 10 mL) for 16 hours after being previously ignited for 2 hours at 550 °C (Anderson and Ingram, 1993). The soil samples (0.5 g) were sequentially extracted with different chemicals of different strengths. The P fractions were separated into four pools according to their lability (Boitt et al., 2018b; Cross and Schlesinger, 1995), the details are provided in the Figure 4.1. A prewash with 0.5 *M* NaCl salt (5 mL, centrifuged for 5 min at 3500 rpm) was included between sequential steps to avoid the effect high levels of exchangeable Ca can have on P extraction and distribution (Perrott, 1992). The P_i in alkaline soil extracts was determined using the method of Dick and Tabatabai (1977b) with the modification of He and Honeycutt (2005), while in acid soil extracts, the P_i was analyzed as described by Murphy and Riley (1962). The total P per extract was measured using Inductively Coupled Plasma Optical Emission Spectrophotometry (ICP-OES: Varian 720-ES ICP-OES, Varian, Melbourne, Australia). The P_o content of the extracts was determined as the difference between total and P_i, all the soil P results were expressed in mg kg^{-1} soil.

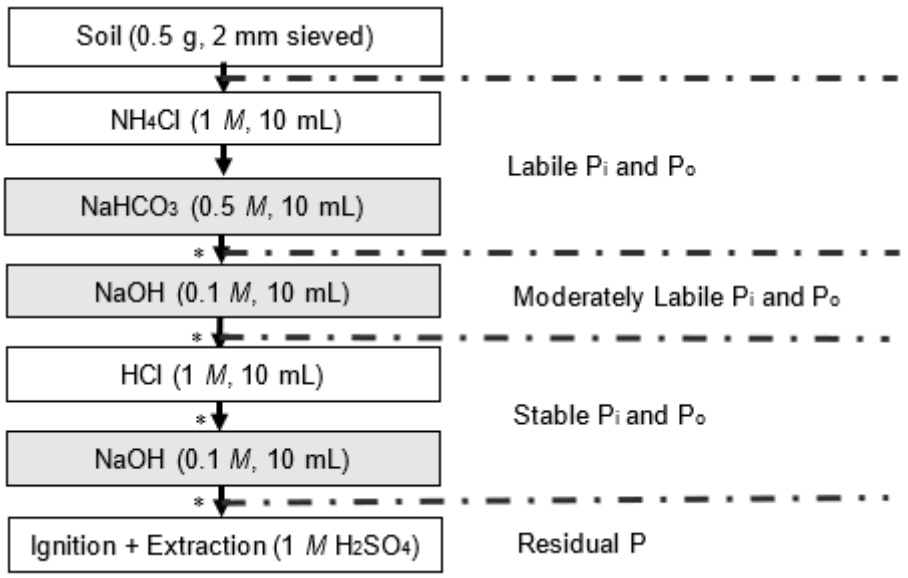


Figure 4.1 Phosphorus fractionation scheme according to Condron et al. (1996); Condron and Newman (2011); Hedley et al. (1982); Perrott (1992). Grey boxes indicate fractions with P_i and P_o forms, dot-dashed lines separate different P pools with different lability, asterisks indicate the pre-treatment with 0.5 M sodium chloride.

4.2.6 Plant and other soil analysis

The roots and shoots were separated and thoroughly washed. Then labelled on a pot basis. All samples were oven-dried at 65 °C for 48h, after which dry weight was determined. The oven-dried shoot samples underwent acid digestion (Nitric acid (HNO_3 69%)-Hydrogen Peroxide (H_2O_2 30%), 1:1 v/v) using a microwave digester (CEM MARS XpressTM, CEM Corp. USA) (NIST, 1995). The digest solution was analysed for total P using Inductively Coupled Plasma Optical Emission Spectrophotometer (ICP-OES: Varian 720-ES ICP-OES, Varian, Melbourne, Australia). Shoot P uptake was calculated as the product of P concentration and shoot dry weight.

The bulk and rhizosphere soils were air-dried and analysed for: pH (1:2.5 soil: deionized water ratio) using a pH probe (SevenEasy pH meter, Mettler Teledo, USA), and exchangeable Al was measured in 1 *M* KCl (1:10 soil: extractant ratio; Rayment and Lyons, 2011) extract using Inductively Coupled Plasma Optical Emission Spectrophotometry (ICP-OES: Varian 720-ES ICP-OES, Varian, Melbourne, Australia).

4.2.7 Statistical analysis

The data were analyzed using Minitab® statistical software version 18.1 (Minitab, Inc., State College, Pennsylvania, USA). The lupin species and soil pH treatments were considered as fixed factors. A two-sample t-test was carried out to test the significance of the differences between the effects of lupin species and soil pH levels separately on rhizosphere properties (Enzyme activities, microbial P, organic anions, exchangeable Al, pH and P fractions). Two-way ANOVA was carried out to test the significance of the main effect of each fixed factor on DM yields, root: shoot ratio and shoot P uptake, and to test

whether an interaction between the factors was significant. The effects were significant when P≤ 0.05. A correlation analysis (Pearson) was used to examine relationships between rhizosphere properties, enzyme activities, organic anions, microbial P and P fractions, soil pH and exchangeable Al. Multiple linear regression (backward elimination, $\alpha < 0.05$) was used to determine the most important rhizosphere variables (Acid and alkaline phosphomonoesterases, phosphodiesterase, total organic anions, rhizosphere pH, microbial P and exchangeable Al) contributed to shoot P uptake regardless of species and soil pH levels. The variables were standardized by subtracting the mean then dividing by the standard deviation (see standardized coefficients in Appendix C: Table C.1 and standardized regression equation 4.2).

4.3 Results

4.3.1 Plant yield and P uptake

Shoot and root DM yields between blue and Russell lupins varied significantly (Table 4.2). The average blue lupin shoot yield across both pH treatments was 1.25 g DM pot^{-1}, approximately twice that of Russell lupin with an average of 0.68 g pot^{-1}. The average root DM yields and root:shoot ratios of the two species were similar ($p > 0.05$, Table 4.2).

No significant differences were observed between the effects of soil pH levels on shoot and root DM yield of Russell lupin. In contrast, increasing soil pH from 5.3 to 6.0 has significantly increased the shoot DM yielded of blue lupin. The root:shoot ratio decreased with pH increase by 10% and 3.5% for blue and Russell lupins, respectively.

The average shoot P uptake of blue lupin (1.85 mg pot^{-1}) was higher ($p < 0.001$) than that of Russell lupin (0.88 mg pot^{-1}). The shoot P uptake of the two lupin species was affected differently by soil pH increase; blue lupin increased its shoot P uptake by 6%, whereas that of Russell lupin decreased by 23% at pH 6.0 compared to pH 5.3. The interaction species × pH was barely not significant ($p = 0.053$, Table 4.2).

Table 4.2 Dry matter yields (g pot^{-1}) and shoot P uptake (mg pot^{-1}) of blue lupin (*L.angustifolius*) and Russell lupin (*L.polyphyllus*) after a growth period of 11 weeks, as affected by soil pH change through liming, values are means ± SE of n = 4.

	Shoot yield (g DM pot^{-1})	Root yield (g DM pot^{-1})	root: shoot ratio	Shoot P uptake (mg pot^{-1})
L. angustifolius				
pH 5.3	1.15 ± 0.01	0.83 ± 0.09	0.72 ± 0.08	1.80 ± 0.05
pH 6.0	1.34 ± 0.06	0.86 ± 0.05	0.65 ± 0.03	1.91 ± 0.06
p value	0.025	0.772 n.s.	0.421 n.s.	0.270 n.s.
L. polyphyllus				
pH 5.3	0.70 ± 0.08	0.59 ± 0.06	0.86 ± 0.07	0.99 ± 0.14
pH 6.0	0.65 ± 0.04	0.55 ± 0.01	0.83 ± 0.10	0.76 ± 0.02
p value	0.602 n.s.	0.697 n.s.	0.820 n.s.	0.169 n.s.
p-value				
Species	<0.001	0.004	0.059 n.s.	<0.001
pH	0.238 n.s.	0.926 n.s.	0.500 n.s.	0.489 n.s.
Species × pH	0.053 n.s.	0.622 n.s.	0.764 n.s.	0.073 n.s.

n.s. not significant

4.3.2 Soil P fractions

The major P pool in the rhizosphere and bulk soils of the two lupins was the moderately labile P (P_i+P_o), while labile P (P_i+P_o) was the lowest portion (Table 4.3). Organic P forms comprised the largest fractions within both the labile and moderately labile P pools regardless of soil pH level and/or lupin species. Labile P_i was significantly higher in the rhizosphere soil compared to the bulk soil for blue lupin regardless of soil pH. However, for Russell lupin, it was significantly higher at pH 6.0 only. In the rhizosphere soil, labile P_i increased significantly with increasing soil pH for both species, while no significant differences were observed in the bulk soils. The labile P_o fraction decreased with soil pH increase ($p < 0.05$) in the rhizosphere and bulk soils of both lupin species. In contrast, moderately labile P_o increased with soil pH by up to 5% and 6% in the rhizosphere soil of blue and Russell lupins respectively. However, when comparing the difference between bulk and rhizosphere soil, moderately labile P_o was lower ($p < 0.05$) in the rhizosphere soil compared to bulk soil at pH 5.3 regardless of lupin species. However, at pH 6.0 the difference between the two soils was significant only for blue lupin. Moderately labile P_i remained relatively constant ($p > 0.05$) in the rhizosphere soil of both species. An increase in moderately labile P_i fraction was observed in the rhizosphere soil compared to the bulk soil regardless of pH for both species. Stabile P_i decreased significantly in the rhizosphere compared to the bulk soil regardless of soil pH for both species. Stable P_o content of rhizosphere soil increased significantly compared to bulk soil for both species regardless of soil pH. Increasing soil pH from 5.3 to 6.0 increased stable P_o by 19% and 11.5% in the rhizosphere of blue and Russel lupins respectively. There was a significant decrease in residual P pool in the rhizosphere of both species compared to their bulk soils irrespective of soil pH.

Regardless of soil pH, the labile P_i in the rhizosphere soil of Russell lupin was higher ($p < 0.05$) compared to blue lupin, while labile P_o was higher ($p < 0.05$) for blue lupin against Russell lupin. Moderately labile P_o was significantly higher in the rhizosphere of Russell lupin compared to blue lupin at pH 5.3 only. Moreover, residual P in the rhizosphere of blue lupin was higher ($p < 0.05$) compared to Russell lupin at pH 6.0 only.

Table 4.3 Effect of soil pH change through liming from 5.3 to 6.0 on the mean (n = 4) quantities (mg kg^{-1} ± SE) of P fractions, sequentially extracted from the bulk and rhizosphere soils of *L. angustifolius* and *L. polyphyllus* after a growth period of 11 weeks under glasshouse conditions.

	L. angustifolius			*L. polyphyllus*		
	Rhizosphere soil	Bulk soil	*p*-value	Rhizosphere soil	Bulk soil	*p*-value
Labile P_i						
pH 5.3	16.4 ± 0.1 B	14.3 ± 0.2	P<0.001	17.9 ± 0.4 A	16.2 ± 0.8	n.s.
pH 6.0	17.9 ± 0.1 B	15.7 ± 0.6	0.013	21.1 ± 0.5 A	18.1 ± 0.4	0.003
p-value	<0.001***	n.s.		0.003**	0.067	
Labile P_o						
pH 5.3	60.1 ± 1.6 A	42.5 ± 0.5	0.002	52.3 ± 1.9 B	39.2 ± 1.0	0.001
pH 6.0	54.8 ± 0.5 A	35.2 ± 0.8	<0.001	43.6 ± 1.5 B	30.8 ± 0.4	<0.001
p-value	0.018	<0.001		0.011	<0.001	
Moderately labile P_i						
pH 5.3	92.3 ± 4.1 A	82.5 ± 1.7	n.s.	97.7 ± 0.98 A	91.8 ± 2.4	n.s.
pH 6.0	92.5 ± 4.7 A	83.4 ± 1.2	n.s.	100.2 ± 0.68 A	91.0 ± 2.0	0.005
p-value	n.s.	n.s.		n.s.	n.s.	
Moderately labile P_o						
pH 5.3	210.4 ± 1.3 B	231.9 ± 2.0	P<0.001	221.4 ± 2.3 A	233.7 ± 2.8	0.014
pH 6.0	220.3 ± 4.6 A	236.2 ± 1.9	0.018	235.4 ± 9.62 A	230.8 ± 3.4	n.s.
p-value	n.s.	n.s.		0.253	0.531	
Stable P_i						
pH 5.3	98.3 ± 3.2 A	123.5 ± 8.6	0.034	107.2 ± 4.7 A	131.6 ± 6.4	0.022
pH 6.0	91.1 ± 1.2 A	116.4 ± 3.4	0.040	96.8 ± 5.2 A	125.6 ± 4.4	0.006
p-value	n.s.	n.s.		n.s.	n.s.	
Stable P_o						
pH 5.3	43.5 ± 3.9 A	30.5 ± 1.3	0.052 n.s.	46.0 ± 2.4 A	29.2 ± 0.8	0.007
pH 6.0	53.6 ± 1.8 A	33.4 ± 1.0	0.030	52.0 ± 5.3 A	29.7 ± 0.9	0.026
p-value	0.021	n.s.		n.s.	n.s.	
Residual P						
pH 5.3	101.3 ± 0.5 A	106.6 ± 1.1	0.005	102.2 ± 1.0 A	109.8 ± 1.6	0.029
pH 6.0	103.8 ± 0.7 A	107.4 ± 1.3	0.050	100.3 ± 0.7 B	109.3 ± 1.0	0.029
p-value	0.026	n.s.		n.s.	n.s.	

Different uppercase letters (A and B) indicate significant differences between the two lupins for each P fraction in the rhizosphere soil, according to a two-sample t-test at 5%.

4.3.3 Rhizosphere soil pH and exchangeable Al

The pH was lower (p <0.05) in the rhizosphere of blue lupin when compared to the bulk soil regardless of soil pH level. At pH 5.3, the rhizosphere pH decreased by 0.4 and 0.2 units for blue and Russell lupins respectively, compared to the bulk soil, while at pH 6.0, the rhizosphere pH decreased by 0.5 and 0.1 units for blue and Russell lupins, respectively. Significant differences were found between the rhizosphere pH under pH 5.3 and pH 6.0 for both species (Appendix C: Figure C.1).

The amount of exchangeable Al was higher in the rhizosphere soil compared to the bulk soil for the two lupin species regardless of soil pH level (Figure 4.2). However, the differences between bulk and rhizosphere soils were significant ($p < 0.001$) only for blue lupin. The concentration of exchangeable Al decreased ($p < 0.01$) with soil pH increase in the rhizosphere and bulk soils for both species. For example, passing from pH 5.3 to pH 6.0, the exchangeable Al concentration in the rhizosphere soil of blue and Russell lupins decreased by 72 and 88%, respectively. Likewise, the exchangeable Al in the bulk soil decreased by 88 and 91% for blue and Russell lupins, respectively.

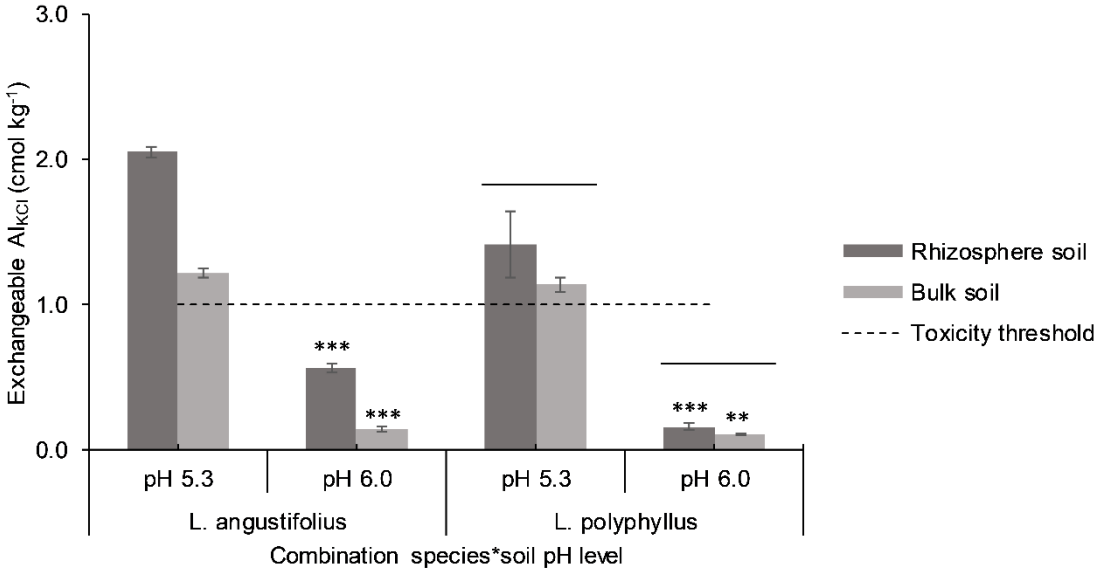


Figure 4.2 Exchangeable Al in the rhizosphere and bulk soils of L. angustifolius and L. polyphyllus at the end of the experiment as affected by soil pH increase from 5.3 to 6.0 through liming. Asterisks indicate the level of significance in the difference between the two soil pH levels in terms of exchangeable Al within each lupin species for rhizosphere and bulk soils separately, according to a two-sample t-test at 5%. Line over bars indicates the differences between bulk and rhizosphere soils which were not significant within each soil pH level, according to a two-sample t-test at 5%. Dotted dash line indicates the lower end of the Al toxicity threshold range for legumes according to Edmeades et al. (1983).

Rhizosphere soil pH and exchangeable Al showed similar correlation strengths with most of the rhizosphere properties (Table 4.4). However, these two parameters moved in opposite directions. For instance, labile P_i correlated positively with pH and negatively with exchangeable Al in the rhizosphere of both species. Inversely, labile P_o correlated negatively with pH and positively with exchangeable Al. Moderately labile P_o fraction was found to be significantly correlated with pH and exchangeable Al only in the rhizosphere of blue lupin. Residual P was significantly correlated with pH and exchangeable Al only in the rhizosphere of blue lupin. Acid phosphomonoesterase was the only enzyme significantly correlated with exchangeable Al in the rhizosphere of blue lupin. Further, no significant correlation was found between pH and enzymes activity in the rhizosphere of blue lupin. However, for Russell lupin, both phosphomonoesterases and phosphodiesterase were significantly correlated with pH and

exchangeable Al following opposite trends. Microbial P was strongly correlated with rhizosphere pH for both species. Yet, the linear relationship between microbial P and exchangeable Al was significant only for blue lupin (Table 4.4).

Table 4.4 Correlation coefficients (Pearson's *r*) between exchangeable Al, pH , P fractions, microbial P, enzymes activity and total organic anions in the rhizosphere of L.angustifolius and L. polyphyllus after 11 weeks of growth under glasshouse condition across two different pH levels.

	pH	Exchangeable Al	Labile P_i	Labile P_o	Moderately labile P_i	Moderately labile P_o	Stable P_i	Stable P_o	Residual P	Microbial P	AcPME	AlPME	PDE	TOAs
L.angustifolius														
pH	1.00	**-0.99****	**0.95*****	**-0.76***	-0.13	**0.76***	-0.65	**-0.73***	**0.76***	**0.80***	-0.60	-0.44	0.28	0.23
Exchangeable Al		1.00	**-0.98*****	**0.76***	0.35	**-0.97*****	0.57	-0.64	**-0.76***	**-0.85***	**0.78***	0.68	-0.39	-0.10
Labile P_i			1.00	-0.69	0.10	0.66	-0.57	0.59	**0.82***	**0.90****	-0.44	-0.24	0.53	-0.37
Labile P_o				1.00	0.26	-0.58	0.67	-0.64	-0.41	**-0.78***	0.63	0.50	-0.29	0.47
Moderately labile P_i					1.00	-0.57	-0.1	-0.05	0.37	0.10	0.01	0.42	0.22	-0.20
Moderately labile P_o						1.00	-0.34	0.45	0.52	0.50	-0.16	-0.20	0.34	-0.47
Stable P_i							1.00	**-0.95*****	-0.50	-0.61	**0.83***	0.27	-0.10	-0.36
Stable P_o								1.00	0.54	0.50	**-0.87****	-0.41	-0.10	0.10
Residual P									1.00	0.60	-0.30	0.13	0.48	0.27
Microbial P										1.00	-0.42	-0.20	0.63	**0.65****
AcPME											1.00	0.69	0.35	0.38
AlPME												1.00	0.41	**0.61***
PDE													1.00	**0.51***
TOAs														1.00
L. polyphyllus														
pH	1.00	**-0.89****	**0.86****	**-0.83***	0.58	0.59	-0.52	-0.37	-0.54	**0.84****	**-0.83****	**-0.78***	**0.94****	0.36
Exchangeable Al		1.00	**-0.76***	**0.99*****	-0.73	-0.50	0.34	-0.37	0.29	-0.63	**0.86***	**0.91****	**-0.83***	0.33
Labile P_i			1.00	**-0.76***	0.55	0.50	-0.47	0.44	-0.43	**0.80***	**-0.82***	-0.66	**0.81***	0.46
Labile P_o				1.00	-0.68	-0.46	0.27	-0.28	0.28	-0.52	**0.85****	**0.88****	**-0.80***	-0.38
Moderately labile P_i					1.00	0.23	-0.20	0.30	0.14	0.65	-0.52	**-0.85****	0.42	0.14
Moderately labile P_o						1.00	-0.46	0.42	0.1	0.46	-0.34	-0.52	0.48	0.10
Stable P_i							1.00	**-0.89****	0.42	-0.69	0.45	0.34	-0.30	0.23
Stable P_o								1.00	-0.10	0.55	-0.29	-0.42	0.10	-0.10
Residual P									1.00	-0.41	0.57	0.10	-0.64	-0.20
Microbial P										1.00	-0.67	-0.64	0.68	0.12
AcPME											1.00	0.62	**-0.82***	-0.10
AlPME												1.00	-0.68	-0.38
PDE													1.00	0.50
TOAs														1.00

AcPME acid phosphomonoesterase, AlPME alkaline phosphomonoesterase, PDE phosphodiesterase, TOAs total organic anions.
Asterisks indicate the significance level (*$p < 0.05$, **p <0.01, ***p <0.001).

4.3.4 Phosphatase activity

Acid phosphomonoesterase activity was greatest among soil phosphatases, followed by alkaline phosphomonoesterase and phosphodiesterase (Figure 4.3). Additionally, enzyme activities were greater in the rhizosphere soil of blue lupin compared to Russell lupin. The activity of acid phosphomonoesterase decreased (blue lupin: $p > 0.05$; Russell lupin: $p < 0.01$) by up to 9% in the rhizosphere soil of blue lupin and by up to 13% in the rhizosphere of Russel lupin, when soil pH increased from 5.3 to 6.0 (Figure 4.3a). Similarly, alkaline phosphomonoesterase activity decreased (blue lupin: $p = 0.055$, Russell lupin: $p = 0.015$) by up to 6% and 21% respectively, following soil pH increase (Figure 4.3b). Whereas, phosphodiesterase activity showed an opposite trend to that of phosphomonoesterases, increasing by up to 19% and 38% for blue and Russell lupins respectively (Figure 4.3c).

Acid and alkaline phosphomonoesterases were negatively correlated with labile P_i (blue lupin: acid ($r = - 0.44$, $p > 0.05$, Table 4.4), alkaline ($r = - 0.24$, $p > 0.05$); Russel lupin: acid ($r = - 0.82$, $p = 0.012$, Table 4), alkaline ($r = - 0.66$, $p > 0.05$). Whereas, labile P_o was positively correlated with both acid (blue lupin: $r = 0.63$, $p > 0.05$; Russell lupin: $r = 0.85$, $p = 0.008$) and alkaline phosphomonoesterase (blue lupin: $r = 0.50$, $p > 0.05$; Russell lupin: $r = 0.88$, $p = 0.004$). Phosphodiesterase was positively correlated with labile P_i (blue lupin: $r = 0.53$, $p > 0.05$; Russell lupin: $r = 0.81$, $p = 0.013$) and negatively correlated with labile P_o (blue lupin: $r = - 0.29$, P> 0.05; Russell lupin: $r = - 0.80$, $p = 0.017$; Table 4.4). Acid phosphomonoesterase was the only phosphatase enzyme significantly correlated with other P fractions in the rhizosphere soil of blue lupin (Table 4.4), specifically with stable P_i ($r = 0.83$, $p = 0.011$) and stable P_o ($r = -0.87$, $p = 0.005$). However, these correlations were weak ($p >0.05$) for Russell lupin.

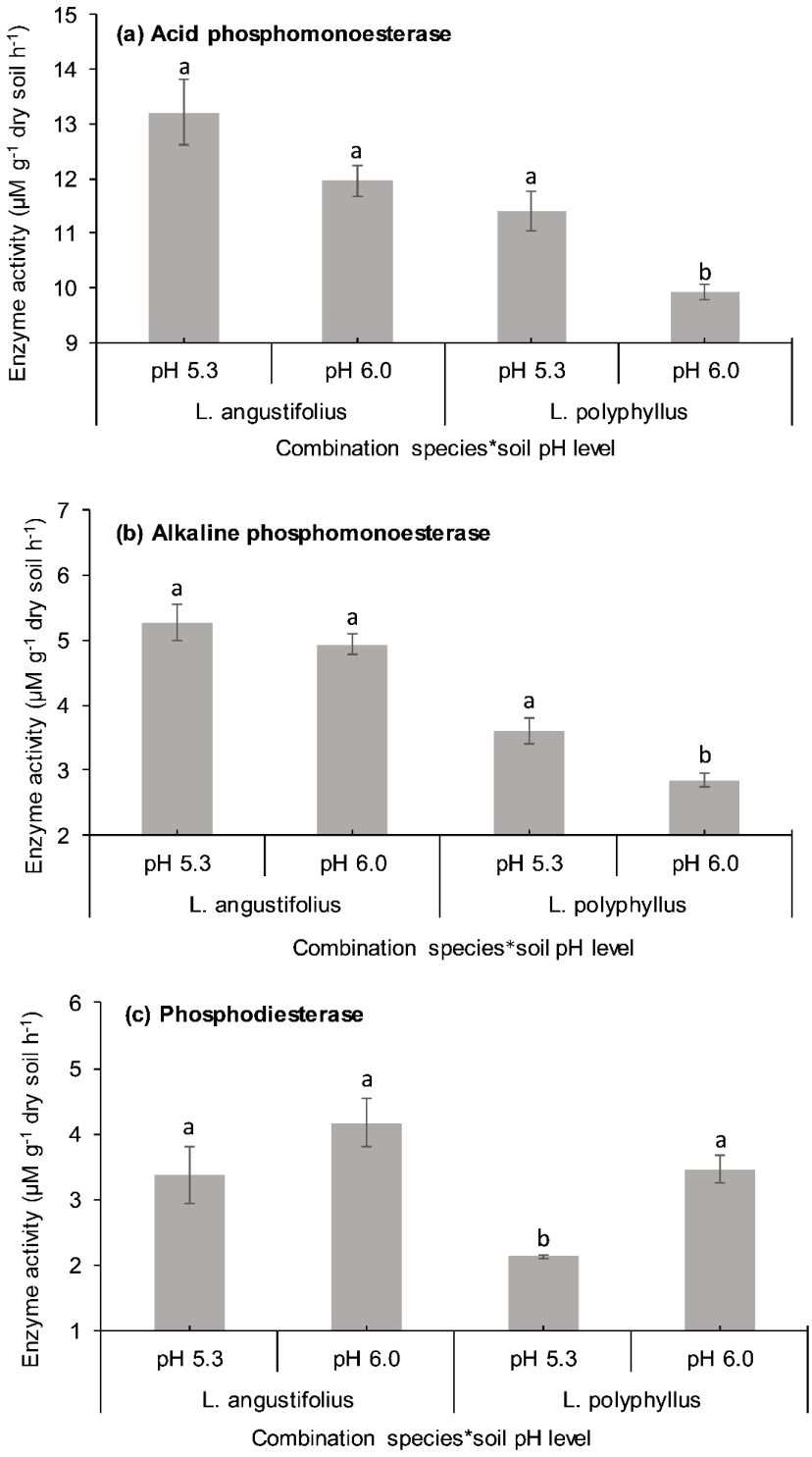


Figure 4.3 Activities of phosphomonoesterases, both acid (a) and alkaline (b) and phosphodiesterase (c) in the rhizosphere soil of two different lupin species after 11 weeks of the growth period, as affected by soil pH increase from 5.3 to 6.0 through liming. Different lowercase letters above the bars indicate significant differences between the two soil pH levels within each lupin species, according to a two-sample t-test at 5%.

4.3.5 Microbial biomass P

Increasing soil pH from 5.3 to 6.0, increased significantly microbial P in the rhizosphere soil of both species (Figure 4.4). Blue lupin had higher (p <0.001) microbial P concentration in the rhizosphere compared to Russell lupin regardless of soil pH level; the microbial P for blue lupin was 16% and 17% higher than Russell lupin under pH 5.3 and pH 6.0, respectively. The interaction soil pH×species was not significant.

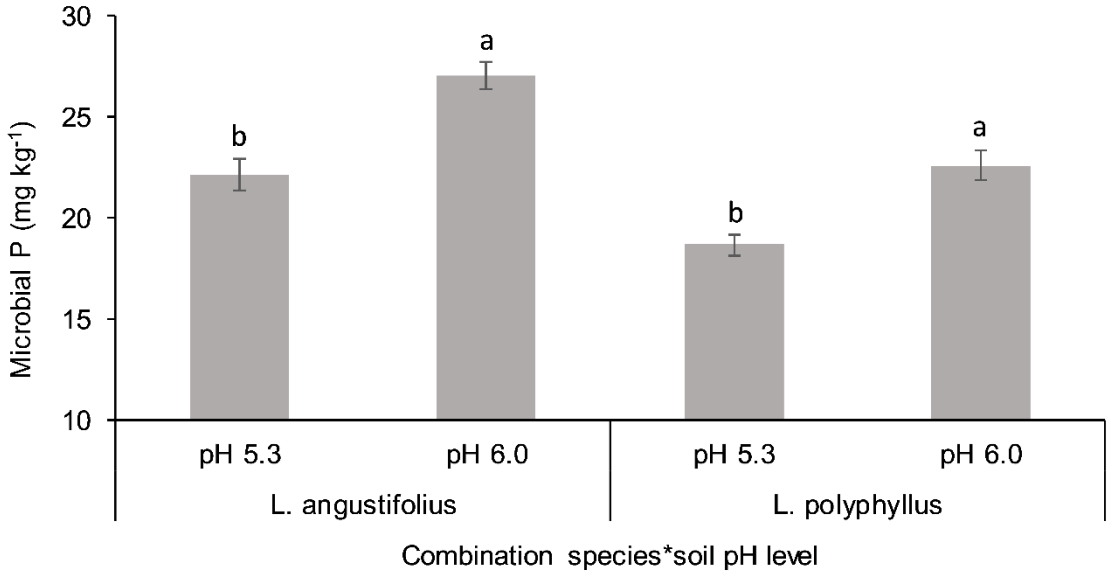


Figure 4.4 Microbial biomass P (mg kg^{-1}) in the rhizosphere soil of *L. angustifolius* and *L. polyphyllus* after 11 weeks growth period, as affected by soil pH increase from 5.3 to 6.0 through liming. Different lowercase letters above the bars indicate significant differences between the two soil pH levels within each lupin species, according to a two-sample t-test at 5%.

In the rhizosphere of blue lupin, microbial P was strongly and positively correlated with labile P_i (r = 0.90, p = 0.002, Table 4.4) and negatively correlated with labile P_o (r = - 0.78, p = 0.024). However, in the rhizosphere of Russell lupin, it was significantly correlated with labile P_i fraction only (r = 0.80, p = 0.015, Table 4.4). No significant correlation was found between microbial P and enzyme activities for both species (Table 4.4). Though, phosphodiesterase showed a strong positive correlation with microbial P in the rhizosphere of both species (blue lupin: r = 0.63, p = 0.12, Russel lupin: r = 0.68, p = 0.071). Additionally, a strong and significant positive correlation (r = 0.65, p < 0.01) was found between microbial P and total organic anions in the rhizosphere of blue lupin only (Table 4.4).

4.3.6 Organic anions production

Six organic anions (pyruvate, malonate, fumarate, acetate, citrate, and malate) were detected in the rhizosphere soil of both lupin species, while succinate was detected only in the rhizosphere soil of blue

lupin. The total organic anions produced in the rhizosphere of blue lupin across soil pH levels were 2-fold higher (p = 0.001) than Russell lupin (Figure 4.5). Citrate and malate were the highest organic anions among others exuded in the rhizosphere of both species. Moreover, significant differences were found between blue and Russell lupin in the concentrations of citrate (p = 0.047), malate (p < 0.001), fumarate (p < 0.001), and malonate (p = 0.001) across soil pH levels. However, no significant differences were found for acetate and pyruvate between the two species. The total organic anions released under pH 6.0 increased by 18% and 25% for blue and Russell lupins, respectively, compared to pH 5.3. Besides, citrate and malate anions under pH 6.0 increased by 24% and 8%, respectively, compared to pH 5.3 in the rhizosphere of blue lupin, while in the rhizosphere of Russell lupin there was an increase of 21 % in citrate and a decline (- 4%) in malate exudation (Figure 4.5).

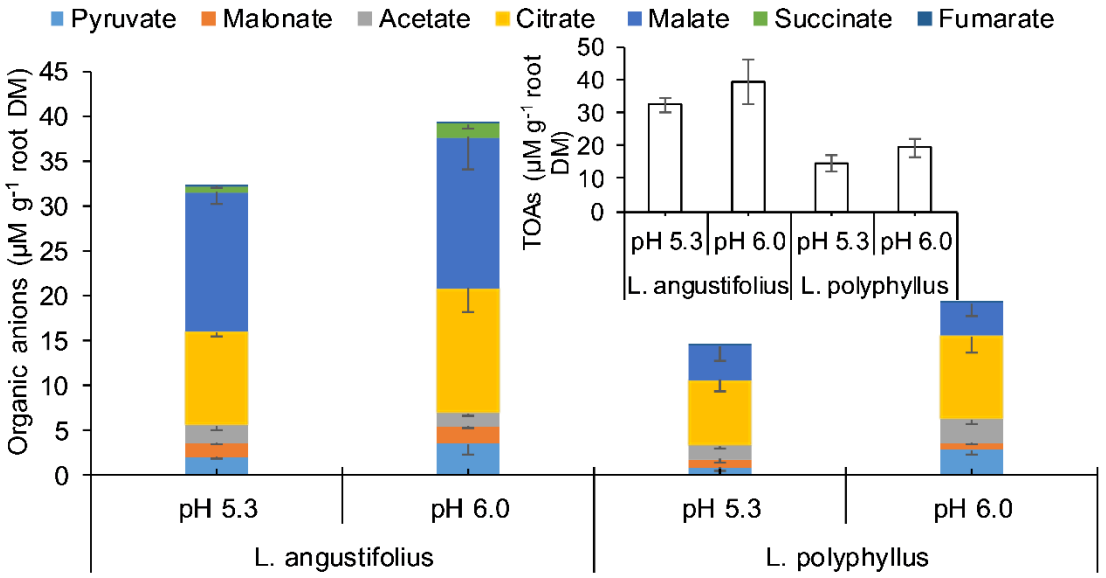


Figure 4.5 Concentrations of organic anions (µM g^{-1} root DM) in the rhizosphere of *L. angustifolius* and *L. polyphyllus* after 11 weeks growth period, as affected by soil pH increase from 5.3 to 6.0 through liming. Error bars represent standard error (SE) of four replicates (n = 4).

A strong positive relationship (r^2 = 0.72) was found between total organic anions and shoot P uptake regardless of lupins and soil pH levels as shown in Figure 4.6. Additionally, the multiple regression analysis conducted on the data of the two lupins together showed that the total organic anion concentration (*TOAs*) was the most important factor which impacting P uptake. Approximately 71% of the variation in shoot P uptake, regardless of plant species, was explained by standardized equation 4.2:

Shoot P uptake (mg pot^{-1}) = 1.34 + 0.46 TOAs (n = 16; p < 0.001) (4.2)

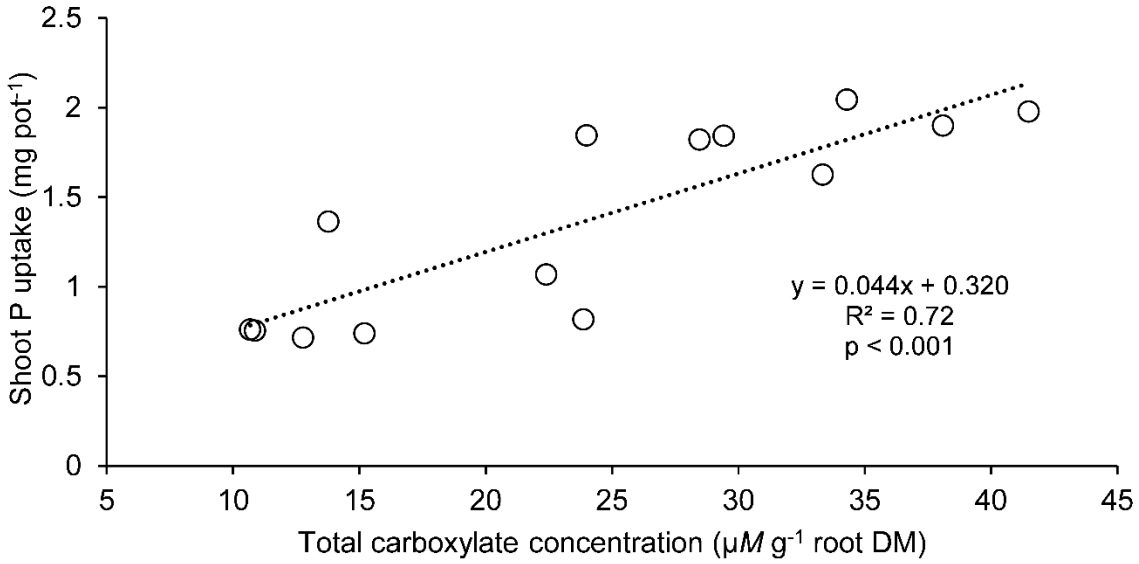


Figure 4.6 Relationship between total organic anions (TOAs) and shoot P uptake regardless of lupin species and soil pH levels.

4.4 Discusssion

4.4.1 Plant yield and P uptake

The greater shoot P uptake of blue lupin compared with Russell lupin indicates that the former was able to take up more soil P than the latter where the level of available soil P was low. This could be related to the higher root biomass of the blue lupin, increasing its capacity to explore a larger soil volume (Lynch, 2007). Generally, under limited P availability, plants increase their root-to-shoot ratio in order to enhance P acquisition (Hermans et al., 2006; Ramaekers et al., 2010). Thus, the slight decrease in this ratio with pH increase for both lupin species could be due to the increased P_i availability in their rhizosphere soils. However, the shoot P concentrations in both lupin species (ranged 0.12–0.16%) were below the optimum range (0.2–0.3%) for lupins (Li et al., 2008; Müller et al., 2015). Russel lupin produced more root per unit of shoots compared to blue lupin, though was not reflected in terms of shoot P uptake. Moreover, labile phosphorus in the rhizosphere of Russell lupin was higher than blue lupin, but again, was not reflected in terms of shoot P uptake. Further, the P dynamics in the rhizosphere of the two species were relatively consistent compared to the bulk soil. These results indicate that Russell lupin probably has a lower P requirement compared to blue lupin. This hypothesis is in line with Davis (1991), who found that two perennial lupins (*L. polyphyllus* and *L. arboreus*) grown in an acid soil (pH_{water} 5.3) with an Olsen P of 5 mg kg^{-1}, showed no response to a range of P fertilizer rates (0 to 800 kg ha^{-1}). However, in our experiment the plants could have been pot-bound. Also, only two P levels were used.

Organic anions are well recognized as an important strategy for mobilizing P in rhizosphere soil, and thereby enhancing P uptake (Sun et al., 2020; Wang and Lambers, 2020). This was confirmed in the present study where total organic anions were the most important factor that affected shoot P uptake across the two species. Organic anions can also improve P nutrition indirectly through Al detoxification (Chen and Liao, 2016). Therefore, the higher P uptake by blue lupin compared to Russell lupin could be attributed to its higher release of organic anions.

The positive correlation found between total organic anions concentration (µM g^{-1} rhizosphere soil dry weight) and root DM (r = 0.65, p = 0.017) regardless of lupin species and pH may explain the higher exudation of organic anions in the rhizosphere of blue lupin. However, the increase in shoot P uptake at pH 6.0 compared to pH 5.3 for blue lupin only coincided with an increase of total organic anions, while root DM remained unchanged. This result stresses the importance of investigating root morphological traits along with root exudation in order to identify which root structure is mostly related to organic anions exudation (McKay Fletcher et al., 2020), we hypothesize that soil pH change can indirectly influence organic anions secretion by altering root morphology (Robles-Aguilar et al., 2019). On the other hand, higher uptake of Ca^{2+} at pH 6.0 after liming has presumably simulated protons extrusion from roots which is supposed to be responsible for organic anions release (Neumann and Römheld, 2007).

The enhanced shoot DM yield of blue lupin at pH 6.0 compared to pH 5.3, may be attributed to the increase in P uptake. Moreover, the improved rhizosphere soil acidity being within the optimal pH for narrow-leaf lupin growth (5–5.5 according to Tang et al. (1992)) with a significant reduction in exchangeable Al (being below toxicity threshold: 1–2 cmol kg^{-1} according to Edmeades et al. (1983)) could have contributed to increasing shoot DM yield of blue lupin. On the other hand, the relatively higher pH in the rhizosphere of Russell lupin at soil pH 6.0 could have affected root nodulation, causing a decline in nitrogen supply to the plant and therefore reducing the yield (Jessop and Mahoney, 1982; Tang et al., 1992). In consistence with our results, Hendrie et al. (2018) found that Russel lupin yield was unresponsive to lime application.

4.4.2 Rhizosphere pH and exchangeable Al related to organic anions exudation

In this study, blue lupin exhibited reduced rhizosphere soil pH relative to the bulk soil. This was obviously due to the acidification of the rhizosphere, which is reported to be mediated by several factors, including environmental stresses such as P deficiency and Al toxicity (Berenji et al., 2017; Hinsinger et al., 2003). The differential uptake of anions and cations by plant roots has been considered the major source of the H^+ flux into the rhizosphere soil (Jaillard et al., 2003; Tang and

Rengel, 2003). However, the contribution of organic acids in rhizosphere acidification has been reported to be minor (Wang and Lambers, 2020). In maize, for example, the contribution of exuded organic acids to acidification was < 0.5% (Petersen and Böttger, 1991). Conversely, the activation of plasma membrane H^+-ATPase (H^+-pumping) has been reported to be related to citrate exudation in white lupin (*Lupinus albus*) (Yan et al., 2002) and purple lupin (*Lupinus pilosus*)(Ligaba et al., 2004) under P deficiency. Whereas, Zhu et al. (2005) found that cations (K^+, Mg^{2+}, and Na^+) were also involved as counterions for citrate release in cluster roots of *Lupinus albus*. In our study, it was not possible to separate the quantitative contribution of each of these processes to rhizosphere pH decrease. Nevertheless, the weak relationships found between total organic anions and rhizosphere pH (Table 4.4) support the hypothesis that organic anions exudation and rhizosphere acidification are two separate processes that might be spatially coordinated.

The higher exchangeable Al concentration in the rhizosphere of blue lupin can be explained by the increase in Al solubility due to acidification (Calba et al., 2004). The relatively higher pH in the rhizosphere of Russell lupin reduced Al activity (Wang et al., 2006b). Thus, less exchangeable Al was found in the rhizosphere of Russell lupin compared to blue lupin. However, shoot Al uptake of Russell lupin was identical to that of blue lupin (Appendix C: Table C.2). These results indicate that blue lupin was likely more tolerant to high soil exchangeable/Al toxicity. This could be attributed to its higher secretion of organic anions which is widely established as an Al-tolerance mechanism (Zhang et al., 2019b). Organic anions secreted by roots into the rhizosphere can externally chelate monomeric Al^{3+} into non-toxic complexes (Chen and Liao, 2016). This hypothesis is supported by the strong and positive correlations found between blue lupin shoot Al concentration and the concentrations of individual organic anions extracted in the rhizosphere (Appendix C: Table C.3): malate (r = 0.89, p < 0.01), pyruvate (r = 0.80, p <0.05), and total organic anions (r = 0.80, p < 0.05), referring to an Al-induced exudation of organic anions. Further, the obtained quantities of citrate and malate which are known to be released in large amounts relative to other organic anions under Al stress by Al-resistant species (Ma, 2000; Ma et al., 2001; Zheng et al., 1998) could be considered as an additional evidence of the Al-exclusion mechanism adopted by blue lupin. Though, it cannot be ruled out that P deficiency was also involved in these responses.

4.4.3 The response of phosphatase to rhizosphere pH elevation

The predominance of acid phosphomonoesterases is obviously a consequence of the acid soil used in this study (Harrison, 1983; Juma and Tabatabai, 1978). The $\Delta activity/\Delta pH_{rhizosphere}$ ratios were: - 1.2, - 0.3 and 0.8 for acid, alkaline phosphomonoesterases, and phosphodiesterase respectively, in the rhizosphere of blue lupin and - 1.5, - 0.8 and 1.3 respectively, in the rhizosphere of Russell lupin. These

ratios suggest that the order of sensitivity of the enzymes to rhizosphere pH increase following bulk soil pH increase from 5.3 to 6.0 was as follows: acid phosphomonoesterase # phosphodiesterase # alkaline phosphomonoesterase. The inhibition effect of soil pH increase on acid phosphomonoesterase activity was in accord with Ekenler and Tabatabai (2003); Margenot et al. (2018). However, this behaviour did not necessarily reflect the generally accepted pH optima for acid phosphomonoesterase (Niemi and Vepsäläinen, 2005).

Controversial findings were reported regarding the response of alkaline phosphomonoesterase to soil pH increase. Contrary to our results, Acosta-Martinez and Tabatabai (2000); Ekenler and Tabatabai (2003); Wang et al. (2006a) reported that alkaline phosphomonoesterase activity was positively correlated with soil pH increase. Whereas, Margenot et al. (2018) found that this enzyme did not change under an unfertilized soil in response to increasing liming application rates, furnishing a stepwise pH gradient from 4.7 to 6.4. The observed decrease in phosphomonoesterases activity in our study can be explained by the repressive effect of the increased P_i availability (Nannipieri et al., 2011; Spiers and McGill, 1979) at pH 6.0 as the release of these enzymes by microorganisms and plants is determined by their need for orthophosphate (Skujiņš and Burns, 1976).

The opposite trend found between phosphomonoesterases and phosphodiesterase in response to rhizosphere soil pH increase suggests that liming could change the relative role of phosphatases in the rhizosphere of lupins. This difference in the influence of pH change on phosphatases may partly be due to the shift in the microbial communities (Paul and Clark, 1996); bacterial phosphatases had higher pH optima than fungal phosphatases (Tabatabai, 1994; Turner and Haygarth, 2005). The higher phosphodiesterase activity measured at pH 6.0 indicates that they are probably originated from bacteria and actinomycetes, which are more abundant in neutral soils (Alexander, 1977). Moreover, Hayano (1977); Turner and Haygarth (2005) reported that the activity of phosphodiesterase in acid soils was likely limited by adsorption to soil constituents, similar observations were reported by Leprince and Quiquampoix (1996) for other phosphatases. Although it reduces enzyme activity, sorption can also stabilize enzymes to the extent that much of the activity measured in soil assays are from the stabilized enzymes (Kiss et al., 1975; Skujiņš and Burns, 1976). Therefore, the observed increase in rhizosphere pH after liming could have mobilized some of the stabilized phosphodiesterase in our soils (Allison, 2006). Moreover, the strong positive correlation found between phosphodiesterase and microbial P (r = 0.74, p = 0.002) regardless of lupin species, indicates that the additional increase in phosphodiesterase at pH 6.0 could have been mediated by the substrate (diester P) loading into the rhizosphere soil due to microbial cells death. On the other hand, this relationship

appears to support the hypothesized use of phosphatase by microorganisms to scavenge P under P limitation (Margenot et al., 2018; Oberson et al., 2001).

4.4.4 Phosphorus dynamics associated with rhizosphere properties

Our results showed that liming influenced rhizosphere pH, phosphatase activities, organic anions exudation, and subsequently P distribution in the rhizosphere. We observed greater P uptake by blue lupin (+ 50%) than Russell lupin, which should be translated into larger differences in terms of P chemistry in the rhizosphere. Instead, some consistent patterns between the two species were observed such as the fact that labile P_i increased significantly following soil pH increase in the rhizosphere of both species. This could be due to the effect of pH increase on P_i desorption (Barrow et al., 2020b; Sato and Comerford, 2005). The sorption of P_i has also been reported to be reduced by pH increase due to a decrease in surface positive charge, thus an increase in solution P (Barrow et al., 2020a; Nobile et al., 2020). This view is supported by the positive relationship found between pH and labile P_i (Table 4.4) in the rhizosphere of both lupins. It is supported also by the negative relationship found between labile P_i and exchangeable Al (Table 4.4) because P associated with Al is known to be released by desorption reaction at higher pH (Le Mare and Leon, 1989; Penn and Camberato, 2019). Compared with Russell lupin, less moderately labile P_i was accumulated in the rhizosphere of blue lupin. This can be attributed to the mechanisms of ligand exchange and/or chelation of metal ions through organic anions exudation which could have been also contributed to P release from sparingly soluble Al, Fe and Ca phosphate (Gerke et al., 1994; Rose et al., 2010).

The increased P_i availability at pH 6.0 compared to pH 5.3 in the rhizosphere, could have resulted from the mineralization of labile P_o which was significantly depleted in the rhizosphere soils of both species following pH increase. A possible explanation for P_o mineralization in this study might be related to the enhancement of microbial activity at higher pH (Condron and Goh, 1990; Kiflu et al., 2017) providing that the depletion occurred also in the bulk soil when pH was increased. Turner and Haygarth (2005) suggested that phosphodiesterase is the rate-limiting step that regulates labile P_o mineralization. This agrees with the inverse relationships observed between phosphodiesterase and labile P_o in the rhizosphere of Russell lupin (r = - 0.80, p= 0.017), implying that the mineralization of labile P_o at pH 6.0 compared to pH 5.3 in the present study, probably results from the additional release of phosphodiesterase at pH 6.0. This suggests that the decrease in phosphomonoesterases relative to phosphodiesterase may not necessarily impact the mineralization of labile P_o in the rhizosphere.

The higher labile P_i concentration in the rhizosphere of Russell lupin compared to blue lupin could be due to low P utilization by Russel lupin referring to its low shoot P uptake. The higher labile P_i in the rhizosphere of both species compared to the bulk soil is in accordance with previous reports (Sugihara et al., 2016; Sun et al., 2020).

The accumulation of labile and stable P_o in the rhizosphere compared to the bulk soil, regardless of lupin species, can be attributed to several factors. For instance, it is well documented that roots supply organic carbon which plays an important role in stimulating microbial growth and activity in the rhizosphere (Helal and Sauerbeck, 1989; Toal et al., 2000), which in turn promotes P immobilization by soil microbes (Wu et al., 2007). This is supported by the significant positive correlation (r = 0.65, p = 0.007) found between microbial biomass P and total organic anions – a source of carbon for microbes (Hütsch et al., 2002; Pausch and Kuzyakov, 2018) – irrespective of soil pH and lupin species. Similar relationships were reported in previous studies (Brookes et al., 1984; Chen et al., 2002).

The accumulation of moderately labile P_o and stable P_o in the rhizosphere of both lupins at pH 6.0, relative to pH 5.3, is in line with the results of Li et al. (2015) who found an accumulation of moderately labile Po in the rhizosphere of fababean (*Vicia faba L.*) and maize (*Zea mays L.*) grown in acid soils treated with OH^- to increase soil pH. This reflects the enhancement of microbial activity at higher pH, indicating that P_i was immobilized into organic fractions by microorganisms (Condron et al., 2005). The implication of P immobilization in increasing soil P_o content has been confirmed by several workers (Condron and Goh, 1989; George et al., 2006). Additionally, the observed decrease of phosphomonoesterases in the rhizosphere of both lupin species following pH increase likely contributed to P_o accumulation. For instance, we found that acid phosphomonoesterase was correlated strongly and inversely with stable P_o (r = - 0.87, $p < 0.01$) in the rhizosphere of blue lupin.

Stable P_i was assumed to represent the fraction of calcium phosphate and occluded P within Fe and Al oxides (Cross and Schlesinger, 1995). In the present study, stable P_i fraction depleted significantly in the rhizosphere compared to bulk soil regardless of lupin species. This is likely to result from the root-induced pH decrease which is supposed to dissolve mainly calcium phosphate (Hinsinger, 2001). This result indicates that calcium supply via liming at a relatively moderate rate (2 to 3 t ha^{-1}) is not necessarily increasing Ca-P compounds in the soil at least in the short term. The depletion of residual P in the rhizosphere relative to the bulk demonstrates that both species can utilize that recalcitrant P part. The increased residual P in the rhizosphere of blue lupin at pH 6.0 could be due to P_o accumulation.

4.5 Conclusion

This study was able to reveal the short-term effect of lime-induced pH elevation on P cycling processes and P dynamics simultaneously in the rhizosphere of two lupin genotypes grown on an acid grassland soil. Lime application increased labile P_i in the rhizosphere of both species. This partly resulted from labile P_o mineralization which likely resulted from microbial activity enhancement at higher pH. Another inference, drawn from this experiment, is that the decrease in phosphomonoesterases relative to phosphodiesterase following pH increase does not necessarily impact the mineralization of labile P_o in the rhizosphere. In parallel, elevated pH in the rhizosphere has promoted extractable P_o accumulation mainly as moderately labile and stable forms due to phosphomonoesterases activity reduction and microbial P immobilization. We compared blue and Russell lupins, for the first time, and we found that the higher shoot P uptake of blue lupin compared to Russell lupin is explained mostly by its higher root biomass and higher exudation of organic anions. However, our research was limited to one soil and a comparatively short evaluation period. Therefore, for future research we recommend evaluating more soils with different P fertilities across different plant growth stages. We also recommend using a bigger pot size to avoid any negative effect that small pot size could have on plant growth.

Chapter 5

Soil pH Effects on Phosphorus Mobilization in the Rhizosphere of *Lupinus angustifolius*

(Published paper)

5.1 Introduction

Phosphorus (P) is an essential macro-nutrient that often limits the productivity in natural and agricultural ecosystems (Hou et al., 2020; Mogollón et al., 2018). Phosphorus availability and mobility are low in most soils, especially acid soils where P availability is mainly limited by adsorption reactions due to low pH and high concentrations of Al/Fe oxides and hydroxides (Gessa et al., 2005; McDowell and Condron, 2000) as well as sorption to clays and organic matter (Asomaning, 2020). Soil microorganisms also immobilize P into organic forms which may further constrain P availability to plants (Richardson and Simpson, 2011). Liming is a commonly used agricultural practice on acid soils to maintain an appropriate pH for plant growth and decreasing Al phytotoxicity (Bouray et al., 2020; Morton and Moir, 2018). However, reports of lime-induced pH modification effects on P availability are (1) inconsistent (2) focussed mainly on soil chemical changes and (3) often limited to the bulk soil (Azeez et al., 2020; Curtin and Syers, 2001; Haynes, 1982; Margenot et al., 2018; Mkhonza et al., 2020; Simonsson et al., 2018). However, plant P acquisition is mainly determined by many processes in the vicinity of the roots; that is, the rhizosphere (George et al., 2011). Thus, tackling the pH-P availability relationship from a rhizosphere perspective would better help to understand the effects of soil pH increase via liming on P bioavailability and utilization by plants.

Plants can increase P acquisition by utilizing various biophysical and chemical mechanisms such as alteration in root system architecture, production of extracellular enzymes (phosphatases), secretion of organic anions and acidification of the rhizosphere (Hinsinger et al., 2011). Legumes like blue lupin (*Lupinus angustifolius*) are known for their ability to thrive under P-limited environments (Lambers et al., 2013). Unlike white lupin (*Lupinus albus*), blue lupin does not form cluster roots (Wang et al., 2008) and has been reported to exude carboxylate at a rate lower than that of white lupin (Hocking and Jeffery, 2004). Furthermore, when compared to white lupin, blue lupin has a less extensive root system, consisting of a dominant taproot with a relatively large number of primary lateral roots and few secondary roots (Clements et al., 1993). There is also no evidence that this species develops effective mycorrhizal associations (Lambers and Teste, 2013). Yet, blue lupin had a superior ability to

access P from the sparingly soluble forms compared with other non-cluster root lupin species (Pearse et al., 2007). Thus, this species may have evolved other adaptive strategies for their P-acquisition efficiency, such as root-morphological changes, and increased exudation of organic anions (Chen et al., 2013; Pearse et al., 2006). However, responses of these adaptations to lime-induced pH elevation, have been poorly investigated. It is imperative to quantify these responses to better understand to what extent plants can acquire P in limed soils under different pHs.

Zymography is a new technique for quantitative visualization of enzyme activities in two dimensions (2D) in-situ (Razavi et al., 2019). It has been applied in the rhizosphere for various purposes. For example, it has been used to study the effects of plant growth (Ma et al., 2018a), root morphology (Ma et al., 2018a; Ma et al., 2018b) and root exudate composition (Zhang et al., 2019a) on spatial and temporal patterns of enzyme activities in the rhizosphere of many plant species. The DGT technique has been widely used to measure labile solutes in soils and sediments, based on hydrogels with homogeneously distributed analyte-selective binding phases (Davison and Zhang, 2016). It has numerous advantages over the chemical soil extraction methods: (1) it relies on diffusion for solute uptake and thus mimics a key mechanism for nutrients uptake by roots (2) it has been shown to correlate with plant uptake of phosphorus (Degryse et al., 2009; Mason et al., 2013) through sampling similar pools of the available nutrient (Six et al., 2013), and (3) DGT is the only technique capable of generating quantitative, sub-mm scale 2D images of P. Two dimensional measurements of labile P with DGT have been performed through analysis by laser ablation-inductively coupled plasma-mass spectrometry (LA−ICP−MS) (Santner et al., 2010). However, LA-ICP-MS is expensive, not available to many laboratories, time-consuming and does not differentiate between dissolved organic P and inorganic P (Vogel et al., 2019). Ding et al. (2013) developed a colorimetric technique that can be used for the submillimeter-scale imaging of labile P in combination with DGT using Computer Imaging Densitometry (CID) which has the potential to reduce costs and analysis times for high-resolution imaging of P dynamics at the root-soil interface.

Zymography and DGT have significantly expanded our knowledge of nutrients and contaminants dynamics in natural soil-rhizosphere-plant systems. Hummel et al. (2021) demonstrated that combining the two techniques is technically feasible. Coupling these techniques to simultaneously examine P and enzymes in the rhizosphere under different soil pHs could generate high-resolution analysis, thereafter, bringing new insight on how soil pH is modulating P mobilization in rhizosphere and plant P acquisition. In this study, we sought to (1) use colorimetric DGT with zymography to study the effects of soil pH increase through liming on acid phosphatase activity and labile P distribution patterns in the rhizosphere of blue lupin grown in two contrasting acid pasture soils, (2) examine the

effects of soil pH increase on root morphological and physiological traits involved in P mobilization and acquisition by blue lupin. We hypothesized that increasing soil pH to near neutral (pH 6.3) would increase P availability and promote enzyme activity in the rhizosphere, alter root morphology and increase the exudation of organic anions.

5.2 Material and methods

5.2.1 Soil and plant preparation

Two contrasting pasture soils: Mt Grand soil sampled from Mt Grand station (44°40’19.49’’S, 169°19’5.66’’E), a commercial sheep and beef high-country farm operated by Lincoln University, located in central Otago district, New Zealand and Millers Flat soil collected near Millers Flat Village, central Otago district, New Zealand (45° 37' 18.24"S, 169° 34' 58.38"E), from a permanent pasture with typical low fertility high country pasture species, comprising browntop, fescue tussocks and poa grass species. Both soils were sampled from the upper 15 cm, air-dried, and sieved (2 mm mesh). The soils were characterized using the analyses listed in Table 5.1. The main differences between the two soils were: total organic P content, resin P, exchangeable Al concentration and initial pH.

Lime ($CaCO_3$, lab-grade) was applied to a subset of moist soil at a rate of 4.2 and 13.7 t ha^{-1} for Mt Grand and Millers Flat soils respectively, to raise the soil pHs from their initial values (Table 5.1) to a target value of 6.5, but the actual pH value is 6.3. This pH target was selected based on the classic understanding of maximum chemical P availability at near pH 6.5 as reported in many studies reviewed by Penn and Camberato (2019). The soils were packed in the rhizoboxes (internal dimensions: 15 × 30 × 2.5 cm) to achieve a consistent bulk density throughout of 1.1 g cm^{-3}. Sixteen rhizoboxes used in the experiment (2 soil pH levels × 2 soil types × 4 replicates for each pH-soil type combination).

Table 5.1 Results of soil chemical and particle-size distribution before the establishment of the experiment.

	Mt Grand	Millers Flat	By method of
Initial pH	5.3	4.7	Blackmore et al. (1987)
Exchangeable Al_{KCl} (cmol kg^{-1})	1.13	10.55	Rayment and Lyons (2011)
Exchangeable Al_{CaCl2} (mg kg^{-1})	4.7	31.7	Hoyt and Nyborg (1972)
Olsen P (mg kg^{-1})	7	8	Olsen et al. (1954)
P retention (ASC, %)	20	52	Blackmore et al. (1987)
Resin P (mg kg^{-1})	14	29	Saggar et al. (1990)
inorganic P (mg kg^{-1})	243	181	Bowman and Moir (1993); Dick and
Organic P (mg kg^{-1})	401	216	Tabatabai (1977a); Turner et al. (2005)
Sulphate sulphur (µg g^{-1})	3	5	Watkinson and Kear (1994)
Organic matter (% w w^{-1})	5.1	7.9	Blackmore et al. (1987)
Total N (% w w^{-1})	0.27	0.30	(Dumas combustion method using an
Total C (% w w^{-1})	2.96	4.60	Elementar Vario Max Cube Analyser)
CEC (meq 100 g^{-1})	11	16	Brown (1943)
Ca (meq 100 g^{-1})	3.4	1.2	Rayment and Higginson (1992)
Mg (meq 100 g^{-1})	0.65	0.52	
K (meq 100 g^{-1})	0.30	0.33	
Na (meq 100 g^{-1})	< 0.02	0.07	
Base saturation (%)	38.3	12.9	
Particle-Size distribution (%)			ISSS Classification
Clay (0.05–2 µm)	3	6	
Sand (20–2000 µm)	50	40	
Silt (2–20 µm)	47	54	

ISSS International Society of Soil Science

The soils in the rhizoboxes were watered and incubated for a week to allow the dissolution of lime before sowing. Blue lupin (*Lupinus angustifolius*) seeds were germinated on moist tissue paper for 48 hours and thereafter one seedling was planted in each rhizobox (Plate 5.1b). The rhizoboxes were distributed randomly in a climate chamber and kept at a controlled temperature (24 °C day/16 °C night) and a daily photoperiod of 14 h with a light density of 300 µmolm^{-2} s^{-1} (Plate 5.1a). During the growth period, the rhizoboxes were kept inclined at an angle of 45–50° so that the roots grew along the detachable cover of the rhizobox. The rhizoboxes were covered with aluminium foil to limit photochemical reduction phenomena in the rhizosphere and biofilm formation on the front plate (Plate 5.1c). A layer of 10-µm thick polycarbonate membrane (0.2 µm pore size, GVS Group, Sanford, USA) was placed between the soil and detachable cover to protect the soil and roots from physical damage during the rhizobox opening. Watering ports in one of the walls of the rhizoboxes allowed uniform irrigation during the plant growth. Soil moisture was maintained at 70% of field capacity (FC) by watering rhizoboxes to a specific weight with tap water every two days. Four weeks after planting, regions of interest (ROIs) were identified in each rhizosphere: these were defined as the areas where lateral roots are clearly visible, partially buried in the soil and positioned at the same level as soil surface without being overlapped. The distribution of labile P and phosphatase activity was then

determined across the ROIs of the separate plant rhizospheres using DGT and zymography, respectively (see below). A week later, the shoots from each rhizobox were harvested and oven-dried at 65 °C for 48 h to estimate aboveground biomass. Shoot samples underwent acid digestion (Nitric acid (HNO_3 69%)-Hydrogen Peroxide (H_2O_2 30%), 1:1 v/v) using a microwave digester (CEM MARS XpressTM, CEM Corp. USA) (NIST, 1995). The digest solution was analyzed for total P using an Inductively Coupled Plasma Optical Emission Spectrophotometer (ICP-OES: Varian 720-ES ICP-OES, Varian, Melbourne, Australia). Shoot P uptake was calculated as the product of P concentration and shoot dry weight.

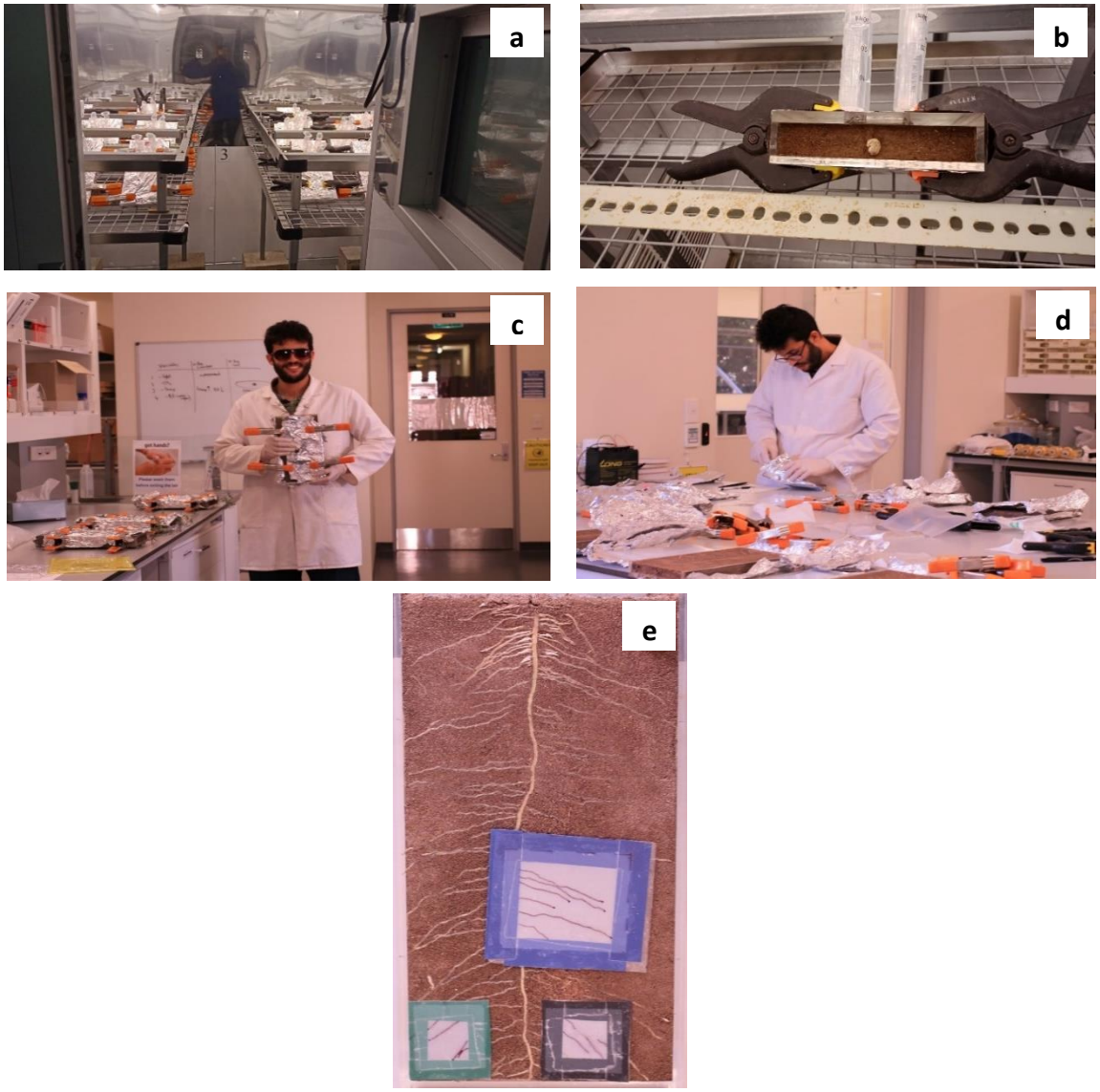


Plate 5.1 Climate chamber (a), transplantation of blue lupin seedling into the rhizobox (b), rhizoboxes transfered into the laboratory for DGT and zymography manipulation (c), zymography filters harvesting after deployment (d) and soil-root interface with DGT assembly deployed (e).

5.2.2 Organic anions and root morphology measurements

At harvest, rhizosphere soil adjacent to the visible lateral roots axes was collected. The sampling targeted the soil 10–20 mm from the root tip and within 2–4 mm from the root surface. The roots where the DGT was deployed were excluded. Bulk soil was sampled 30–50 mm away from roots. The small amount of the collected rhizosphere soil per rhizobox (< 2 g) was used for organic anions extraction (see below). Subsequently, the roots were carefully lifted out of the soil and gently shaken to remove the loosely adhering soil around the roots and discarded, the soil adhering around the remaining roots was carefully sampled using a soft brush and defined as "rhizosphere soil" (Wang et al., 2016). The rhizosphere and bulk soils collected in this step were used to measure pH and exchangeable Al.

The roots were then washed free of soil and scanned with a CanoScan LiDE 210 scanner (Canon, Japan). Subsequently, the roots were oven-dried at 65 °C for 48 h to estimate belowground biomass. Root morphological traits, such as total root length, root surface area and average root diameter were acquired from the scanned root images in WinRHIZO regular V. 2009 software (Regent Instruments Inc., Quebec, Canada). The specific root length (SRL) was calculated based on the root length per unit root dry weight (m g^{-1}). The root length measurements were partitioned into five diameter classes: < 0.5, 0.5–1, 1–1.5, 1.5–2, and > 2 mm. The relative diameter class length (rDCL) = DCL/total root length, was computed for each of the five diameter classes (yielding a proportion of root length to normalize disparity between plant sizes).

Organic anions were determined for both rhizosphere and bulk soils according to Mimmo et al. (2008) with some modifications. Specifically, the air-dried soils were extracted with 25 mM KH_2PO_4 (4 h, pH 2.35 adjusted with H_3PO_4) using 1:5 (w:v) ratio, followed by filtration using a cellulose acetate syringe filter (pore size 0.45 µm, filter diameter: 28 mm). A subsample (100 µL) of the supernatant was analyzed using High-Performance Liquid Chromatography (Shimadzu Corporation, Kyoto, Japan) as described by Shi et al. (2011). The analytical standards were prepared by dissolving known amounts of L-malic acid, malonic acid, citric acid, shikimic acid, succinic acid, pyruvic acid, DL-lactic acid, acetic acid, and fumaric acid (all from Sigma-Aldrich, UK) in 25 mM KH_2PO_4 (pH 2.35) matrix solution. The HPLC data was processed using Lab solutions LCMS software (Shimadzu Corporation, Japan).

5.2.3 Soil zymography

The visualization of acid phosphatase activity in the soil was conducted using direct zymography (Sanaullah et al., 2016). Nylon membrane filters (0.45 µm pore size, 145 mm diameter, Pall Corporation, Michigan, USA) were soaked for 10 mins in an artificial chemical phosphate substrate: 4-methyllumbelliferyl phosphate disodium salt (4-MUP, Sigma Aldrich, UK) dissolved in a modified

universal buffer (MUB, pH 6.5) to a concentration of 12 mM and subsequently oven-dried at 30 °C for 10 min. Phosphate substrate in the membranes diffuses into the soil and is enzymatically hydrolyzed, producing a fluorescent substrate: 4-methyllumbelliferone (4-MU), which then diffuses back to the membrane (Razavi et al., 2019). Under ultraviolet (UV) light, the released fluorescent substrate can be visualized. The rhizoboxes were opened from the detachable cover and the saturated membranes were deployed directly to the soil surface for 1 h, during which they were covered with aluminium foil. After incubation, the membranes were carefully lifted off the soil surface and any attached soil particles were gently removed using a soft brush. The membranes were oven-dried for 4 min at 30 °C and placed under UV light (365 nm) in a UVP DigiDoc-It imaging system (UVP, Upland, California, USA) comprised of a lightweight hood with UV blocking viewpoint and a UV transilluminator. Images were taken using Canon Powershot G7 10 MP digital camera equipped with a 6x image-stabilized optical zoom (Canon, Tokyo, Japan) which was connected to a computer and controlled using a capture software (Doc-ItMLS version 6.3.3, UVP, California, USA). The camera settings and the conditions of the imaging were the same for all rhizoboxes.

A calibration line (Appendix D: Figure D.1) was prepared as described by Giles et al. (2018). The nylon membranes were cut into strips (4 cm^2, n = 3) and soaked in a known concentration of 4-MU (0, 35, 70, 135, 200, 400, 600 and 800 µ*M*). The 4-MU substrate was dissolved in 100 µL dimethyl sulfoxide (DMSO) and diluted with universal buffer (MUB, pH 10) to the desired concentration. The amount of 4-MU per unit area was calculated from the volume of solution taken up by the membrane and its size, then normalized by the incubation time (1 h). All measurements of phosphatase activity in 2D images are presented in units of pmol mm^{-2} h^{-1}.

5.2.4 DGT imaging

Chemical imaging of labile P was conducted using the DGT technique. Zirconium oxide (ZrOH) precipitated in a hydrogel was prepared according Guan et al. (2015) to serve as the DGT binding gel. This gel was chosen for its high binding capacity for phosphate and neutral colour (Ding et al., 2013; Ding et al., 2010; Guan et al., 2015). Each DGT binding gel sheet (approx. size: 4 × 5 cm) was mounted under a layer of 10-µm-thick nuclepore membrane (0.2 µm pore size, Nuclepore Track-Etched Membrane, Whatman, UK) and onto a pre-cleaned acetate sheet using vinyl electrical tape, which also sealed the four edges of the gel-mebrane assembly. The detailed description of the DGT assembly (diffusion layer + ZrOH gel + acetate) set up is given in the Appendix D.

When the deployment of zymography membranes was completed, the soil moisture increased to~ 80-85% FC and the rhizoboxes were put back in the climate chamber for 24 h, after which the rhizoboxes were brought into the lab, laid flat and opened. Water was added to the region of interest (ROI) within

the area previously targeted for zymography until a thin film of water was visible at the soil surface. The DGT gel assembly was deployed for 24 h under slight pressure to ensure thorough contact with the soil underneath. After deployment, DGT gel was harvested by gently removing the assembly from the soil surface, rinsing with a jet of high purity water and then carefully cutting the nuclepore membrane along all four edges using a PTFE razor blade. The gels were immediately subjected to a heat treatment in hot water (85 °C) for five days to further bind the P onto the precipitated ZrOH. The gels were then stained using the molybdenum blue method as described by Ding et al. (2013) and finally scanned using CanoScan LiDE 210 scanner at a resolution of 600 dpi, corresponding to a pixel size of 42×42 µm. The mass of P accumulated on each DGT gel was calculated using a seven-point calibration curve that established the relationship between greyscale intensity and mass of P accumulated in per area of gel (calibration range: *4.4 – 1103.9* ng cm^{-2}) (see Appendix D, Figure D.2). The mass of P bound on the gel was used to calculate the average diffusive flux of P into the DGT gel during the deployment (forthwith, "DGT-P flux").

5.2.5 Image processing and analysis

The images were processed and analysed using ImageJ (https://imagej.net.). Zymograms and DGT images were scaled (resolution: 65 µm and 42 µm, respectively) and aligned with the help of root photographs as described by Hummel et al. (2021). The P images were evaluated firstly by visual inspection: images where no changes in P fluxes in the rhizosphere compared to the bulk soil were visible and/or with air-bubbles (formed during DGT assembly deployment or preparation) were discarded, then successful chemical images where single lateral roots are entirely visible and did not overlap with others were selected for further analysis.

For each rhizobox replicate, phosphatase activities and P fluxes in the bulk soils were determined in ten and five 3 × 3 mm areas respectively, away from any obvious root structures. Phosphatase activity and P flux next to the each of the previously identified roots in each replicate were measured using a single 1 × 6 mm profile (phosphatase activity: 16 × 93 pixels; DGT-P: 24 × 143 pixels). The profiles were established across the roots between 0.5 and 1.5 cm from root tip in Mt Grand soil and between 0.5 and 1 cm in Millers Flat soil for both phosphatase activity and P flux. The profiles of acid phosphatase activity and P flux combined were carried out successfully for three different roots in Mt Grand soil for each soil pH separately. In Millers Flat soil five root were successfully analyzed at pH 4.7, while at pH 6.3 only one single root was successful. At the root tips, the profiles were established for DGT-P fluxes only using three different roots in Mt Grand soil for each soil pH, while in Millers Flat soil five roots were used at pH 4.7 and three roots at pH 6.3. The root images were straightened in ImageJ to assist with the profile analysis. The zone of increased acid phosphatase activity was defined as the

perpendicular distance from the root axis where acid phosphatase activity was at least 30% higher than the activity in the bulk soil (Ma et al., 2018a). The zone of P mobilization was defined as the perpendicular distance from the root axis to where DGT-P flux was at least one standard deviation above the mean flux in the bulk soil, while the zone of P depletion was defined as two standard deviations below the mean flux in the bulk soil. The center of the root was set as the mid-point between the two visible root limits. The root diameter was measured manually in ImageJ at the locations where the profiles were taken.

5.2.6 Statistical analysis

A two-sample t-test was used for identifying the significant differences between the effects of the two pH in each soil. One-way ANOVA with Tukey's test as post-hoc test ($p < 0.05$) was carried out to identify the differences between the combinations: soil type-pH using Minitab® statistical software version 18 (Minitab, Inc., State College, Pennsylvania, USA). Multiple linear regression (backward elimination, $\alpha < 0.05$) was used to determine the most important variables (total organic anions, all root traits including fine root and thick root lengths, bulk soil DGT-P, rhizosphere pH and exchangeable Al) that contributed to shoot P uptake regardless of soil type and pH (see Appendix D, Table D.2 and Equation D.1). The variables were standardized by subtracting the mean then dividing by the standard deviation. A correlation analysis (Pearson) was used to examine relationships between the variables. Significance was assumed at the 5% level throughout, unless stated otherwise.

5.3 Results

5.3.1 Root morphology, plant growth and P uptake

Total root length, root surface area and root biomass were higher in the Mt Grand soil than in the Millers Flat soil at both pHs ($p < 0.001$) (Table 5.2). In Mt Grand soil, the soil pH increased from 5.3 to 6.3 decreased root total length, root surface area and root DM yield by 12, 6.6 and 20.6%, respectively, but the differences were not significant. In Millers Flat soil a significant increase was observed in root total length, surface area and average diameter when pH increased from 4.7 to 6.3, but root yield remained unchanged. The specific root length (SRL) was higher in Millers Flat soil compared to Mt Grand ($p < 0.05$). A decrease in SRL was observed in Millers Flat soil at pH 6.3 compared to pH 4.7 ($p < 0.05$), whereas in Mt Grand soil a slight but not significant increase in SRL with pH was observed.

Table 5.2 Root morphological traits, dry matter (DM) yields and shoot P uptake of *Lupinus angustifolius* grown in two contrasting soils (different acidities and P fertilities): Mt Grand and Millers Flat, at two different pHs per soil (Mt Grand: pH 5.3 and 6.3, Millers Flat: pH 4.7 and 6.3). Different letters indicate significant differences within each row ($p < 0.05$ after Tukey's test).

	Mt Grand		Millers Flat	
	pH 5.3	pH 6.3	pH 4.7	pH 6.3
Total root length (cm)	1182.6 ± 82.9 a	1040.3 ± 112 a	334.1 ± 44.3 b	491.5 ± 95.2b
Root surface area (cm^2)	372.4 ± 14.2 a	347.9 ± 22.0 a	94.9 ± 16.0 c	185.3 ± 28.8 b
Average root diameter (mm)	1.0 ± 0.06ab	1.1 ± 0.06ab	0.9 ± 0.04b	1.2 ± 0.04a
Specific root length (m g^{-1} root DM)	1.2 ± 0.09 b	1.4 ± 0.10 b	2.3 ± 0.11 a	2.0 ± 0.29 ab
Root yield (g DM $rhizobox^{-1}$)	0.97 ± 0.05 a	0.77 ± 0.05 a	0.15 ± 0.07 b	0.28 ± 0.03 b
Shoot yield (g DM $rhizobox^{-1}$)	1.88 ± 0.07 a	1.54 ± 0.11 a	0.45 ± 0.15 b	0.51 ± 0.28 b
Shoot P concentration (g kg^{-1} DM)	1.47 ± 0.04 a	1.06 ± 0.16 b	0.50 ± 0.04 c	0.59 ± 0.04 c
Shoot P uptake (mg $rhizobox^{-1}$)	2.75 ± 0.12 a	1.60 ± 0.21 b	0.26 ± 0.06 c	0.26 ± 0.04 c

For each soil-pH combination, the percentage of total root length that belongs to each root diameter class– the relative diameter class length (rDCL)– is shown in Figure 5.1. At the initial soil pH (pH 5.3 for Mt Grand and pH 4.7 for Millers Flat), the fine roots (Ø ≤ 0.5 mm) accounted for almost 38% and 41% of total root length, respectively. Increasing soil pH to 6.3 reduced the share of fine root length in both soils, yet this reduction was more pronounced ($p < 0.01$) in Millers Flat soil (-18% versus - 8% in Mt Grand). Conversely, the share of thick roots (Ø > 2 mm) length increased slightly by 2% and 4% with pH increase in Mt Grand and Millers Flat soils, respectively.

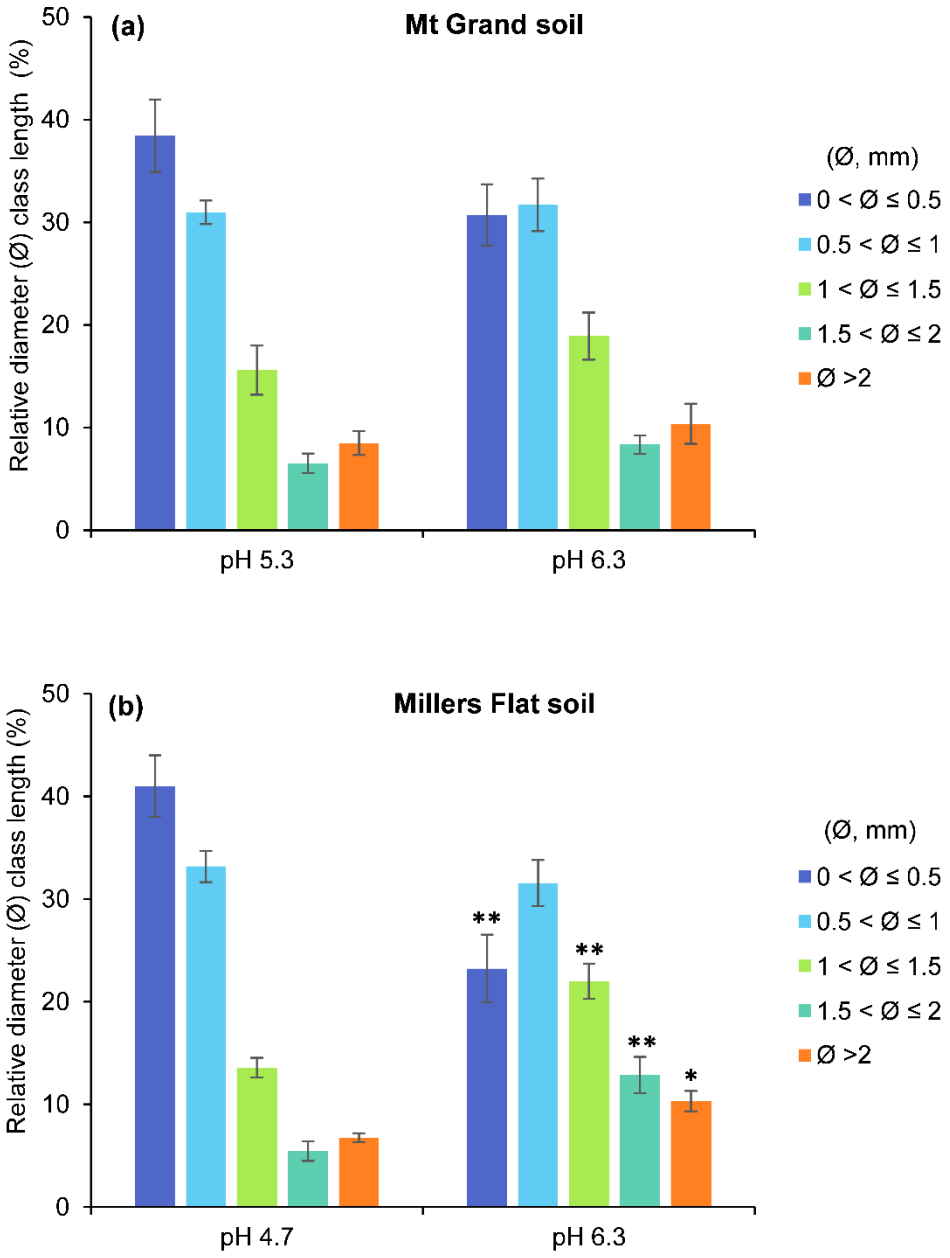


Figure 5.1 Mean relative diameter class length (rDCL(%)= DCL/total root length) of *Lupinus angustifolius* grown in two contrasting soils (different acidities and P fertilities): Mt Grand and Millers Flat, at two different pHs per soil (Mt Grand: pH 5.3 and 6.3, Millers Flat: pH 4.7 and 6.3). Bars: means of four replicates (± SE). Asterisks: significant differences (* $p < 0.05$, ** $p < 0.01$ after two-samples t-test) between the two soil pHs per diameter class.

The shoot yield in Mt Grand soil was higher compared to Millers Flat ($p < 0.001$). Similarly, shoot P concentration and P uptake were significantly higher in Mt Grand compared to Millers Flat (Table 5.2). The shoot yield was not affected ($p > 0.05$) by soil pH change in either soil. In contrast, shoot P concentration and P uptake were higher at pH 5.3 compared to pH 6.3 in Mt Grand soil ($p < 0.05$ and $p < 0.01$), while no difference was found in Millers Flat between the pH levels. Regardless of soil type

and pH, shoot P concentration and P uptake were significantly correlated with all root traits (total root length, fine root length, thick root length, surface area, root DM yield and total organic anions) except SRL and average root diameter (Appendix D: Figure D.3). Fine root length showed the strongest positive correlation (Shoot P concentration: r = 0.89, p < 0.001; Shoot P uptake: r = 0.91, p <0.001; Appendix D: Figure D.3). Also, a strong positive polynomial relationship was found between both shoot P uptake and P concentrations, and fine root length (Figure 5.2). Additionally, a decrease in other shoot nutrient (magnesium (Mg), manganese (Mn), Zinc (Zn) etc.,) concentrations has been observed in both soils with soil pH increase (Appendix D: Table D.1)

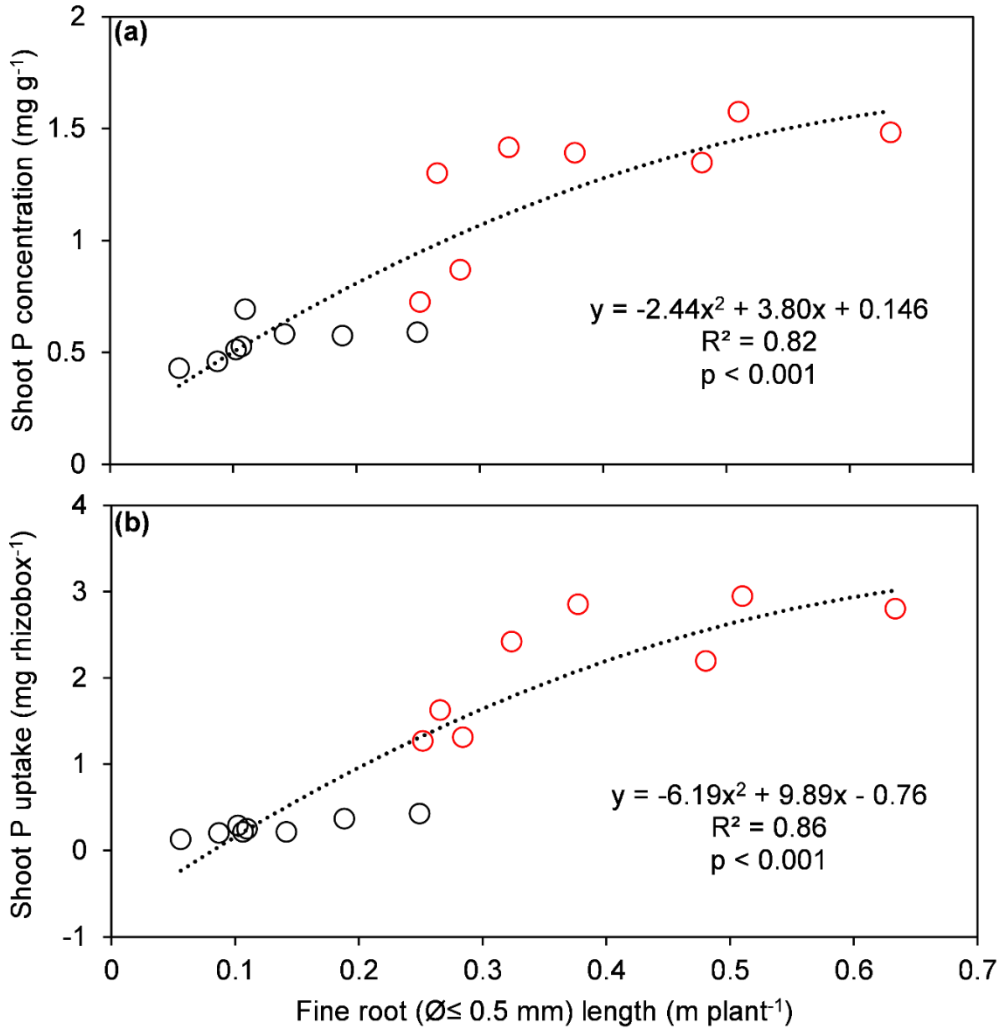


Figure 5.2 Relationship between both (a) shoot P concentration (mg g^{-1}) and (b) P uptake (mg $rhizobox^{-1}$), and fine root (Ø ≤ 0.5 mm) length (m $plant^{-1}$) across soil type×pH combinations. Red circles represent Mt Grand soil and black circles represent Millers Flat soil.

5.3.2 Soil pH, soil exchangeable Al and organic anions exudation

The rhizosphere pH decreased ($p < 0.001$) by 0.95 and 0.38 units compared to the bulk soil at pH 5.3 and pH 4.7 in Mt Grand and Millers Flat soils, respectively (Appendix D: Figure D.4). However, at pH 6.3, no difference was found between rhizosphere and bulk soils regardless of soil type. The exchangeable Al measured in the rhizosphere was between 0.2 and 3.5 $cmol_c\ kg^{-1}$ higher compared to the bulk soil regardless of soil type and pH ($p < 0.05$), except at pH 4.7 in Millers Flat soil, where no difference was observed between the bulk and rhizosphere soils (Figure 5.3). Average soil exchangeable Al across soil pHs was 78% and 89% higher in Millers Flat bulk and rhizosphere soils, respectively compared to Mt Grand soils. Soil pH increase from the initial value to pH 6.3 decreased exchangeable Al in the bulk and rhizosphere soils regardless of soil type.

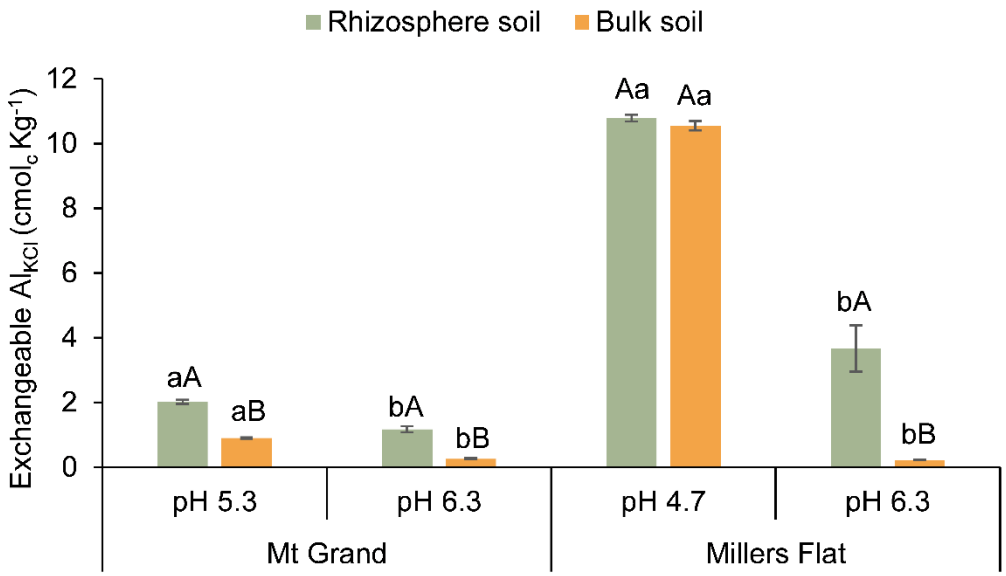


Figure 5.3 Bulk and rhizosphere exchangeable Al concentrations ($cmol_c\ kg^{-1}$)of two contrasting soils (different acidities and P fertilities: Mt Grand and Millers Flat) after 5 weeks growth of *Lupinus angustifolius*. Bars show the mean (± SE) of 4 replicates. Lower-case letters indicate significant differences ($p < 0.05$ after two-sample t-test) between the two pHs within each soil (Mt Grand: pH 5.3 and 6.3, Millers Flat: pH 4.7 and 6.3) for bulk and rhizosphere soils separately. Capital letters indicate significant differences ($p < 0.05$ after two-sample t-test) between bulk and rhizosphere soils per pH condition.

Citrate and malate were the dominant organic anions detected in Mt Grand soil. However, in Millers Flat soil citrate was the dominant anion, while malate was not detected. Lactate, malonate, and fumarate were also detected at low concentrations in both soils. Further, maleate was detected in Millers Flat soil only and pyruvate in Mt Grand soil only. Total organic anions extracted in the rhizosphere soil were greater ($p < 0.01$) in Mt Grand soil compared with Millers Flat soil regardless of pH (Figure 5.4).

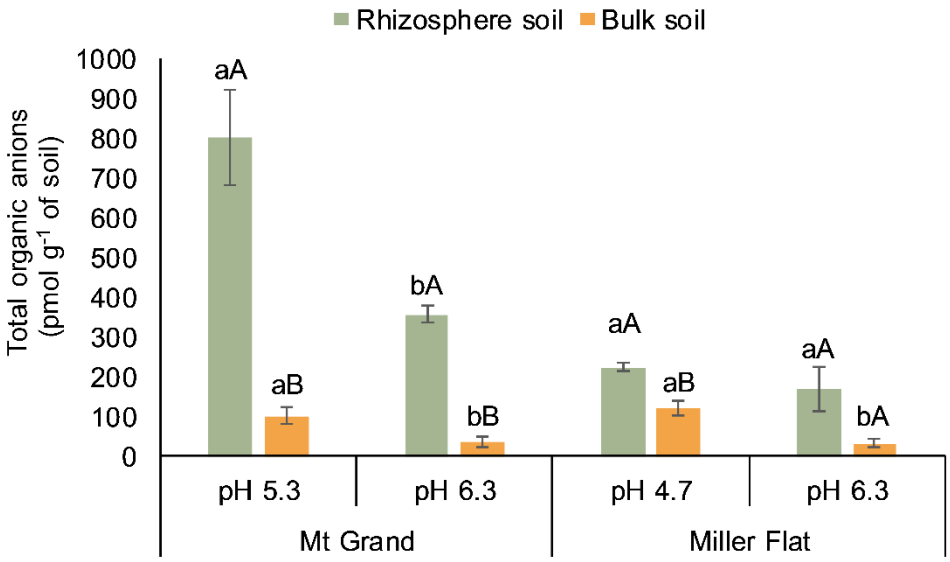


Figure 5.4 Total organic anions (pmol g^{-1} of soil) extracted in the bulk and rhizosphere soils of *Lupinus angustifolius* grown in two contrasting soils (different acidities and P fertilities): Mt Grand and Millers Flat, at two different pHs per soil (Mt Grand: pH 5.3 and 6.3, Millers Flat: pH 4.7 and 6.3) for 5 weeks. Bars show the mean (± SE) of 4 replicates. Lower-case letters indicate significant differences ($p < 0.05$ after two-sample t-test) between the two pHs within each soil for bulk and rhizosphere soils separately. Capital letters indicate significant differences ($p < 0.05$ after two-sample t-test) between bulk and rhizosphere soils in each pH condition.

The total organic anions concentrations in the rhizosphere were always higher than in the bulk soil regardless of soil type and pH ($p < 0.05$). At pH 6.3, the release of organic anions decreased in both bulk and rhizosphere soils when compared to the initial pH, regardless of soil type. Across all soil type-pH combinations, total organic anions in the rhizosphere were strongly correlated with all root morphological traits except root average diameter (Appendix D: Figure D.3). Also, a strong positive polynomial relationship was found between both shoot P uptake and P concentrations, and total organic anions in the rhizosphere (Figure 5.5).

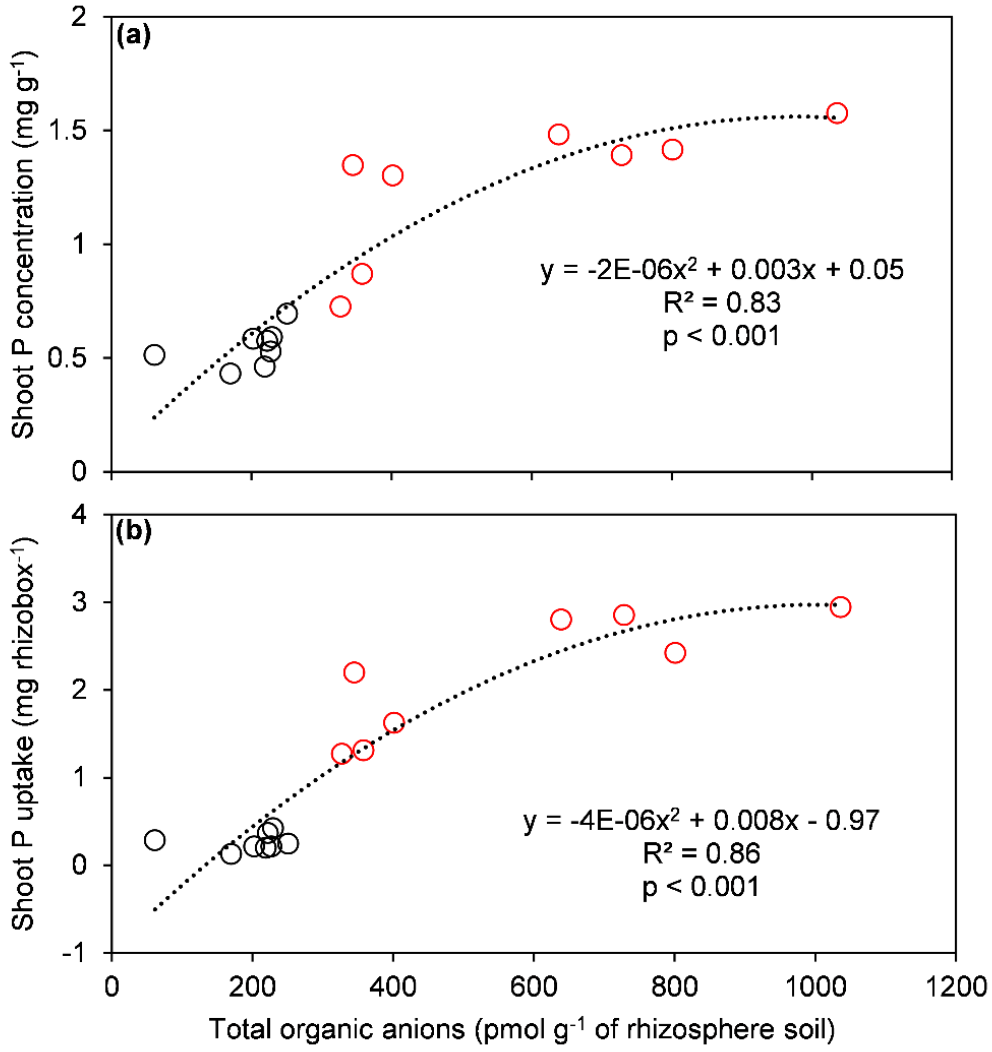


Figure 5.5 Relationship between total organic anions (pmol g^{-1}) exuded in the rhizosphere and both shoot P concentration (a) and P uptake (b) across soil type-pH combinations. . Red circles represent Mt Grand soil and black circles represent Millers Flat soil.

5.3.3 Acid phosphatase activity and P flux distribution in the rhizosphere

Acid phosphatase activity in the bulk soil was not affected by soil pH change in Mt Grand soil (p > 0.05), whereas a significant increase was observed in Millers Flat soil (Appendix D: Figure D.5a). In Mt Grand soil, P flux increased in the bulk soil with soil pH increase (p < 0.001) . Contrarily, a decrease was observed in Millers flat soil with soil pH increase (p < 0.001) (Appendix D: Figure D.5b). The average acid phosphatase activity in the bulk soil, across pH levels, was higher (p < 0.001) in Millers flat soil (19.20 ± 0.48 pmol mm^{-2} h^{-1}; mean ±SE) compared to Mt grand soil (7.40 ± 0.20). In contrast, the average P flux (9.96 ± 0.44 pg cm^{-2} s^{-1}) was greater (p < 0.001) in Mt Grand soil across pH levels compared to Millers Flat (7.32 ± 0.31).

Profiles of acid phosphatase and DGT-P fluxes from the root center to the surrounding soil (Figure 5.6) showed that acid phosphatase activity was co-occuring with P mobilization in Mt grand soil (Figure 5.6a and b; Figure 5.7). However, in Millers Flat soil the higher acid phosphatase activity was co-localised with P depletion (Figure 5.6c and d; Figure 5.7). The rhizosphere extent of acid phosphatase in Mt grand soil was > 2 mm from the root center at pH 5.3, while at pH 6.3 was only 0.83 mm. Similarly, in Millers Flat soil the rhizosphere extent of acid phosphatase decreased from 1.90 mm at pH 4.7 to 1.35 mm at pH 6.3. Phosphorus mobilization extent in Mt Grand soil was 0.39 mm from the root center at pH 5.3 and 0.59 mm at pH 6.3. Furthermore, the P depletion extent in Millers Flat soil was 0.97 mm from root center at pH 4.7 and 0.42 mm only at pH 6.3. Similar to the bulk soil, the DGT-P flux in the rhizosphere (Figures 5.6 and 5.8) was higher at pH 6.3 compared to pH 5.3 in Mt Grand soil, whereas in Millers Flat, higher fluxes were observed at pH 4.7 compared to 6.3.

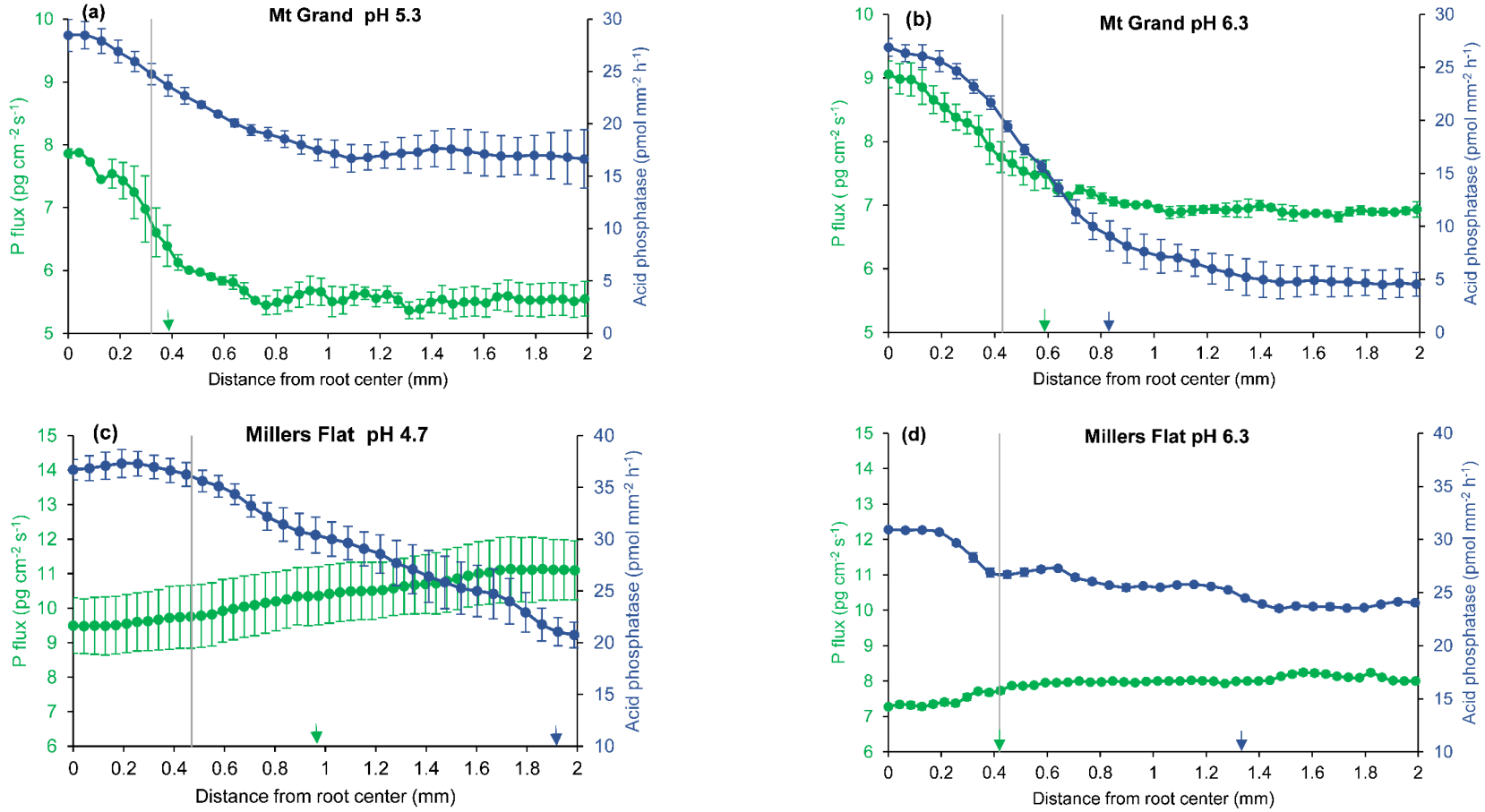


Figure 5.6 Profiles of acid phosphatase activity (pmol mm^{-2} h^{-1}) and P fluxes (pg cm^{-2} s^{-1}) distribution as a function of the distance from the root center towards the surrounding soil: (a) Mt Grand soil at pH 5.3, (b) Mt Grand soil at pH 6.3, (c) Millers flat soil at pH 4.7 and (d) Millers Flat soil at pH 6.3. Vertical grey lines: the position of the average root radius. Small vertical arrows show the zones of acid phosphatase activity (blue) and P depletion/mobilization(green). Error bars indicate the standard errors of acid phosphatase activity and P fluxes for three different roots in Mt Grand soil, while in Millers flat soil five roots were used at pH 4.7 and a single root at pH 6.3. Error bars in (d) indicate the standard errors in 1 mm thick profile (16 pixels).

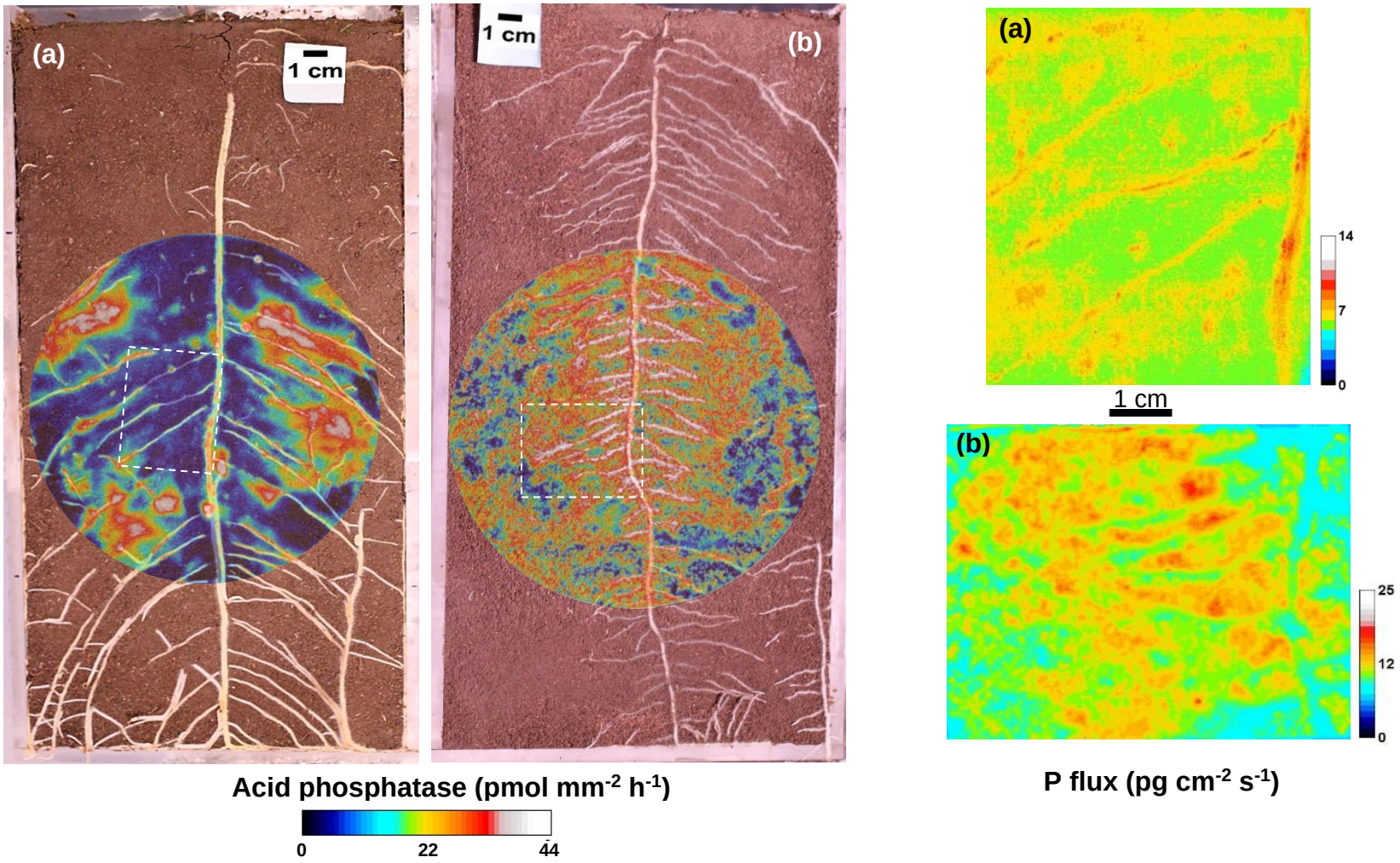


Figure 5.7 Examples of contrasted spatial distribution patterns of labile P in the rhizosphere of *Lupinus angustifolius* (right): (a) P mobilization in Mt Grand soil pH 5.3 versus (b) P depletion in Millers Flat soil pH 4.7. Examples of roots grown in rhizoboxes and the spatial distribution of acid phosphatase activity (left), the zymograms displayed as a transparent overlay, the white dashed rectangles indicate DGT region of interest.

Phosphorus fluxes into the DGT at the root tips in both soils were greater than the corresponding bulk soil at pH 6.3, while at the lower pH it was difficult to discern a meaningful difference (Figure 5.8a). For instance, in Mt Grand soil, at pH 6.3 the DGT-P flux was 24% higher at the root tips center compared to 2 mm distance from it, while at pH 5.3, this difference was only 7%. On the other hand, in Millers Flat soil, DGT-P flux at root tips center behaved differently between the two investigated soil pHs (Figure 5.8b); it increased by 18% and decreased by 8% at pH 6.3 and 4.7, respectively at the root tips center compared to 2 mm distance from it.

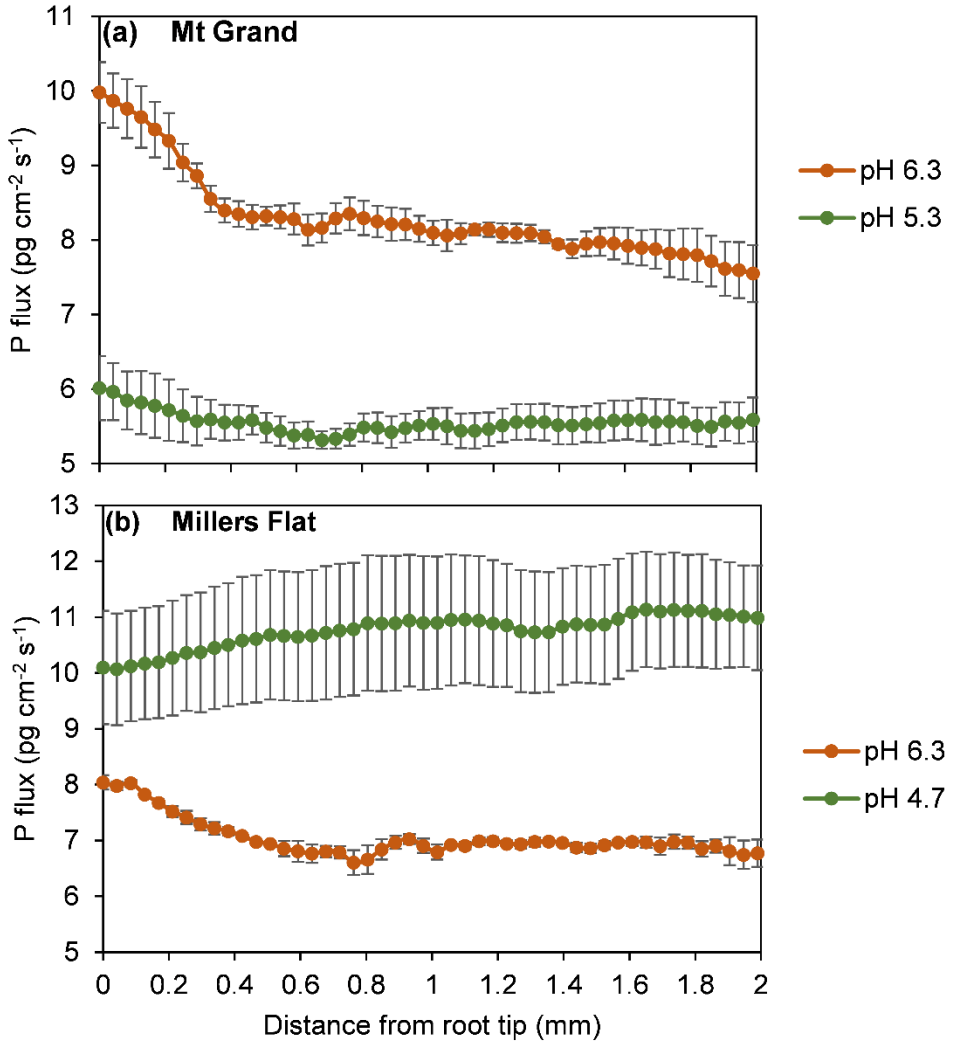


Figure 5.8 DGT-P fluxes as a function of distance from the centre of the root tip : (a) Mt Grand soil pH 5.3 and 6.3, and (b) Millers Flat soil pH 4.7 and 6.3. Error bars indicate the standard errors of P fluxes for three different roots (n = 3) in Mt Grand soil. Three roots were also used in Millers Flat soil pH 6.3, while five roots (n = 5) were used in Millers Flat soil pH 4.7.

5.4 Discussion

5.4.1 Root morphological and physiological adaptations to soil pH increase

Plants have evolved several strategies to acquire P under low P conditions (Lambers et al., 2006), including root-morphological changes (Hammond et al., 2004) and the exudation of low molecular weight organic anions (Pearse et al., 2006). In our study, shoot P concentrations were below the optimum range (2-3 g kg^{-1}) for lupins (Li et al., 2008; Müller et al., 2015), confirming soil P deficiency conditions. Exudation of organic anions by blue lupin was likely partly induced by P deficiency, as shown by the strong correlation of shoot P concentration and P uptake with total organic anions in the rhizosphere as well as by the resulting positive polynomial relationship. For instance, in Mt Grand soil, the significantly higher DGT-P (available P) in the bulk soil at pH 6.3 compared to pH 5.3 has coincided with a significant decrease in total organic anions exudation. However, in Millers Flat soil, despite the significantly decreased DGT-P in the bulk soil following soil pH increase, shoot P concentration, P uptake and total organic anions did not change. This result implies that factors other than P and pH were probably controlling P nutrition and plant growth in this soil, which is evidenced by the significant differences in shoot P uptake and yield observed between this soil and Mt Grand soil at the same pH of 6.3 with a comparatively similar DGT-P in the bulk soil.

Exchangeable Al concentrations in Millers Flat soil were very high, especially in the rhizosphere, exceeding the toxicity threshold (1-2 cmol Kg^{-1}) suggested by Edmeades et al. (1983) for legumes and 3 mg kg^{-1} by Moir et al. (2016). Aluminium toxicity is known to affect uptake and translocation of water and nutrients, altering plant metabolisms and growth (Singh et al., 2017). Therefore, higher exchangeable Al concentrations in Millers Flat soil likely inhibited root growth and impeded P mobility across the soil-root interface (Gessa et al., 2005) hindering P uptake by blue lupin. However, although exchangeable Al in the rhizosphere soil was significantly reduced at pH 6.3 compared to pH 4.7, shoot P uptake and plant growth did not improve. This could be explained by the fact that the high lime rate applied to this soil to raise pH to 6.3 decreased nutrient availability (Barman et al., 2014; Scanlan et al., 2017) as reflected by the significantly reduced concentrations of Mn, Zn, B, Fe, K and S in blue lupin shoots at pH 6.3 compared to pH 4.7 (Appendix D: Table D.1). Similarly, shoot nutrient (Mn, Zn, Cu, Mg, K and P) concentrations significantly decreased at pH 6.3 compared to pH 5.3 in Mt Grand soil. These results question the suitability of near-neutral soil pH for blue lupin cultivation. The higher uptake of Mn at pH 5.3 in Mt Grand soil is believed to be due to the higher secretion of organic anions. This occurs because the carboxylates mobilize not only soil inorganic and organic P, but also a range of micronutrients, including Mn (Lambers et al., 2015). This is confirmed by the strong correlation found between total organic anions and Mn concentration in blue lupin shoots ($r = 0.91$, $p < 0.001$) across all combinations of soil type-pH. Furthermore, recently Wang and Lambers (2020) underlined leaf Mn concentration as an easily-measurable proxy for carboxylates in the rhizosphere. Thus, low Mn

concentration in lupin shoot at pH 6.3 in our soils, compared to their initial pH, indicates that higher liming rates might have neutralized the solubility effects of organic acids, thus lowering the uptake of P and other elements. This view agrees with Valentinuzzi et al. (2015). Additionally, higher supply of Ca could adversely affect P mobilisation via organic anions exudation, because of "Ca-aided co-adsorption" mechanism described by Duputel et al. (2013).

In addition to organic anions exudation, the higher P acquisition by blue lupin in Mt Grand soil compared to Millers Flat soil could be associated with greater root length and root surface area, which allowed exploration of a larger soil volume. This is supported by the strong correlation found between P uptake and: total root length (r = 0.86, p < 0.001,Appendix D: Figure D.3) as well as root surface area (r = 0.86, p < 0.001, Appendix D: Figure D.3) regardless of soil type and pH. Interestingly, the higher specific root length in Millers Flat soil was not reflected in terms of P uptake in comparison with Mt Grand soil, suggesting that this root trait may not be related to the mechanism of P acquisition. Moreover, the benefits of root-specific length in terms of additional P uptake are still a matter of debate (Robles-Aguilar et al., 2019; Zobel et al., 2007) . We hypothesize that the higher specific root length (SRL) in Millers Flat soil was due to the reduction of root biomass (root DM vs. SRL: r = - 0.87, p < 0.001, Appendix D: Figure D.3) because of low pH and associated aluminium stress. The increase in specific root length of blue lupin at acidic pH was confirmed recently by Robles-Aguilar et al. (2019). As an alternative to the traditional diameter-based root classification where fine roots have most often grouped in a single pool, commonly ≤ 2 mm, we have assigned a smaller diameter cut-off (Ø ≤ 0.5 mm) to explicitly link the contribution of more adsorptive fine roots (McCormack et al., 2015) to P uptake. Fine roots are crucial for water and nutrient uptake (Iversen et al., 2017; Wang et al., 2019). For instance, in maize, fine roots have been proven to be directly linked to P acquisition (Ø≤ 0.2 mm, (Wen et al., 2017)) and (Ø ≤ 0.6 mm, (Zhang et al., 2012)). However, little is known about lupins. Our study was the first to show that, regardless of soil type and pH, blue lupin fine root (Ø ≤ 0.5 mm) length was strongly correlated with shoot P uptake/ P concentration more than any other root trait included in this experiment (Appendix D: Figure D.3, Figure 5.2). Consequently, the lower shoot P uptake/P concentration at pH 6.3 compared to pH 5.3 in Mt Grand soil was possibly due to the reduction of fine root length. Also, the slightly lower total root length and surface area could have contributed. Likewise, the significantly lower fine root length in Millers Flat soil at pH 6.3 compared to pH 4.7 could to some extent explain the unresponsiveness of shoot P uptake/P concentration to soil acidity improvement after liming, because fine roots acquire nutrients better than thick roots (McCormack et al., 2015). By multiple regression analysis, the importance of fine root length in explaining the variability in shoot P uptake was further evidenced (Appendix D: Equation D.1, Table D.2). On the other hand, in a decreasing order of importance, the following root morphological traits: fine root length, total root length, surface area, and thick root length were found to be strongly and positively correlated with

total organic anions concentration in the rhizosphere (Appendix D: Figure D.3). This result confirms and extends previous findings demonstrating multiple coordination and trade-offs among physiological and morphological traits involved in P acquisition in crop species (Honvault et al., 2020). However, further efforts are needed to improve the incomplete knowledge about the relevance of this strategy in blue lupin. In summary, our results demonstrated, for the first time, that root morphological and physiological traits involved in P acquisition by blue lupin were negatively affected at near-neutral pH. This would help explaining the general agronomic recommendation that blue lupins prefer acid soils.

5.4.2 Effect of soil pH change on P availability and phosphatase activity spatial patterns in the rhizosphere

Profiles of DGT-P fluxes next to lateral roots revealed two contrasting patterns of P mobilization between the two investigated soils (Figure 5.6 and 5.7): P depletion in Millers Flat soil versus P mobilization in Mt Grand soil. These contrasting patterns could be because the total organic P content of Mt Grand soil was twice that of Millers Flat soil. Thus, more labile P is expected to be sourced from organic P hydrolysis via phosphatases (Condron et al., 2005). Furthermore, the higher release of organic anions in the rhizosphere of blue lupin in Mt Grand soil compared to Miller Flat soil might have contributed to shaping these patterns, because organic anions allow for displacement of inorganic and organic P from bound or precipitated forms through the chelation of Al^{3+}, Fe^{2+} and Ca^{2+} (Wang and Lambers, 2020). They also constitute a source of carbon for soil microbes (Hütsch et al., 2002). In Mt Grand soil, at pH 5.3, the higher rhizosphere extent of acid phosphatase activity compared to pH 6.3 suggests that more organic P was likely mineralized increasing P availability for the plant, hence explaining the greater shoot P uptake at pH 5.3. However, at pH 6.3, the DGT-P in the rhizosphere was higher compared to pH 5.3, but not reflected in term of P uptake. This could partly be due to the alteration of root morphological and physiological traits following soil pH increase as previously discussed. Moreover, the observed increase in DGT-P at pH 6.3, whether in the rhizosphere soil or the bulk soil, could be attributed to the effect of pH increase on desorption of inorganic P (Barrow et al., 2020b; Penn and Camberato, 2019). Also, the sorption of inorganic P is reduced at higher pH due to a less positively charged soil that repel phosphate anions and thus increase soil solution P (Barrow et al., 2021; Nobile et al., 2020). Additionally, lime amendment has been found to enhance organic P decomposition (Condron and Goh, 1989; Kiflu et al., 2017). Recently, Wan et al. (2020) confirmed that pH directly influences the growth of organic P-mineralizing microbes, thus affecting organic P mineralization.

The higher P mobilization extent in the rhizosphere of blue lupin in Mt Grand soil, at pH 6.3 compared to pH 5.3, can be explained by the root size. Thus, after normalization of P mobilization extent by root radius, P mobilization extents were identical between the two pHs ($p > 0.05$, Appendix D: Table D.3).

Therefore, we suggest that root radius and other traits, such as root hairs should be considered in future DGT imaging studies, especially when comparing crops of different rooting systems. For instance, Ma et al. (2018b) visualized the spatial distribution of enzyme activity in the rhizosphere of various plant species including *Lupinus polyphyllus*. They found that root radius strongly affects phosphatases activity per root surface area. This implies that labile P distribution patterns in the rhizosphere would also be affected by root radius, given that P availability and phosphatase activity are associated.

The combination of zymography and DGT techniques in this study demonstrated the co-localization between P availability and acid phosphatase activity in the rhizosphere of blue lupin. Co-localized P depletion and elevated phosphatase activity in Millers Flat soil agree with a recent study conducted on the same lupin species (Hummel et al., 2021). Depletion of DGT-P along the roots in this soil is in line with previous studies (Kreuzeder et al., 2018; Santner et al., 2012), suggesting that the released P was taken up by the roots or/and rhizosphere microbes. However, the extremely low shoot P concentration/shoot P uptake in Millers Flat soil indicates that other mechanisms possibly contributed to creating such a spatial pattern: the higher P retention in Millers Flat soil (52% versus 20% in Mt Grand soil) due to high Al content had likely affected the solubility of P and its diffusion in the rhizosphere soil resulting in a slow replenishment of the depleted P, thus causing a low utilization of P by plants (Degryse and McLaughlin, 2014; Volf and Rosolem, 2020). Furthermore, the resulting decrease in DGT-P in both rhizosphere and bulk soils when Millers Flat soil pH increased can only be attributed to the lime effect because lime per se can reduce the mobility of P in the soil depending on soil type. For example, Curtin and Syers (2001); Eslamian et al. (2020) demonstrated that lime-induced P retention was due to high Ca supply, through its effect on surface electrostatic potential or by co-adsorbing with phosphate. Also, lime addition likely hydrolyzed the initial exchangeable Al and provided a surface for P adsorption. This hypothesis was confirmed in several studies as reviewed by Penn and Camberato (2019). Therefore, the decreased P depletion extent at pH 6.3 compared to pH 4.7 possibly resulting from restrictive effects of lime on soil P solubility and bioavailability.

The observed decrease, although small, in P flux at the root tips at pH 4.7 compared with an increase at pH 6.3 in Millers Flat soil could either be (1) because Al toxicity effects on root tips were alleviated after liming, external supply of Ca has also been found to ameliorate Al toxicity (Rengel, 1992), (2) P deficiency effect because P availability was restricted at pH 6.3. Both (1) and (2) might have triggered the localized release of protons and citrate resulting in enhanced solubilization of the adsorbed or/and precipitated P (McKay Fletcher et al., 2020; Siao et al., 2020). Likewise, P flux at root tips in Mt Grand soil was more pronounced at pH 6.3 than pH 5.3. Again, this could be due to the role of lime in reducing toxic Al concentrations. Moreover, P desorption following liming likely facilitated root access to certain P forms. In summary, our results demonstrated that the lower uptake of P at pH 6.3 compared to pH

5.3, in Mt Grand soil, is not necessarily due to low P availability in the rhizosphere, but rather seemed to be affected by the alteration of root traits, in particular organic anions and fine root length. Also, the limitation of other nutrients at higher pH could have partly contributed to this, because several studies have proven that P interacts synergistically with other elements to modulate P absorption by plants (de Souza Cardoso et al., 2020; Zhao et al., 2020).

5.5 Conclusion

For the first time, we combined zymography and colorimetric DGT techniques, and analysis of root traits to understand how lime-induced pH elevation affects P acquisition by blue lupin grown in two contrasting pasture soils (different acidities and P fertilities). We found that (1) the spatial distribution patterns of P availability and acid phosphatase activity in the rhizosphere of blue lupin were mainly driven by soil properties, (2) shoot P uptake was strongly correlated with fine root length and total organic anions in the rhizosphere, regardless of soil type and pH (3) liming reduced the length of fine roots, the exudation of organic anions in the rhizosphere and the rhizosphere extent of phosphatase activity, and (4) across all combinations of soil type × pH, variation in shoot P uptake/P concentration were mostly explained by exchangeable Al concentration in the rhizosphere, rhizosphere pH and fine root length. Taken together, our results demonstrate that increasing soil pH to near neutral (pH 6.3) using lime does not improve P acquisition by blue lupin. Also, the liming effects on P availability are driven by soil properties. These findings challenge the classical view that legume P availability is maximized at near-neutral soil pH, but may instead be species-specific, perhaps depending on the adaptation of individual species to low P environments. The results and information generated in this study are valuable for future effective utilization of lime and improving the productivity of blue lupin in acid P deficient soils.

Chapter 6

Surface Liming Effect on Phosphorus Biochemistry and Dynamics in Extensive Acid Grassland

(Published paper)

6.1 Introduction

Liming is a commonly used agricultural practice to increase soil pH and thus the availability of nutrients such as phosphorus (P) for pastures (Holland et al., 2018). Elevated soil pH increases the available inorganic P (P_i) by desorption reactions, and by decreasing P sorption because the soil adsorbing surfaces become more negative as pH increase and thus less attractive to P species (Barrow, 2017; Barrow et al., 2020b), mainly divalent phosphate ions (HPO_4^{2-}) (Barrow, 1984). Lime-induced pH elevation also reported to have similar effects on soil P sorption/desorption (Barrow, 1984; Haynes, 1982). Nonetheless, reports of liming effects on soil P availability have been inconsistent (Curtin and Syers, 2001; Haynes, 1982; Margenot et al., 2018; Mkhonza et al., 2020). Also, most of the cited studies were based on laboratory and/or controlled experiments which may not be relevant for open fields systems where liming effects may be influenced by depth and environmental factors (rainfall, temperature).

Phosphorus availability in acid soils is mostly restricted by adsorption reactions between P ions and metal (e.g. Al/Fe) oxides and hydroxides, clay minerals and organic matter (Asomaning, 2020; Sims and Pierzynski, 2005), therefore P availability is considered to be maximized around pH 4.5 and 6.5 where the degree of P fixation by Al, Fe and Ca is minimized (Penn and Camberato, 2019; Price, 2006). In contrast, Barrow (2017); Barrow et al. (2020b) argued that raising soil pH to 6 or 7 is not likely to increase P availability to plants and that P desorption and P uptake by plants occur with a much lower pH optimum. However, these two contrasting views were based on abiotic processes (precipitation and adsorption/desorption reactions; see Barrow (2021)) to assess the effects of pH on P availability, which may be similarly mediated by biological processes since organic P could account for 30 to 65% of soil total P (Condron et al., 2005). The pH increase following a liming event could enhance organic P mineralization because P cycling enzymes (phosphatases) are sensitive to pH change, they also have distinct pH optima (Turner and Blackwell, 2013; Turner, 2010). Moreover, Wan et al. (2020) demonstrated that pH drives the growth of organic P-mineralizing microbes.

Nonetheless, the importance of pH in regulating the soil organic P pool is poorly understood since few studies, if any, have separated the effect of pH from other soil properties (Condron and Goh, 1990;

Condron et al., 1993; Simonsson et al., 2018; Turner and Blackwell, 2013). Phosphodiesterase is the most likely rate-limiting step in organic P mineralization (Condron et al., 2005). However, it is far less investigated compared to phosphomonoesterases. The activity of acid phosphomonoesterase being the dominant enzyme in acid soil is commonly observed to decrease with pH increase (Hui et al., 2013), while controversial findings were reported regarding the alkaline phosphomonoesterase (Margenot et al., 2018; Wang et al., 2006a). Therefore, liming could have a substantial impact on P availability by altering phosphatase activity. Independent of its effects on soil microbial composition, lime could also directly impact phosphatase activity by mobilizing the stabilized enzymes due to sorption (Allison, 2006; Skujiņš and Burns, 1976). This may mask the individual effect of microbial biomass on enzyme activity. Accordingly, with total activity (e.g. absolute activity), it is not possible to ascertain whether the observed differences in the soil enzyme activity are due to the difference in microbial biomass or for a different reason (de Medeiros et al., 2015; Raiesi and Beheshti, 2014). Expressing enzyme activity per unit of microbial biomass P (the so-called specific activity) is one way to accommodate this issue.

Soil acidity and low P availability are among the major limitation to pastures (especially the legume component) establishment and persistence in New Zealand (NZ) upland grasslands, popularly called "high and hill and country'' (Maxwell et al., 2013; Moir et al., 2016). Thus, lime application is often critical, but not always practised, due to on-farm financial constraints (high cost of aerial application). However, lime solubility in the soil is low (Hendrie et al., 2018). Moreover, NZ hill and high country are generally drylands and often have a short, moisture limited production season (Moir et al., 2000). This could further restrict the effectiveness of liming in these typical environments and thereafter limit its reaction with soil P. In this context, we conducted a field experiment run for 18 months in a long-term (60 years+) permanent grassland with significant historical inputs of single superphosphate. We aimed to evaluate the impact of liming on P biochemical processes and dynamics. To produce different pH conditions (5.4–7.0), we applied four different lime rates (0, 2, 5 and 10 t ha^{-1}). To evaluate the extent of lime reactivity and movement down the soil profile (0–7.5 cm) we split the profile into two different depths 0–3 cm and 3–7.5 cm. The following hypotheses were raised:

(1) Liming improves soil P availability by enhancing organic P mineralization.
(2) Liming alters phosphatase activity and enhances microbial activity.

6.2 Material and methods

6.2.1 Experimental site and design

The experiment was established on 23rd November 2018 at Mt Grand Station, a 2131-ha commercial sheep and beef high country farm operated by Lincoln University, located in the Central Otago district, New Zealand (44°40'19.49''S, 169°19'5.66''E). The paddock (60 ha) on which the experimentation was

undertaken was on a south-facing hillside (shady aspect) of moderate-steep (20-25°) slope at 600 masl. The soil at the site is classified as a Brown soil (NZ classification after Hewitt (2010); the United States Department of Agriculture classification: Dystrudepts (USDA, 2014)), vegetated with naturalized grasses, naturalized adventive clovers, native tussocks and remnant native shrubs (Plate 6.1). Soil fertility status was characterized using the analyses listed in Chapter 4, Table 4.1. The climate is continental-like with hot dry summers and cold frosty winters. Long-term average annual rainfall (60 years) is 703 mm, with high annual and monthly variability (Maxwell et al., 2016). The daily air-temperature and rainfall in the site during the experimentation period (November 2018 to June 2020) are given in Figure 6.1.

Plate 6.1 Field trial set up.

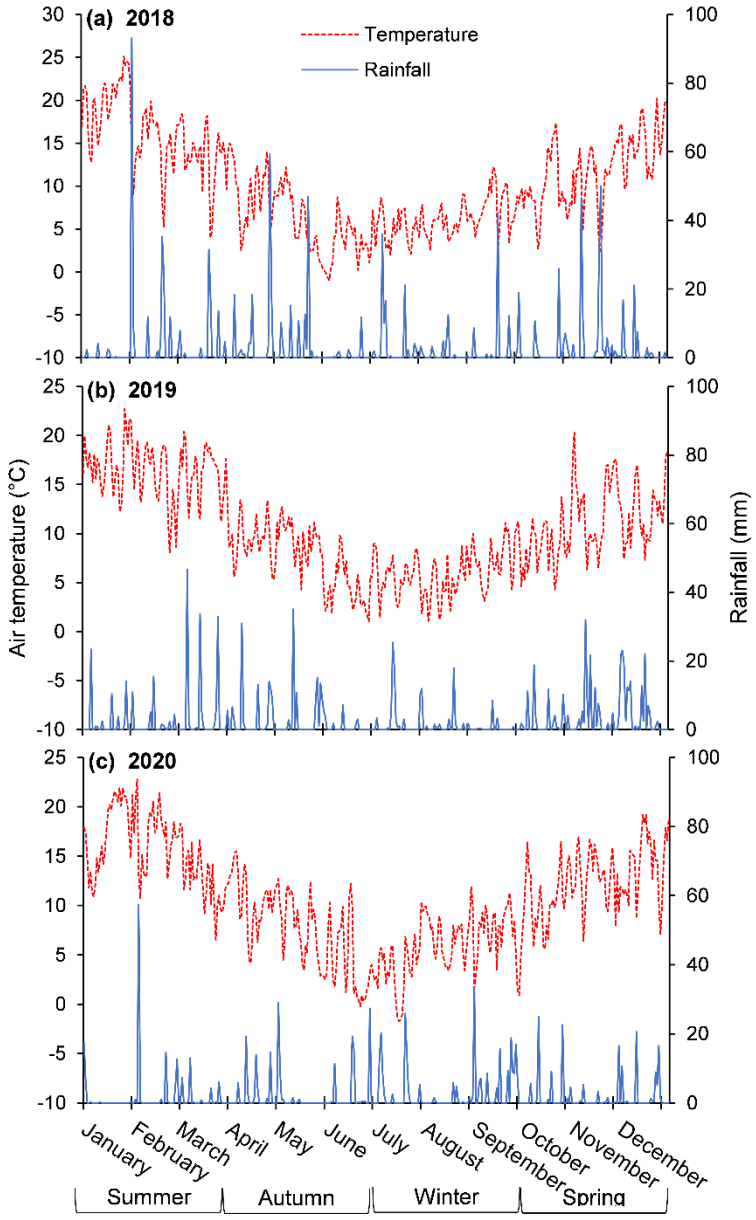


Figure 6.1 Daily air temperature (°C) and rainfall for three years: (a) 2018, (b) 2019 and (c) 2020 including the experimentation period (November 2018 to June 2020), at Mt Grand station, central Otago, New Zealand.

The developed grassland trial site had received regular applications of single superphosphate since the advent of aerial topdressing in the 1940s. Applications were every 2–3 years from the 1950's onwards at the site, at a mean approximate rate of 100 kg SSP $ha^{-1}yr^{-1}$. The experiment consisted of twelve plots (5×5 m) across slope, arranged in randomized blocks with three replicates per treatment. The treatments consisted of four rates of agricultural lime (90% $CaCO_3$): 0, 2, 5 and 10 t ha^{-1} which were selected to produce a range of pH conditions (5.4–7.0). The lime was applied once at the establishment of the experiment directly on the soil surface and evenly distributed within plots. The plots did not receive any fertilizer inputs or irrigation. Salt (NaCl) was applied twice during the study at low rates (≈50 kg ha^{-1}) as a stock management tool to increase grazing pressure to reduce the annual grass weeds (Tozer et al., 2013).

6.2.2 Soil sampling and analysis

The soil sampling was conducted on three occasions (April 2019, November 2019, and June 2020). A soil core sampler (25 mm diameter) was used to randomly collect fifteen sub-samples from the 0–7.5 cm depth within each plot-replicate to make up the composite sample per replicate. At the third sampling, the soils cores were split into two depths: upper topsoil 0–3 cm and 3–7.5 cm, and the soils were analysed for each depth separately to assess how soil pH changed in the topsoil layer (0–7.5 cm) after 18 months and therefore evaluate to what extent lime was mobile within that layer. The samples were sieved to 4 mm, homogenized, and stored at 4 °C. Plant material and stones were removed from samples prior to sieving.

The soil samples were analysed for a variety of chemical, biological, and biochemical characteristics. Soil pH was measured with a pH probe (SevenEasy pH meter, Mettler Toledo, USA) using 1:2.5 soil: deionized water ratio. Exchangeable Al was measured in 1 *M* KCl (1:10 soil: extractant ratio) and 0.02 *M* $CaCl_2$ (1:4 soil: extractant ratio) extracts using Inductively Coupled Plasma Optical Emission Spectrophotometry (ICP-OES: Varian 720-ES 283 ICP-OES, Varian, Melbourne, Australia). Total C and total N were determined by combustion using Vario-Max CN Elemental analyser (Elementar GmbH, Hanau, Germany). Anaerobic mineralizable N (AMN) was determined as described by Keeney and Bremner (1966b). Olsen P and resin P were analysed according to Olsen (1954) and Saggar et al. (1990), respectively. The variability in soil moisture was assessed from the samples collected throughout the study period. Gravimetric moisture content was determined by the mass difference before and after drying at 105 °C for 48 h.

Microbial biomass P (MBP) was determined after chloroform fumigation and extraction with 0.5 *M* $NaHCO_3$ (Brookes et al., 1982). To estimate the P_i recovery from the fumigated samples during the extraction, a third set of non-fumigated samples were spiked with 25 mg P L^{-1} (Boitt et al., 2018a). The P recovery from microbial biomass was further corrected by a coefficient (K_{pi}) of 0.4 (Brookes et al., 1982; Hedley and Stewart, 1982). Acid phosphomonoesterase (AcPME), alkaline phosphomonoesterase (AlPME) and phosphodiesterase (PDE) activities were assayed and determined as described by Tabatabai (1994). Enzyme activities were expressed as micromole of *p*-nitrophenol (*p*NP) produced after 1-hour incubation per gram of over dried soil (i.e., absolute activity). The specific activities of the enzymes were calculated by dividing absolute enzyme activities over the microbial biomass P values (de Medeiros et al., 2015; Margenot et al., 2018).

Soil P fractionation was carried out at the last sampling only for the soils collected at 0–3 cm depth, using the method described by Condron et al. (1996) and modified by including the residual P fraction (recalcitrant P); the residual soil (0.25 g) was extracted with H_2SO_4 (6 *M*, 4 mL) for 16 hours after being previously ignited for 3 hours at 550 °C (Gahoonia and Nielsen, 1992). The soil samples (0.5 g) were

subject to a sequential extraction using different chemicals of different strengths: 1 *M* NH_4Cl, 0.5 *M* $NaHCO_3$ (pH 8.5), 0.1 *M* NaOH, 1 *M* HCl and a second extraction with 0.1 *M* NaOH. These P fractions were separated and summed into four pools according to their lability: labile P (P_i and P_o) (NH_4Cl + $NaHCO_3$), moderately labile P (P_i and P_o) (first NaOH), stable P (P_i and P_o) (HCl + second NaOH) (Boitt et al., 2018b; Cross and Schlesinger, 1995). To avoid the effect high levels of exchangeable Ca could have on P extraction and distribution, a prewash with 0.5 *M* NaCl salt (5 mL, centrifuged for 5 min at 3500 rpm) was included between the sequential extractions (Perrott, 1992). Total P in the soil extracts was analysed using Inductively Coupled Plasma Optical Emission Spectrophotometry (ICP-OES: Varian 720-ES 268 ICP-OES, Varian, Melbourne, Australia). For each P pool, P_i was analysed according to Murphy and Riley (1962) and He and Honeycutt (2005) for acid and alkaline soil extracts, respectively, while P_o was determined as the difference between total P and P_i. The quantities of P fractions were expressed in mg kg^{-1} soil.

6.2.3 Statistical analysis

The data were analysed using Minitab® statistical software version 18.1 (Minitab, Inc., State College, Pennsylvania, USA). Lime treatments and soil sampling dates were considered as fixed factors. A two-way ANOVA was carried out to test the significance ($p < 0.05$) of the main effect of each factor and their interactions. One-way ANOVA was carried out to test the significance ($p < 0.05$) of the differences between the effects of treatment levels and sampling dates separately, followed by a comparison using Tukey's post-hoc test in cases of significant differences ($p < 0.05$). A two-sample t-test ($p < 0.05$) was used to identify the significant differences between the two depths: 0–3 cm and 3–7.5 cm. The partial least square (PLS) regression was used to identify the relationship between soil variables (pH, P fractions, microbial P, enzyme activities, Olsen P, resin P, total C, total N, anaerobic mineralizable N, exchangeable Al, and soil moisture) and labile P_i at 0–3 cm depth 18 months after lime application, thus revealing the relative importance of the different variables in constructing the pattern of co-variation. Additionally, each soil variable will have a loading score showing if its effect is high or low with respect to labile P_i. The optimum number of PLS components corresponds to the first minimum for the prediction error from the full cross-validation (leaves out only one sample at the time). All variables used in the PLS analysis were cantered and scaled to unit variance.

6.3 Results

6.3.1 Temporal and liming effects on soil properties at 0–7.5 cm depth throughout 18 months

The temporal and lime treatment effects on soil properties at 0-7.5 cm depth throughout 18 months are presented in Table 6.1. By eighteen months post treatment application, lime had increased soil pH ($p < 0.001$). However, soil P availability and related biological processes were not affected by liming at

0–7.5 cm, regardless of sampling date (Table 6.1). No difference was observed in soil pH between 0 and 2 t ha^{-1} in the first year, whereas an increment of 0.2 units was found after 18 months. At 5 and 10 t ha^{-1}, soil pH was higher ($p < 0.05$) compared to 0 and 2 t ha^{-1} irrespective of sampling date. No difference was obtained between 5 and 10 t ha^{-1} with time. Sampling date significantly affected all soil properties across lime treatments. Although the interaction treatment × date effects were not significant regardless of soil properties, the sampling date effects seem to be more pronounced in the treated soils (particularly at 5 and 10 t ha^{-1}) compared to the untreated (0 t ha^{-1}). At 5 and 10 t ha^{-1}, resin P (plant available P) and acid phosphomonoesterase activity were higher ($p < 0.05$) in the third sampling (June 2020) compared to April 2019, while no significant changes were observed between sampling dates at 0 t ha^{-1}. Soil pH and alkaline phosphomonoesterase activity were affected ($p < 0.05$) by sampling date, but at 10 t ha^{-1} only. Microbial biomass P was the only soil property which was affected by sampling date in the absence of lime (0 t ha^{-1}). The KCl-extracted Al increased ($p < 0.01$) with time at 5 and 10 t ha^{-1} only. In contrast, $CaCl_2$-extracted Al remained unaffected by both liming and sampling date. Soil moisture across all treatments was affected ($p < 0.001$) by sampling date. However, when considering each treatment level separately, no significant effect was detected between sampling dates. Similar observations were found for phosphodiesterase activity (Table 6.1).

Table 6.1 Temporal and liming effects on soil pH, bioavailable P, microbial P, enzyme activities, and exchangeable Al at 0–7.5 cm depth over 18 months period. Different upper-case letters denote the significant differences between lime rates for each sampling date separately ($p < 0.05$ after Tukey's HSD test). Different lower-case letters denote the significant differences between the sampling dates within each lime rate separately ($p < 0.05$ after Tukey's HSD test). Asterisks indicate the level of statistical significance (*p <0.05, **p < 0.01, p < 0.001).

Lime (t ha^{-1})	Sampling Date	pH	Olsen P	Resin P	MBP	AcPME	AlPME	PDE	Al_{KCl} (cmol kg^{-1})	Al_{CaCl2} (mg kg^{-1})	MC (%)
			(mg kg^{-1})		μ*M p*NP g^{-1} dry soil						
0	April 2019	5.4B	10.6	18.3	40.2a	12.3	2.6	2.7	0.4	3.7	24.6
	Nov 2019	5.5B	12.1	31.3	24.9b	15.1	3.5	3.7	0.5	4.2	28.7
	June 2020	5.4C	13.3	33.8	39.7ab	14.8	4.2	3.0	0.5	4.5	26.2
	p value	n.s.	n.s.	n.s.	*	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
2	April 2019	5.5B	13.9	22.3	51.6	10.5b	2.6	2.8	0.2	3.0	23.2
	Nov 2019	5.7B	16.4	36.7	36.9	15.2a	3.8	3.8	0.4	4.8	29.0
	June 2020	5.6B	14.5	36.5	47.9	14.0ab	4.7	3.2	0.4	3.5	26.3
	p value	n.s.	n.s.	n.s.	n.s.	*	n.s.	n.s.	n.s.	n.s.	n.s.
5	April 2019	6.1A	9.4b	16.3b	38.5ab	10.9b	2.4b	2.9	0.1b	2.3	22.8
	Nov 2019	6.2A	14.8a	37.0a	35.0b	14.5a	3.5ab	3.9	0.3b	2.5	26.8
	June 2020	5.9A	14.9a	39.3a	57.1a	14.0a	4.4a	3.5	0.6a	4.2	26.5
	p value	n.s.	*	***	*	**	n.s.	n.s.	***	n.s.	n.s.
10	April 2019	6.4Aab	11.1	18.7b	40.1b	9.3b	2.5b	3.0	0.1b	3.2	23.4
	Nov 2019	6.5Aa	14.8	37.0a	33.9b	14.0a	3.5ab	3.9	0.4b	3.9	28.3
	June 2020	6.1Ab	15.8	40.3a	60.4a	14.0a	5.1a	4.3	0.5a	3.7	26.5
	p value	*	n.s.	**	**	*	*	n.s.	**	n.s.	n.s.
Main effect	Treatment	***	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
	Date	**	**	***	***	***	***	***	***	n.s.	***
	Treatment×date	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.

MBP: microbial biomass P; AcPME: acid phosphomonoesterase; AlPME: alkaline phosphomonoesterase; PDE: phosphodiesterase.
Al_{KCl}: 1 *M* KCl-extracted Al; Al_{CaCl2}: 0.02 *M* $CaCl_2$-extracted Al, MC: moisture content.

6.3.2 Soil P biochemistry and dynamics at 0–3 cm depth as effected by liming after 18 months

Liming effects on soil pH were more pronounced at 0–3 cm depth compared to 0–7.5 cm. At 0–3 cm depth, the average pH at 0, 2, 5 and 10 t ha^{-1} were 5.6, 6.1, 6.6 and 7.0, respectively. However, at 3–7.5 cm depth, the soil pH did not change with time regardless of treatment level (varied between 5.2 and 5.3, Figure 6.2a). Also, Olsen P, resin P, anaerobic mineralizable N, microbial P and total C were higher ($p < 0.05$, Figure 6.2b, c, d, e, and h) at 0–3 cm depth compared to 3–7.5 cm regardless of treatment level. At 0–3 cm depth, Olsen P increased by 8%, 13% and 18% at 2, 5 and 10 t ha^{-1} compared 0 t ha^{-1}. Likewise, resin P increased by 6%, 17% and 16%. Further, microbial biomass P increased by 20%, 37% and 38% at 2, 5 and 10 t ha^{-1} compared to 0 t ha^{-1}. The KCl-extracted Al increased ($p < 0.05$, Figure 6.2f) at 5 and 10 t ha^{-1} compared to 0 and 2 t ha^{-1} at 0–3 cm depth, whereas no change was observed between the treatment levels at 3–7.5 cm depth. The $CaCl_2$-extracted Al was not affected ($p >0.05$) by lime at 0–3 cm depth (Figure 6.2g).

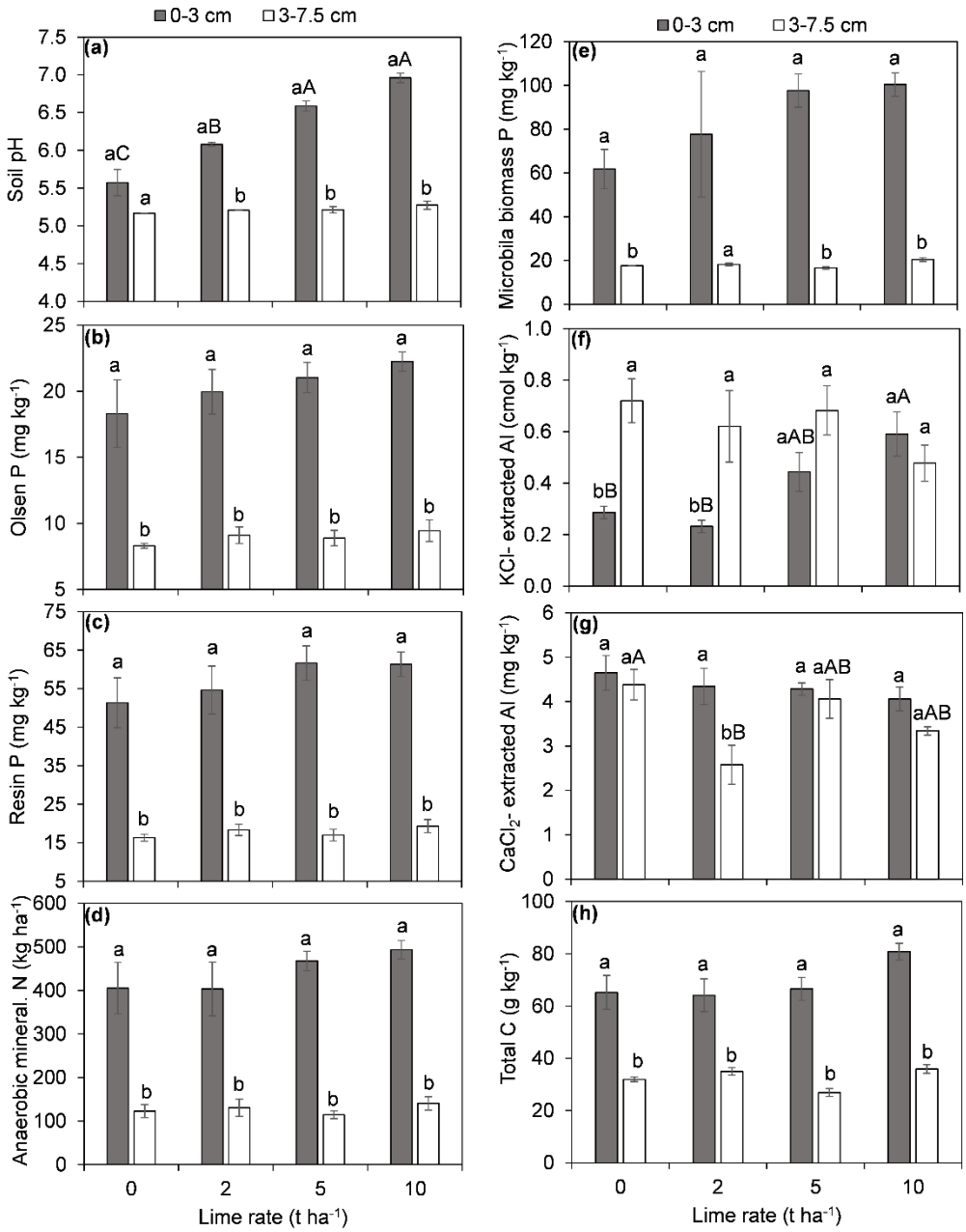


Figure 6.2 Comparison between 0–3 cm and 3–7.5 cm depths, 18 months after lime application, in terms of: (a) soil pH, (b) Olsen P, (c) resin P, (d) microbial biomass P, (e) Al_{KCl} and Al_{CaCl2} as affected by four different lime treatments. Upper-case letters indicate the significant differences between lime treatment levels for each depth separately (after Tukey's HSD test). Lower-case letters indicate the significant differences between the two depths for each treatment level separately (after Tukey's HSD test).

Absolute enzyme activities were significantly higher at 0–3 cm depth compared to 3–7.5 cm regardless of enzyme type and/ or treatment level (Figure 6.3).

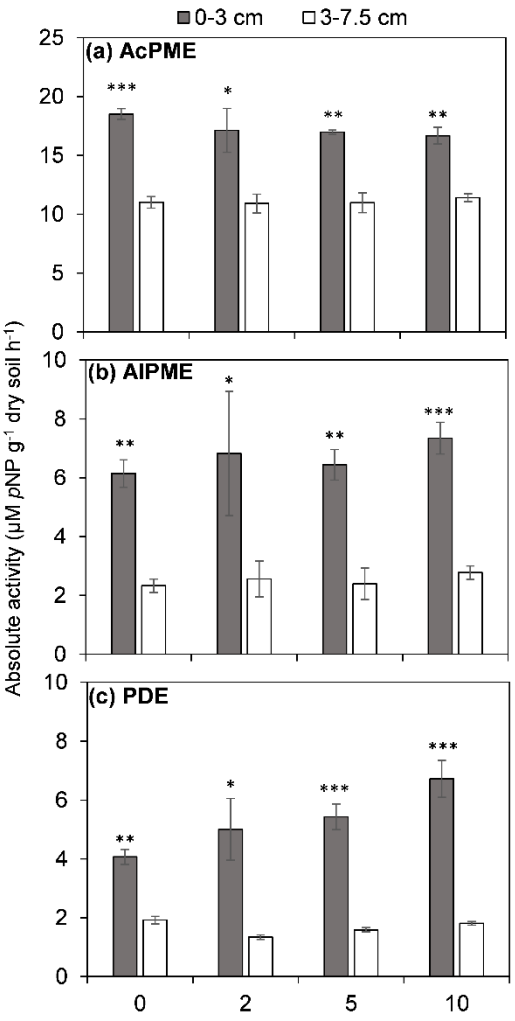


Figure 6.3 Comparison between the two depths: 0–3 cm and 3–7.5 cm, 18 months after lime application, in terms of enzymes activities: (a) acid phosphomonoesterase, (b) alkaline phosphomonoesterase and (c) phosphodiesterase. Asterisks indicate the level of significance (*p < 0.05, **p < 0.01, ***p <0.001, after two-sample t test) in the difference between the two depth per treatment level.

By eighteen months post lime application, P dynamics were affected at 0–3 cm (Table 6.2). Labile Pi increased (p < 0.01) with lime rate increase, while on the contrary labile Po decreased (p < 0.05) with liming. Moderately labile Pi decreased (p < 0.05) at 10 t ha^{-1} only compared to 0 t ha^{-1}. Moderately labile Po decreased by 8%, 16% and 25% at 2, 5 and 10 t ha^{-1}, respectively compared to 0 t ha^{-1}. Although the stable P pool was not significantly affected by liming, an increase in stable Po was observed at 5 and 10 t ha^{-1} compared to 0 t ha-1. Additionally, the amount of residual P accumulated at 10 t ha^{-1} was higher (p < 0.001) compared to the rest of the treatments.

Table 6.2 Soil P fractions (mg kg^{-1}) at 0–3 cm depth 18 months after lime application. Means (n= 3, ± SE) followed by different small letters within a row are significantly different (after Tukey's HSD test). Asterisks indicate the level of statistical significance (*p <0.05, **p < 0.01, p < 0.001).

P fractions	Lime application rate (t ha^{-1})				p value‡
	0	2	5	10	
Labile P_i	26.7 c	35.1 bc	40.5 ab	46.1 a	**
Moderately labile P_i	130.3 ab	148.7 a	129.5 ab	117.4 b	*
Stable P_i	153.5 a	144.2 a	136.1 a	158.6 a	n.s.
Labile P_o	90.6 a	77.7 b	67.5 c	60.5 c	***
Moderately labile P_o	312.1 a	287.2 a	263.4 a	233.8 a	n.s. (0.06)
Stable P_o	83.2 a	63.3 a	125.7 a	101.7 a	n.s.
Residual P	211.1 b	232.5 b	238.9 b	330.1 a	***

n.s. not significant

‡ indicates that p-values show the results of one-way ANOVA

A strong relationship was found between soil pH and soil P fractions, specifically: labile Pi (r^2 = 0.87, p < 0.001; Figure 6.4a), residual P (r^2 = 0.67, p = 0.001; Figure 6.4c) and labile Po (r^2 = 0.88, p < 0.001; Figure 6.4b). The latter showed a negative relationship with pH, while the first two ones were positively related with pH.

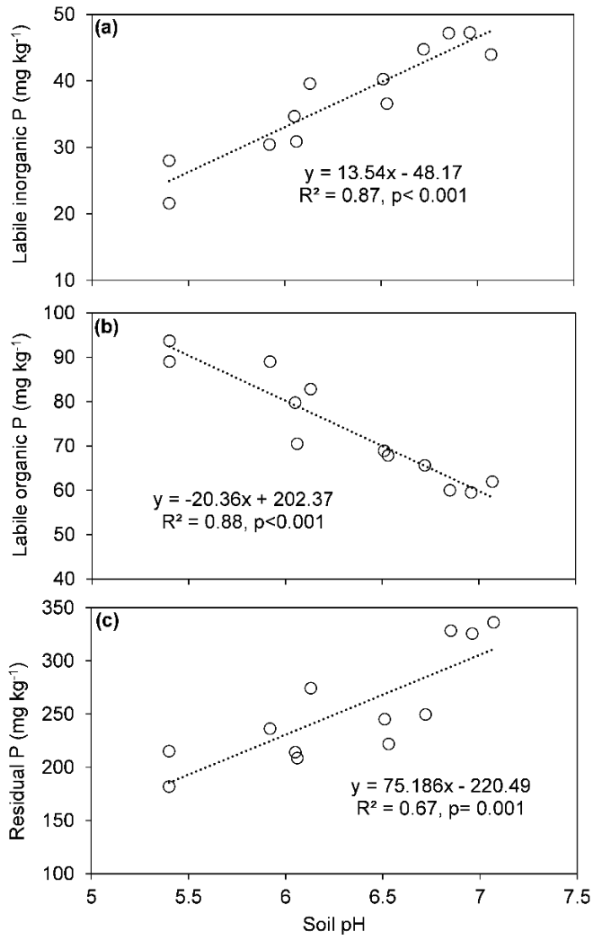


Figure 6.4 Relationship between labile P_i (a), labile P_o (b) and residual P (c), and soil pH at 0–3 cm depth 18 months after lime application.

The absolute activity of acid phosphomonoesterase, at 0–3 cm depth, decreased slightly ($p > 0.05$) in the treated soils compared with the untreated plots (10% was the difference between 0 and 10 t ha^{-1}, Figure 6.5a). Similarly, the absolute activity of alkaline phosphomonoesterase was not affected by liming. However, phosphodiesterase absolute activity increased linearly with lime rate increase being 19%, 25% and 39% higher at 2, 5 and 10 t ha^{-1}, respectively compared to 0 t ha^{-1}. The specific activities of phosphomonoesterases were responsive to liming ($p < 0.05$); they decreased in the presence of lime, whereas phosphodiesterase specific activity remained unchanged (Figure 6.5b).

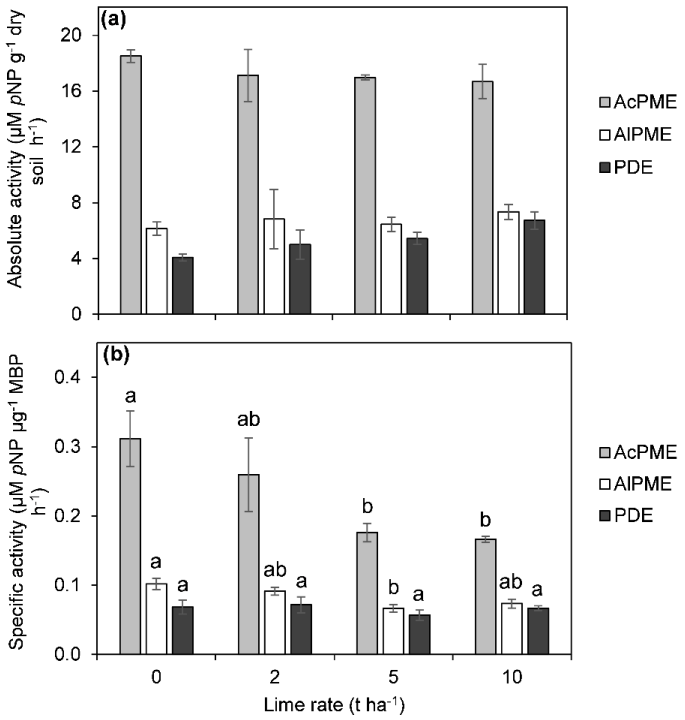


Figure 6.5 Absolute (a) and specific (b) enzyme activities as affected by liming. AcPME: acid phosphomonoesterase, AlPME: alkaline phosphomonoesterase, PDE: phosphodiesterase. Mean activities (n = 3 ± SE) with different lower-case letters are significantly different ($p < 0.05$ after Tukey's HSD test) for each enzyme separately.

At 0–3 cm depth, microbial biomass P was strongly and positively related to soil pH ($r^2 = 0.66$, $p < 0.05$; Figure 6.6a), labile P_i ($r^2 = 0.73$, $p < 0.01$; Figure 6.6b), total C ($r^2 = 0.61$, $p < 0.01$; Figure 6.6c) and anaerobic mineralizable N ($r^2 = 0.73$, $p < 0.001$; Figure 6.6d). The relationships between microbial P and enzyme absolute activities were enzyme-type dependent; microbial P was strongly and positively related with alkaline phosphomonoesterase ($r^2 = 0.7$, $p < 0.001$; Figure 6.7b) and phosphodiesterase ($r^2 = 0.65$, $p < 0.01$; Figure 6.7c). Contrarily, a week relationship was found between microbial biomass P and acid phosphomonoesterase ($r^2 = 0.22$, $p = 0.12$; Figure 6.7a).

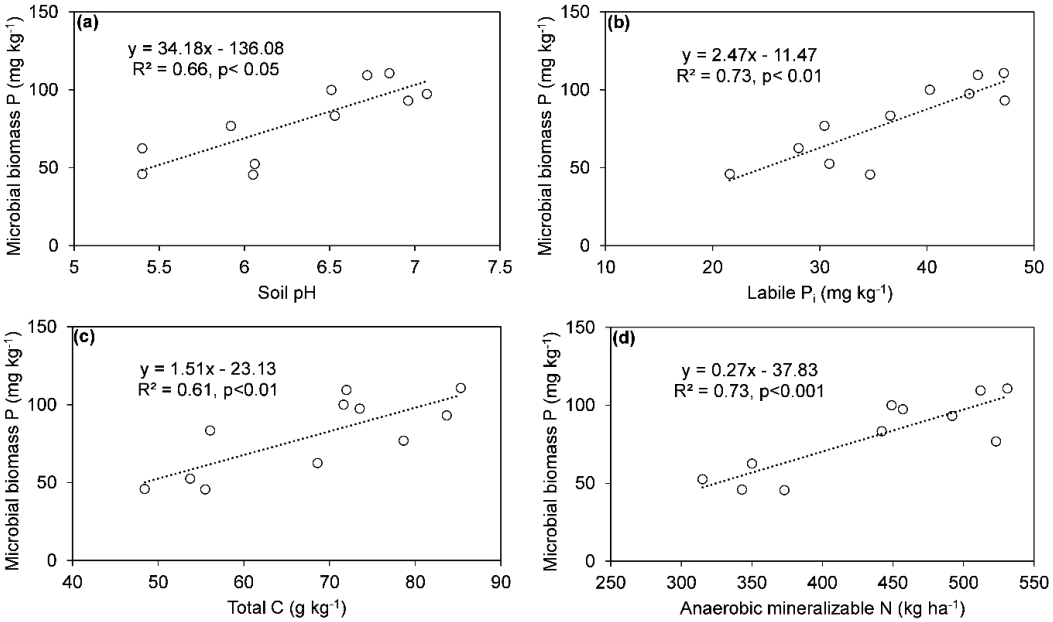


Figure 6.6 Relationship between microbial biomass P and: (a) soil pH, (b) labile P_i, (c) total C and (d) anaerobic mineralizable N in the 0–3 cm depth 18 months after lime application.

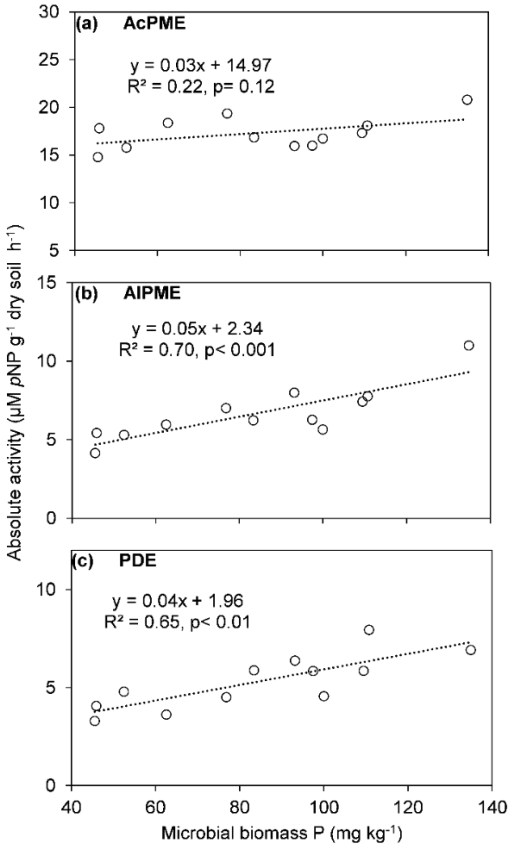


Figure 6.7 Relationship between microbial biomass P and absolute enzyme activities at 0–3 cm depth 18 month after lime application. AcPME: acid phosphomonoesterase, AlPME: alkaline phosphomonoesterase and PDE: phosphodiesterase.

6.3.3 Multivariate analysis at 0–3 cm depth

Most soil variables showed positive loadings within the first component which explained 85% of the variability in labile P_i (Figure 6.8). The absolute activity of phosphodiesterase showed the highest degree of co-variance with labile P_i with a positive loading of 0.26 compared to 0.20 and 0.05 for acid and alkaline phosphomonoesterases, respectively. Also, phosphodiesterase was the only enzyme which significantly affected labile P_i. A clear co-variance was also revealed between soil pH, microbial P, residual P, and labile P_i. Moreover, total C, anaerobic mineralizable N and resin P showed a high degree of co-variance with microbial P (Closely grouped loadings). Furthermore, moderately labile P_i was the only P fraction that was closely varied with exchangeable Al (Al_{CaCl2}). Specific activity of phosphomonoesterases showed a negative correlation with labile P_i, while positively correlated with labile P_o.

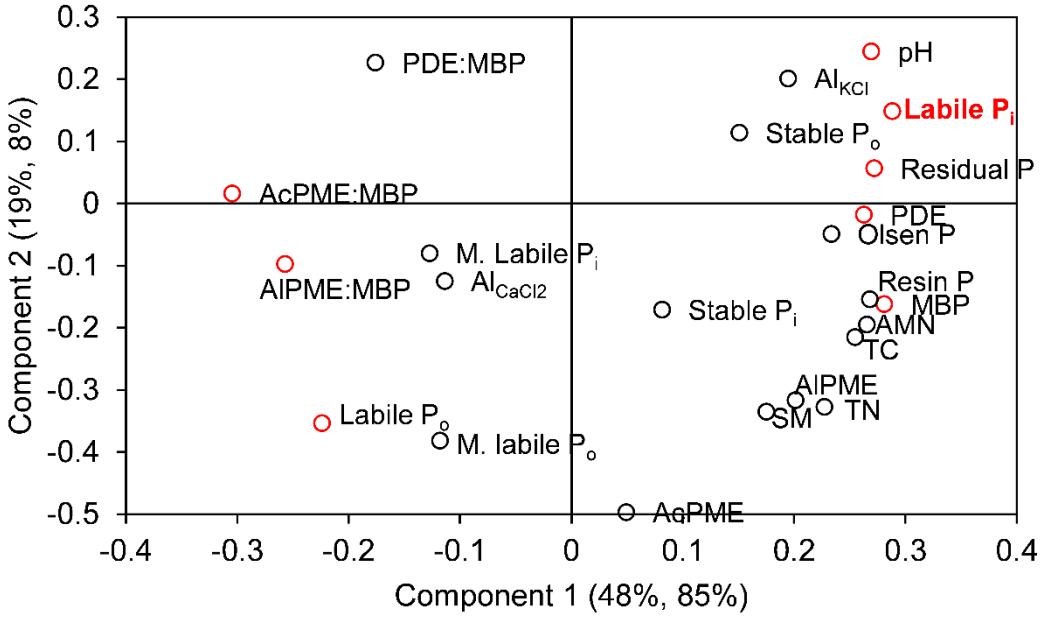


Figure 6.8 Loading plot of PLS regression including all P-related properties at 0–3 cm depth 18 months after lime application. Red circles mark soil variables with significant effect on labile P_i, according to Martens' Uncertainty test based on full cross-validation. TN: total N; AMN: anaerobic mineralizable N; TC: total C; SM: soil moisture; MBP: microbial biomass P; M. labile P_i or P_o: moderately labile P_i or P_o; AcPME: acid phosphomonoesterase; AlPME: alkaline phosphomonoesterase; PDE: phosphodiesterase; Al_{KCl}: KCl-extracted Al, Al_{CaCl2}: $CaCl_2$-extracted Al.

6.4 Discussion

The downward movement of surface applied lime is slow due to its low solubility (Hendrie et al., 2018; Li et al., 2019a; Moir and Moot, 2014) . Therefore, when monitoring soil pH change after liming and its impact on other soil properties (e.g., P biochemistry), sampling depth is an important factor to consider. In our study, at 0–7.5 cm depth, soil bioavailable P, microbial P, and enzyme activities did not respond to liming for 18 months. This could be explained by the fact that liming effects on soil P and biochemical activities were limited to a shallower depth. We report evidence that pH and P-related

processes did not change at the 3–7.5 cm depth with lime rate increase. In contrast, soil pH and P availability increased with liming at 0–3 cm depth. Moreover, P availability, microbial P, and enzyme activities were significantly higher at 0–3 cm depth compared to 3–7.5 cm regardless of lime treatment. This may be explained by the in-depth decline in soil carbon and moisture availability for microbiological activity (Achat et al., 2012; Fierer et al., 2003); total carbon was two-fold lower in the second depth across all lime treatments and moisture content (overall average: 33% at 0–3 cm versus 22% at 3–7.5 cm, $p < 0.001$).

Given that liming effects on soil pH were limited to the top 3 cm, we decided to further investigate P chemistry within this shallow layer; the sequential P fractionation revealed that increasing lime rate from 0 to 10 t ha^{-1} which corresponded to an increase of soil pH from 5.4 to 7.0, increased plant-available P_i (labile P_i) by 42%. Although an increase in plant-available P_i following liming has also been reported previously by Condron and Goh (1990); Condron et al. (1993), this is the first time that such a large difference is observed in the short-term. This could be attributed to two different mechanisms: (1) desorption of the historically applied P when pH increased and/or a decrease in P sorption due to an increase in soil negative charges as pH increases, thus less attractive to phosphate anions (Barrow et al., 2020b; Penn and Camberato, 2019) which could have increased the extractability of P. This view corroborates Simonsson et al. (2018) who conducted desorption experiments on soils obtained from long-term field experiments that have received lime and P fertilizer for 50-76 years. They concluded that liming had a positive effect on the solubility of P added as fertilizer. However, in our study, the decrease in moderately labile P_i, which is assumed to represent the fraction of P_i chemically absorbed to surfaces of Al and Fe oxides (Cross and Schlesinger, 1995), was lower than the observed increase in labile P_i. This leads to the second possible mechanism (2) which is the mineralization of organic P as evidenced by the resulted decrease in labile P_o (- 33%) and moderately labile P_o (- 25%) fractions when soil pH increased from 5.4 to 7.0. This agrees with some previous studies (Condron and Goh, 1990; Condron et al., 1993; Halstead et al., 1963). In contrast, Simonsson et al. (2018) found that liming had no effect on P_o pool; however, they analysed total P_o instead of P_o fractions using ignition method which is unlikely to detect differences in small P_o pools such as labile P_o. Obviously, microbial activity increases at higher pH (Fuentes et al., 2006; Kemmitt et al., 2006). This is supported by the strong relationship found between microbial P and pH. Nonetheless, microbial P was also strongly and positively related to total soil C and anaerobic mineralizable N in our soils. This implies that in addition to its direct effects through pH, liming could indirectly increase soil microbial activity and subsequently organic P mineralization via carbon supply. However, lime effects on soil C stocks are disputable (Paradelo et al., 2015; Vazquez et al., 2019). Although the pasture response to liming was not measured in our study, the average pasture dry biomass (an annual DM accumulation at the site) at the start of the experiment was 3 t ha^{-1} across all plots, composed of 98% grasses and 2% weeds and naturalized

clovers. This annual pasture yield compares well to values measured on the same farm by Maxwell et al. (2016). Moreover, liming has been reported to increase grass production in multiple field trials throughout New Zealand (Morton, 2020). Whereas only few naturalized legume species (e.g., *Trifolium striatum*) showed positive response to lime at the same site used in this study and showed low nutrient requirement for optimum growth (Maxwell et al., 2012; Maxwell et al., 2016).

The resulted increase (+ 36%) in residual P with liming (0 versus 10 t ha^{-1}) could be because of a greater organic matter inputs from pasture residues at 0–3 cm depth. This agrees with the findings of Neto et al. (2021). Furthermore, the resulting increase in stable P_i (+ 3.2%) and stable P_o (+ 18.2%) could be due to the formation of Ca-P compounds. In summary, our study demonstrates that liming strongly affects P dynamics in the historically fertilized soils and consequently increases bioavailable P (Olsen P) being equivalent to fertilizer P input of 8.3–20 kg P ha^{-1} to the surface 3 cm, within a range of 2-10 t ha^{-1} of lime. This result does not agree with the hypothesis suggested recently by Hendrie et al. (2021). The authors investigated the relationship between soil pH and P fractions in 19 extensively farmed soils without being treated with lime and contended that liming is unlikely to increase soil P availability. The pH (water) range of all their soils was narrow (4.7–5.5) which is unlikely to accurately predict the relationship in question. Perhaps it would be better to explore whether P chemistry in these soils would be affected by liming. The results of P fractionations analysis in our study agrees with Hendrie et al. (2021), Chen et al. (2003) and McLaren et al. (2020) showing that past fertilizer P inputs to grassland soils accumulates mainly as moderately labile P_o. This P fraction was affected by liming in our study because we measured a 78.3 mg P kg^{-1} of soil reduction in this P fraction when soil pH increased from 5.4 to 7.0. This amount is almost four times the observed increment in labile P_i. This suggest that the rest of the depleted moderately labile P_o has either being used by pasture and removed off site as animal nutrient transfer in dung (Haynes and Williams, 1993), or transformed into more recalcitrant forms of soil P. More research is required to fully explain these soil P dynamics.

Anaerobic mineralizable N does not only give a quick and precise estimate of N supply from soil to pastures (Reussi Calvo et al., 2018; Sainz Rozas et al., 2008), it is also a good indicator of soil organic matter accumulation (Garcia et al., 2021; Garcia et al., 2020) . This is in line with the strong relationship found between total C and anaerobic mineralizable N (r = 0.85, p < 0.001) in our soils, as such, soil organic matter content might have been increased especially at 5 and 10 t ha-1 where anaerobic mineralizable N was 13% and 18% higher compared to 0 t ha^{-1} being an equivalent input of 62 and 88 kg of N ha^{-1} to the surface 3 cm. Moreover, liming has been found to increase microbial biomass N (Soon and Arshad, 2005). Also, anaerobic Mineralizable N has been shown to be strongly correlated with microbial biomass N (r = 0.91, p < 0.001) according to Stockdale and Rees (1994).This implies that the turnover of the microbial N could also have contributed to increasing mineralizable N in our limed soils. On the other hand, legume residue is known to be highly mineralizable compared to grasses

(Soon et al., 2007). For instance, Soon et al. (2001) found that wheat N uptake and net N mineralization were higher following legume (red clover) residues compared with wheat residues. This suggests that liming could have enhanced the growth and establishment of naturalized adventive clovers in the experimental site. However, this has not been possible to assess through observation because of the dominance of grass at our trial site. Hence the necessity of examining and monitoring the botanical composition in the experimental further at this site.

Our study demonstrates that specific enzyme activities per unit of microbial biomass P were more sensitive to liming than absolute enzyme activities, specifically for phosphomonoesterases. This could be due to an alteration in soil microbial composition. For instance, the pH optima for bacterial phosphatases is higher than that of fungus (Tabatabai, 1994; Turner and Haygarth, 2005). Moreover, Waldrop et al. (2000) found that specific enzyme activity is more closely related to compositional changes in soil microbial communities than total enzyme activity. On the other hand, phosphodiesterase specific activities were not affected by liming, but with a linear increase in absolute activities. This indicates that phosphodiesterase activity and microbial biomass increased at the same rate with liming, suggesting that phosphodiesterase was mostly originated from soil microorganisms, especially bacteria and actinomycetes which are more abundant at neutral soil pH (Turner and Haygarth, 2005). Using multivariate analysis of PLS regression, absolute activity of phosphodiesterase associated positively and significantly labile P_i concentration in our soils. This confirms the earlier suggestion of Turner and Haygarth (2005) that phosphodiesterase is the rate-limiting step in P_o mineralization. This is also additional evidence to support our hypothesis that at least part of labile P_i was sourced from P_o mineralization. On the other hand, specific activities of phosphomonoesterases showed a negative and significant correlation (AcPME: $r = 0.89$, $p < 0.001$; AlPME: $r = 0.80$, $p = 0.001$) with labile P_i which implies a repressive effect of higher P_i availability on the release of phosphomonoesterases (Gatiboni et al., 2021; Nannipieri et al., 2011). In summary, our findings suggest that liming could change the relative role of phosphatases and that the reduction of acid phosphomonoesterase being the dominant enzyme in acid soils may not necessarily affect labile and moderately labile P_o mineralization. To further understand liming impacts on P cycling, a characterization of organic P in the present soil is required, because the strong positive correlation found between phosphodiesterase and microbial P ($r = 0.81$, $p < 0.001$) indicates that the increased phosphodiesterase absolute activity with pH could have been mediated by the substrate (diester P) loading due to microbial cells death.

6.5 Conclusions

This study suggests, for the first time, that the enzyme activity per unit of microbial P may be a suitable indicator for detecting the effect of management practices (e.g., liming) on the influence of microbial biomass changes on phosphatase activities and subsequently P_o mineralization. We suggest that the assessment of liming effects on soil P in the field, at least in the short- term, to be at near surface depth (0–3 cm) to avoid a dilution effect, especially in a dry environment such as New Zealand high country where low soil moisture could significantly affect lime solubility and movement down the soil profile. This study demonstrates that liming could increase soil P availability via labile and moderately labile P_o mineralization in grasslands under field conditions. However, additional field research is required for a range of soils with contrasting acidities and P fertilities.

Chapter 7

General Discussion, Conclusions and Future Research

7.1 General discussion

Legumes play critical dual roles in grazed grassland ecosystems; providing nitrogen inputs and high-quality feed for grazing livestock. However, the establishment and persistence of legumes in NZ hill and high country are restricted by several soil-related factors such as low soil pH, high exchangeable Al concentrations and low P and S fertility (Hendrie et al., 2021; Maxwell et al., 2016; Moir et al., 2016; Morton, 2020; Whitley et al., 2019). Also, the economics of the aerial application of lime and fertilizer to hilly topography has contributed to restraining pastoral farming in these typical dry environments (Craighead, 2005; Edmeades et al., 1985) which play a significant role in the NZ meat and wool industry (Moot et al., 2009; Morris and Kenyon, 2014). Therefore, the application of lime and nutrients is key to sustainable hill country farm systems in New Zealand. In this context, we studied the possibility of utilizing phosphogypsum (PG) as an alternative cheap soil conditioner, which could be used to simultaneously improve soil S & P fertility and alleviate Al toxicity. Comparing PG with soluble fertilizer (PS) using two NZ hill and high-country brown soils (from Glenmore and Molesworth Stations), growing lucerne (*Medicago sativa*) as an indicator crop, demonstrated the fertilizing potential of PG for P and S-deficient acid soils (Chapter 2). For instance, soil P availability increased linearly with PG rate increase in a comparatively similar way as PS fertilizer (Bouray et al., 2020). This indicates the high solubility of total P contained in PG materials and its ability to be easily released into the soil and therefore be available to the plants in the same manner as soluble fertilizers; an application rate of 1, 3, and 9 t ha^{-1} of PG is equivalent to a fertilizer P inputs of 5.4, 16.2 and 48.6 kg P ha^{-1}. However, this study showed that increasing soil P alone is not enough because no substantial change was observed in lucerne P uptake with increasing PG and PS rate increase unless they were combined with lime. This finding highlighted (1) the key role of soil pH in controlling P acquisition by legumes and (2) the necessity of examining the effects of soil pH change using lime on soil P-related processes. Hence, we decided to further investigate the relationship between pH and P availability/uptake by legumes from different perspectives in the following Chapters (4 and 5, and 6).

Another key finding in Chapter 2 was that PG reduced soil exchangeable Al when applied at low rates only (1-3 t ha^{-1}). Several interpretations and explanations were given in this regard, however, analysing soil exchangeable Al alone was not enough to reveal the mechanisms involved. Moreover, the extractable-Al methods are known to solubilize Al from plant-unavailable fractions and provide a potentially misleading indication of Al toxicity status (Marques et al., 2002; Percival et al., 1996). Moreover, the ratio of Ca:Al in soil solution is also important as high Ca in the presence of high Al allows

root elongation to continue (Cunha et al., 2018). Further, Al phytotoxicity is better assessed by the concentration of free Al^{3+} cation in the soil solution rather than the exchangeable Al soil extraction by $CaCl_2$ (Martins et al., 2020; Miotto et al., 2020). Therefore, a separate experiment (Chapter 3) was necessary to understand the effects of PG on Al species distribution in the soil solution and elucidate the mechanism involved in controlling Al activity. In Chapter 3, we used the two soils previously planted with lucerne (Chapter 2) and another two soils (from Molesworth and Lindis Peaks Stations) treated with the same PG rates (0, 1, 3, and 9 t ha^{-1}) and incubated (unplanted) in the laboratory for 60 days. Al speciation was estimated using a geochemical Model (Visual Minteq, Martins et al. (2020); Miotto et al. (2020)). Although we have used different soils (different exchangeable Al concentrations) either planted or incubated and treated with different PG rates, again pH was found to play a major role in controlling Al solubility via Al-OH forms and minerals (amorphous Al $(OH)_3$ and gibbsite). Indeed, PG has been proven to significantly reduce Al^{3+} in the soil solution (especially in the high Al soils such as Molesworth), but only when 1-3 t ha^{-1} were used; higher application rates (above 3 t ha^{-1}) should be avoided as this would further acidify the soil releasing Al into the soil solution. So, at moderate application rates, PG supplied sufficient amounts of calcium to displace Al from soil solid phases into the solution where free Al^{3+} is complexed mainly by SO_4^{2-} and F^-, then transformed into non-phytotoxic forms (Al-SO_4 and Al-F, MacLean et al. (1992); Tanaka et al. (1987)) as evidenced by the resulting positive impact of Al-F on TDM yield of lucerne in our study. However, this disagrees with the finding of Manoharan et al. (2007), in a glasshouse experiment, they found that high concentrations of Al-F complexes had restricted barley root growth at pH< 5. Another mechanism by which PG seemed to restrict Al activity in the soil solution was through precipitation reactions (e.g., alunite formation). Importantly, at 1-3 t ha^{-1}, PG effects on soil pH were generally minimal. This indicates that PG could be used safely for fertilization purposes on moderately acidic soil if used at moderate rates. However, according to Alves et al. (2021), applying PG to soils with low CEC and low subsurface acidity may induce Mg deficiency and therefore affect the yield. Overall, our results demonstrate that PG could contribute to resolving two of the soil constraints in NZ hill and high-country: P & S fertility and Al toxicity. However, PG cannot be used as an alternative for lime and/or chemical fertilizers, but it can be used in combination with lime and/or as a fertilizer supplement.

Mixing PG with lime would come up with an alternative product superior to lime alone or PG alone (Carmeis Filho et al., 2017; Lauricella et al., 2021), a product that would adjust pH, ameliorate soil P and S fertility, and reduce Al concentration all at once. Therefore, we recommend conducting long-term field experiments on a range of NZ acid soils with contrasting Al toxicities to evaluate different lime-PG blends (formulated and tested in advance in the laboratory and by using controlled experiments) in terms of soil fertility improvement and Al toxicity alleviation. Furthermore, this strategy of material blending could also help to increase the solubility of lime and thereafter its

movement down the soil profile. This would in turn help mitigating the subsoil acidity which is a serious threat in NZ acid soils (Whitley et al., 2019). Blending PG with lime would likely allow neutralizing of some of the impurities that PG could contain. However, further research is required to assess the impact of PG utilization in agriculture especially in terms of heavy metals accumulation and radioactivity. Hence, some specific application guidelines need to be established for the agricultural utilization of PG (Chernysh et al., 2021; Wang, 2020).

Chapters 2 and 3 stressed the importance of pH as the ''master variable'' of NZ hill and high-country soil chemistry due to its profound impact on countless chemical reactions involving essential plant nutrients (e.g., phosphorus) and phytotoxic elements (e.g., aluminium). We hypothesized that adjusting soil pH to the biological optimum (5.8-6.3, Edmeades et al. (2016)) using lime could be the key to ensure the persistence of legumes (either naturalized or sown ones) in NZ hill and high pasture swards. For instance, Whitley (2018) demonstrated that liming reduces soil exchangeable Al efficiently to below toxic concentrations (3 mg kg^{-1}) in a range of NZ hill and high country soils. The relationship between pH and exchangeable Al in these soils is illustrated in Figure 7.1.

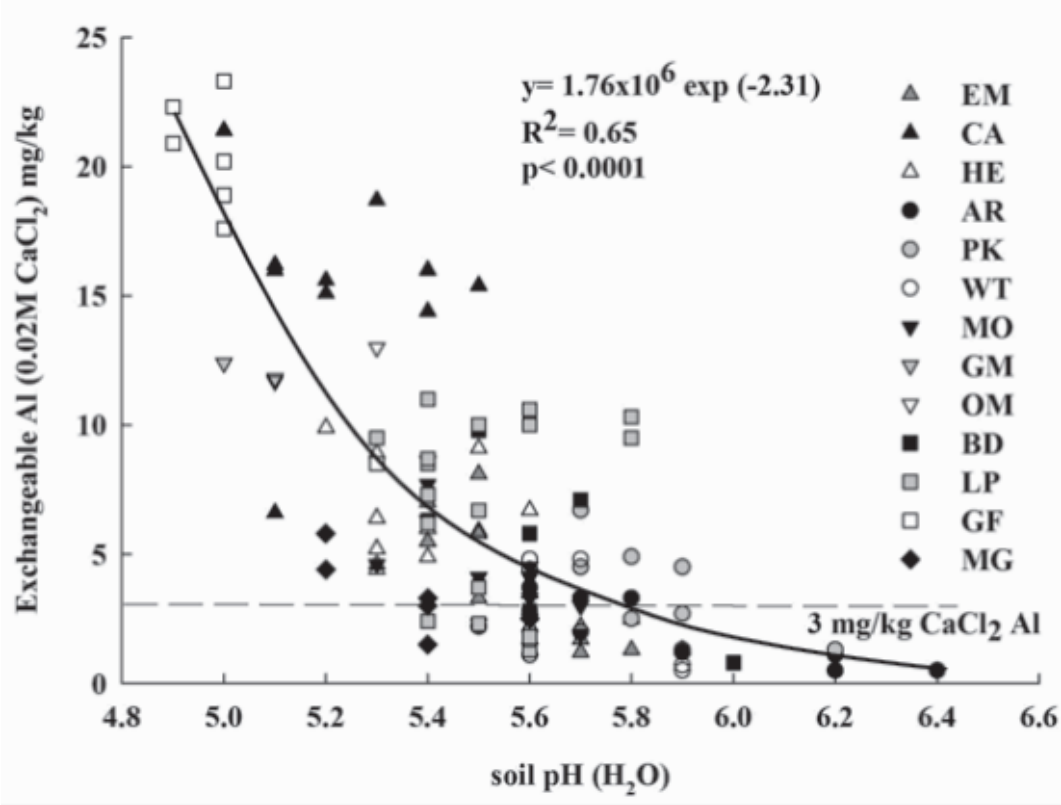


Figure 7.1 Relationship between increasing soil Al with decreasing soil pH for 116 soil samples across 13 hill and high Country farms in New Zealand (Whitley et al., 2016) including in investigated ones in the present research (MO: Molesworth, GM: Glenmore, LP: Lindis Peaks and MG: Mt Grand).

However, less is known about how liming could affect P availability in these soils. Martin-Hendrie (2019) conducted a P fractionation study on 19 hill and high-country soils. He found that only a small proportion (7.2±0.45%) of P contained in these soils is bioavailable, while moderately available P_i

represents 13.3±0.91% and moderately labile P_o accounts for 45.2±1.74% of total P. Providing that (1) moderately labile P is assumed to represent Al and Fe bound P and that (2) organic P represents the dominant share in total P in these soils, we hypothesized that liming could enhance P availability via two different processes: (1) desorption of P from soil adsorbing surfaces, in particular, Al oxides and hydroxides as a result of soil pH increase following liming, and (2) organic P mineralization as a result of higher microbiological activity at higher pH and then a more active rhizosphere zone where plant P acquisition is effectively determined. In this context, we decided to conduct three different experiments at different scales: a glasshouse pot experiment (Chapter 4), a rhizobox experiment (Chapter 5), and a field experiment (Chapter 6). In these experiments, we focused mainly on one soil which is Mt Grand soil (denoted as MG in Figure 7.1) collected from a long-term (60 years+) permanent fertilized pasture in a South Island hill country farm, central Otago. We have used lupins (*Lupinus angustifolius* and *Lupinus polyphyllus*) as bio-indicators of P availability, they were selected because of their capabilities to mobilize P from less available P pools, they are also known to have an active rhizosphere (Lambers and Teste, 2013; Pearse et al., 2006) which would allow seeing responses to environmental changes such as pH change in the short-term. Additional evidence of selecting lupins is their successful establishment in NZ hill and high country soils, specifically Russell lupin (*Lupinus polyphyllus)* (Black et al., 2014; Hendrie et al., 2018; Moot and Pollock, 2014; Ryan-Salter et al., 2014; Scott, 2014). Interestingly, all three experiments were consistent in showing that lime-induced pH elevation increases P availability in the Mt Grand soil. For instance, in the pot experiment, labile P_i in the rhizosphere of blue and Russell lupins increased by 8% and 15%, respectively, when pH increased from 5.3 to 6.0 after 11 weeks of plant growth. Further, in the field experiment, labile P_i increased by 42% eighteen months post lime application, which corresponded to an increase of pH from 5.4 to 7.0. These results were partly due to the mineralization of P_o, in particular labile P_o which decreased by 33% at pH 7.0 compared to pH 5.4. In the field experiment, a decrease in moderately labile P_o has also been observed; it decreased by 25% when soil pH increased from 5.4 to 7.0. These results disagree with Simonsson et al. (2018) who contended that liming did not affect total P_o pool using ignition method which does not give details about different P_o fraction in the soil and could underestimate changes in small P_o pool such as labile P_o fraction.

Phosphorus desorption could also have contributed to increasing soil P availability in our studies following lime application. This view is supported to some extent by the resulted decrease in moderately labile P_i (assumed to represent Al/Fe oxide bound P, Cross and Schlesinger (1995)), especially in the field samples at pH 7.0 compared to pH 5.4. Also, the strong negative correlations found between labile P_i and exchangeable Al in the rhizosphere of both lupins (pot experiment) support the role of desorption processes. However, the present research is mostly focused on examining how P biochemical processes could enhance P bioavailability via liming. Thus, the

contribution of P desorption reactions requires further research to be distinguished from that of biological/biochemical processes. Phosphorus immobilization by soil microbes (microbial biomass P) was also found to increase linearly with liming in both field and pot experiments (Chapters 4 and 6). The stable P pool was less affected by liming, while residual P increases with liming. However, efforts must be deployed to identify the origins of residual P accumulation; it could have resulted from organic matter residue input and/or P_o accumulation or P precipitation due to high Ca supply.

The P_o mineralization in our soils could be related to the observed increase in microbial activity at higher soil pH (Pietri and Brookes, 2008; Robson and Abbott, 1989). It could also be associated with the additional release in the phosphodiesterase enzyme. The opposite trends observed between phosphodiesterase and phosphomonoesterases in response to liming suggest that lime possibility changed the relative role of phosphatases. It could also be due to a change in P_o nature; more phosphodiesters could be sourced from microbial cell death which in turn could mediate additional production of phosphodiesterase. However, to verify this hypothesis, a characterization study of P_o in this soil is necessary using advanced techniques such as NMR and X-ray spectroscopy. Further, our field trial results suggest that specific activity (enzyme activity per unit of microbial P) is more sensitive to liming than the absolute activity (or total activity). Therefore, we recommend using specific activity as an indicator to evaluate the impact of management practice such as liming on the influence of microbial biomass changes on phosphatase activities and subsequently P_o mineralization.

Another key finding from Chapters 4 and 5 is that increasing pH above 6.0 negatively affects blue lupin growth and P uptake due to the alteration of root traits, especially (1) the reduction of organic anions exudation and (2) fine root length reduction. These two traits were found to be highly correlated with P uptake of blue lupin. Specific root length (SRL) of blue lupin increased by 14% with soil pH increase to 6.3 in Mt Grand soil, whereas in Millers Flat soils it decreased by 13% with soil pH increase to 6.3. These coincided with an increase (+ 24%) and a decrease (- 42%) in P availability in Mt Grand and Millers flat bulk soils, respectively. These results agree with Haling et al. (2018) who found that SRL of five cultivars of *Trifolium subterranean* was reduced in low P soils. Similarly, Jeffery et al. (2016) found that SRL of six cultivars of *Trifolium subterraneum* increased with P supply (Figure 7.2). Although this is inconsistent with many other previous studies conducted on legumes (Hill et al., 2006; Pang et al., 2010a), the authors contended that this response was due to the constrained canopy spread of micro-swards of *Trifolium subterraneum* (Jeffery et al., 2017). Furthermore, in our study blue lupin canopy has not been constrained (we only had one plant per rhizobox, and the shoots were allowed to expand beyond the confines of the rhizobox), an increase in SRL has been observed in Mt Grand soil where P availability increased after liming. Interestingly, in Millers Flat soil, SRL was higher ($p < 0.05$) compared to Mt Grand, but it was not reflected in terms of P uptake. Therefore, the benefits of SRL in terms of additional P uptake are still a matter of debate (Robles-Aguilar et al., 2019; Zobel et al., 2007). Russell

lupin growth and P uptake were found to be unresponsive to liming. This indicates that such species are mostly adapted to low pH environments like the hill and high country. However, more investigations are needed to further understand the mechanisms by which these lupin species adapt to low P soils. We have seen that P dynamics in the rhizosphere of both species were relatively similar– both species were able to mobilize some P– however, shoot P content was below the optimum range of 2.7–3.7 mg kg^{-1} suggested by Scanlan (2015) for blue lupin < 80 days. This suggests that P fertilizer application rather than liming is likely to facilitate the establishment of Russell lupin in the hill and high country. For the first time, it has been found that the ability of blue lupin to acquire more P compared to Russell lupin was mainly associated with its higher exudation of organic anions. However, a time-dependent study is required to confirm this including different plant growth stages.

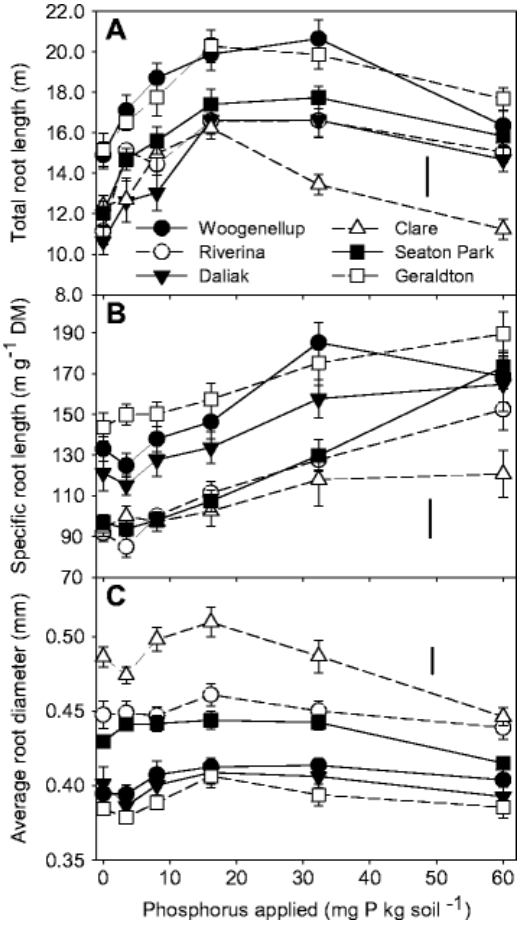


Figure 7.2 Effect of six levels of soil phosphorus (P) application (0, 3.47, 8.07, 16.15, 32.3, 60 mg P kg $soil^{-1}$) on root morphological traits of six cultivars of *Trifolium subterraneum* grown for six weeks in limited-P soil using pots (Jeffery et al., 2016).

In Chapter 5, contrarily to Mt Grand soil, we found that P availability (DGT-P) in Millers Flat soil decreased with liming. This was likely due to the high lime rate applied (13 t ha^{-1}; over-liming problems) to raise pH from 4.7 to 6.3. Also, the chemical properties of such a soil had likely contributed because it contains a very high concentration of exchangeable Al_{CaCl2} (32 mg kg^{-1}) and a medium-high P retention (52%). This result suggests that more work is required to understand P chemistry in this typical soil

under a gradual increase in lime rates. Moreover, we recommend that similar work done in this PhD research be extended for the rest of NZ hill and high-country soils to identify in which areas liming would likely improve P availability. It is also worthwhile to mention that some of our results do not agree with a recent study conducted by Hendrie et al. (2021). The authors contended that liming is unlikely to increase P availability in NZ hill and high country. However, their conclusion has been based on a narrow pH (water) range (4.7–5.5) from 19 soils, which is unlikely to accurately judge lime-induced pH changes effect on P chemistry. Also, the study in question did not use lime or any other treatments to modify pH; they only used the initial soil pH.

7.2 General conclusions and future research

To conclude, this PhD project demonstrates that the use of PG on acid soils is possible. It can be used as a fertilizer supplement (mainly for S, and P depending on the amount used). It can also be used as lime complement to rectify subsoil acidity. However, it is recommended to avoid using high PG rates on acid soil (pH< 5.5) as this would likely acidify the soil and thereafter increase the bioavailability of Al, it could also cause some nutrient imbalances especially Mg deficiency. Therefore, for a sustainable use of PG on acid soil and agriculture in general, guidelines must be established taking into consideration the potential risks that PG impurities might have. Moreover, a specific application recommendation method needs to be developed for NZ acid soils. Additionally, this project has demonstrated that liming enhances the mobilization of historically applied P as much of this unutilized P fertilizer has been fixed by Al/Fe metal oxides into less available P forms. This finding could bring a significant economic benefit to NZ high country farmers. It could also contribute to increasing P use efficiency and therefore managing P resources sustainably. However, the amount of lime to be applied must take into consideration the optimum pH for legumes because the present study showed that lupins for example are more adapted to moderately to slightly acidic soil pH (5.5-6.0). it has been also found that over-liming could negatively affect P availability in some soils (e.g., Millers Flat).

For future research considerations, it is highlighted that:

- Continued long-term monitoring of our field experiment for examinations of liming effects on P_o dynamics and its characterization is necessary taking into consideration the field variability of soil test measures. Further research is also required to investigate how liming will affect P availability in the rest of NZ South Island hill and high-country soils. Furthermore, liming impact on soil C stocks is not clear. Further research is needed this way, seeing the importance of C in mediating several P-related processes.

- Some other grassland species have been shown to be very efficient soil P foragers, in acid low-fertility soils e.g., the grass ‘browntop’ (*Agrostis capillaris*). This merits investigation using a time-

dependent study of seedling development. Moreover, more emphasis on the role of root morphological and physiological (organic anions) traits in P mobilization from acid P-deficient soils is needed for both grasses and legumes, in particular naturalized, adventive clover species (suckling, haresfoot, striated, and cluster) which have shown an interesting adaption to low pH and P-deficient high-country environments. Also, the interaction effects of P and Al on legumes' (non-cluster root lupins and lucerne) P nutrition and acquisition are poorly understood because both P deficiency and Al toxicity could act simultaneously, but it is difficult to distinguish which one is prevailing.

- The role of organic anions in P acquisition by legumes is still poorly understood, a similar thing is true for their involvements in Al detoxification. Also, the current methods of organic anions sampling and analysis have limitations. So, the development of new methods/approaches is solicited such as the use of DGT for an in-situ sampling of citrate (Tiziani et al., 2020). High-resolution imaging techniques (see Chapter 5), if coupled together to visualize P, organic anions, Al (all the three using DGT), enzymes (zymography), and pH (planar optode) in the rhizosphere could bring valuable insights and expand our current understanding of the interaction between soil acidity, P, and plant/microbes processes in the rhizosphere.

- Aluminium chemistry in the hill and high-country soils is still poorly understood, additional speciation studies (using NMR spectroscopy and modelling (e.g., WHAM)) are necessary. The relationships between Al species and legumes (such as lucerne, clovers, and lupins) growth should also be established.

- The Olsen P method underestimates P availability in certain limed soils. However, it is not clear if this is due to the method artifacts (formation of Ca-P compounds due to higher pH (8.5) and high Ca supply from lime) or to the intrinsic soil properties? Resin P and DGT P could probably be an alternative, however, this requires calibration work to be conducted across a large range of soils.

- Radiological characterization of phosphogypsum and the assessment of heavy metal accumulation in the soil using the existing long-term experiments is needed (some countries have used PG in agriculture for long-time such as Brazil and they can be used as experimental platforms).

Appendix A

Supporting Information for Chapter 2

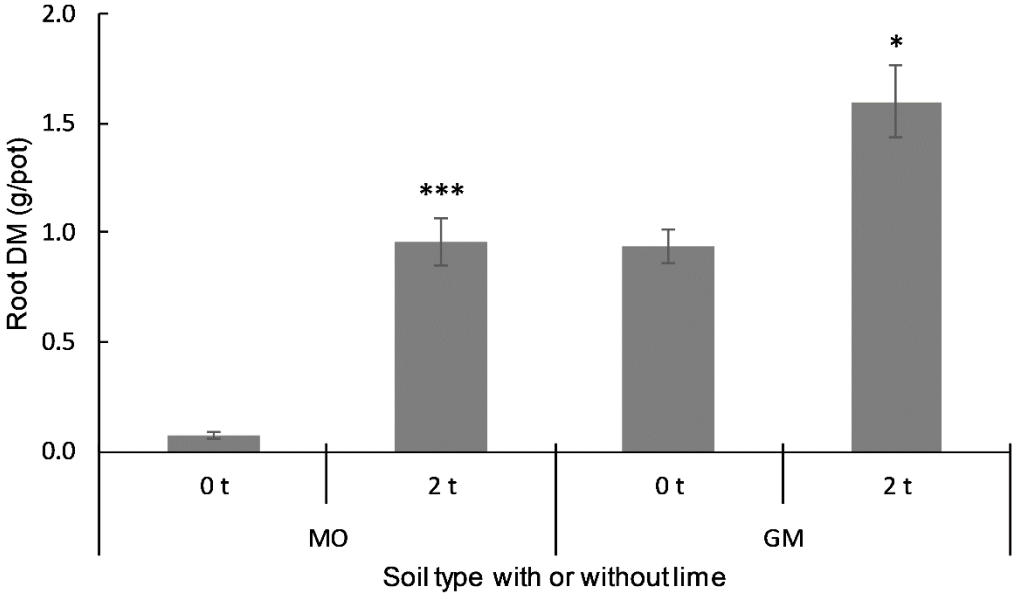


Figure A.1 Lime addition effect on overall average root dry matter (DM) accumulated under each soil (MO and GM indicate Molesworth and Glenmore soils, respectively). Error bars are standard errors (± SE, n = 28), Asterisks indicate the significance of two-sample t-test at 5% for liming (2 t ha^{-1}) effect compared to no lime conditions (0 t ha^{-1}) (* p <0.05, ** p <0.01, *** p <0.001).

Table A.1 The amounts of P removed by the plant (mg P kg^{-1} of soil) after six months of phosphogypsum, soluble fertilizer and lime application (2 t ha^{-1}) to two different soils (Glenmore and Molesworth). Within columns, means followed by the same lower-case letter are not significantly different (Dunnett test at 5%). Within rows, means were compared using a two-sample t-test at 5%.

	Glenmore	Molesworth	p value
Control	22.2	7.1 a	***
Phosphogypsum	19.5	11.4 b	***
Soluble Fertilizer	23.3	9.5 a	*
p value	n.s.	*	

Asterisks indicate significant effect levels (*p < 0.05, **p < 0.01, ***p < 0.001), n.s. not significant. Only the amounts of P removed under liming were reported in this table because in the absence of lime the collected quantities of plant herbage were not sufficient to run the ICP analysis (less than 0.2 g).

Appendix B

Supporting Information for Chapter 3

Table B.1 The separation of Al species into different Al fractions.

Al fraction	Al species included
Al-OH	$AlOH_2^+$, $AlOH_3$ (aq), $AlOH_4^-$, $Al_2OH_2^{4+}$, $Al_3OH_4^{5+}$, $AlOH_2^+$
Al-F	AlF^{2+}, AlF_2^+, AlF_3 (aq), AlF_4^-
Al-SO_4	Al $(SO_4)_2^-$, $AlSO_4^+$
Al-PO_4	$Al_2PO_4^{3+}$, $AlHPO_4^+$
Al-DOM	weakly bounded Al to DOM, Al bound to carboxylic and phenolic groups

Table B.2 Average saturation index (SI, n = 4) per treatment level and per soil for each mineral phase.

Soil	PG rate (t ha^{-1})	Soil amorphous $Al(OH)_3$	Alunite	Crystalline Gibbsite	Diaspore
Molesworth (incubated)	0	0.04	2.15	0.59	1.45
	1	-0.67	1.60	-0.12	0.74
	3	-0.28	4.08	0.33	1.20
	9	-0.24	4.83	0.31	1.18
Molesworth (planted)	0	0.32	3.81	0.89	1.74
	1	-0.11	3.85	0.44	1.31
	3	-0.65	3.84	-0.10	0.77
	9	-1.04	3.33	-0.50	0.38
Glenmore (Planted)	0	0.56	2.69	1.10	1.97
	1	-0.40	2.20	0.15	1.01
	3	-0.72	2.34	-0.17	0.70
	9	-1.01	2.64	-0.45	0.42
Lindis Peaks (incubated)	0	-1.09	-2.78	-0.54	0.32
	1	-1.32	-1.25	-0.77	0.10
	3	-1.30	-0.30	-0.74	0.13
	9	-1.41	0.16	-0.86	0.01

SI were calculated in visual MINTEQ according to SI= log (IAP/K_{eq}), IAP denotes the ion activity product and K_{eq} is the equilibrium constant of the solid phase.
At equilibrium the SI value equals 0 and SI < 0 or SI >0 denotes undersaturation or oversaturation, respectively.

Table B.3 Multiple regression analysis results (Coded Coefficients).

Term	Coef	SE Coef	t-Value	*p*-Value	VIF
Constant	1.437	0.124	11.56	0.000	
Al^{3+}	-0.971	0.177	-5.47	0.000	1.97
Al-F	1.384	0.183	7.56	0.000	2.10
Al-DOC	0.860	0.132	6.49	0.000	1.10

VIF variance inflation factor, SE standard error.

Appendix C

Supporting Information for Chapter 4

Table C.1 Regression standarized coefficients

Term	Coef	SE Coef	t-value	*p*-value	VIF
Constant	1.3424	0.0843	15.92	0.000	
TOAs	0.4592	0.0877	5.23	0.000	1.0

VIF variance inflation factor
SE standard error
TOAs total organic anions

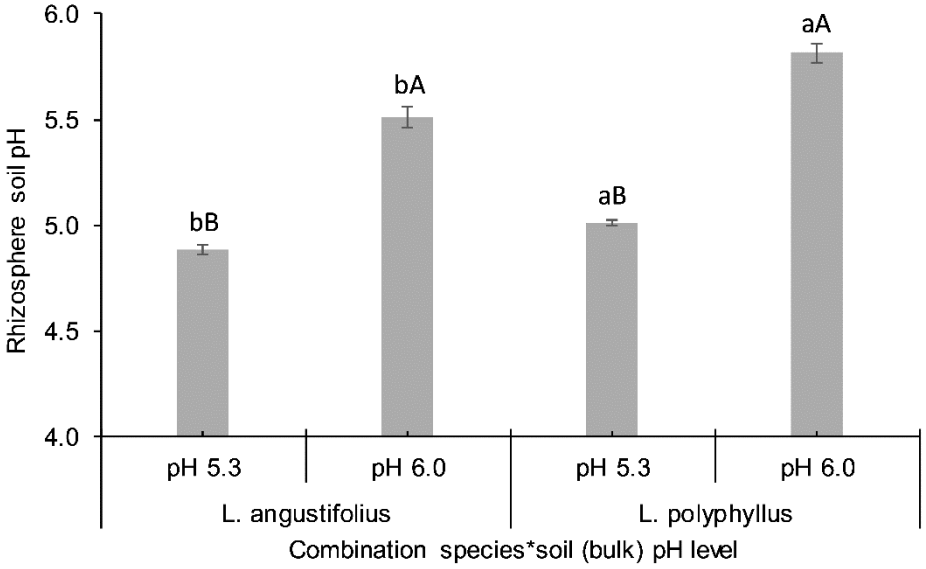


Figure C.1 Soil pH in the rhizosphere of *L.angustifolius* and *L.polyphyllus* at the end of the experiment as affected by soil pH increase from 5.3 to 6.0 through liming, after a growth period of 11 weeks. Lowe-case letters indicate the difference between the two species per soil pH level. Upper-case letters indicate the difference between the two pH levels per species, according to a two-sample t-test at 5%.

Table C.2 Shoot Al uptake (mg pot^{-1} ± SE) and shoot Al concentration (mg kg^{-1} ± SE) of *L. angustifolius* and *L. polyphyllus* after a growth period of 11 weeks in an acid grassland soil under two different soil pH conditions.

	L. angustifolius	*L. polyphyllus*	*p*-value
Shoot Al content (mg pot^{-1})			
pH 5.3	0.24 ± 0.03	0.29 ± 0.08	0.548 n.s.
pH 6.0	0.32 ± 0.11	0.35 ± 0.07	0.808 n.s.
p value	0.470 n.s.	0.126 n.s.	
Shoot Al concentration (mg kg^{-1})			
pH 5.3	0.20 ± 0.03	0.40 ± 0.06	0.059 n.s.
pH 6.0	0.23 ± 0.07	0.53 ± 0.07	0.037
p value	0.699 n.s.	0.232 n.s.	

n.s. not significant.

Table C.3 Pearson correlations between shoot Al concentrations (g kg^{-1}) and organic anions concentrations (µM g^{-1} root DM) for *L. angustifolius* and *L. polyphyllus* separately across two different soil pH levels (5.3 and 6.0).

	Citrate	Malate	Malonate	Acetate	Pyruvate	Fumarate	Succinate	TOAs
L. angustifolius	0.56$^{n.s.}$	0.89**	0.56$^{n.s.}$	0.13$^{n.s.}$	0.80*	0.30$^{n.s.}$	- 0.32$^{n.s.}$	0.80*
L. polyphyllus	-0.01$^{n.s.}$	0.66$^{n.s.}$	0.28$^{n.s.}$	0.1$^{n.s.}$	0.39$^{n.s.}$	0.20$^{n.s.}$	n.a.	0.25$^{n.s.}$

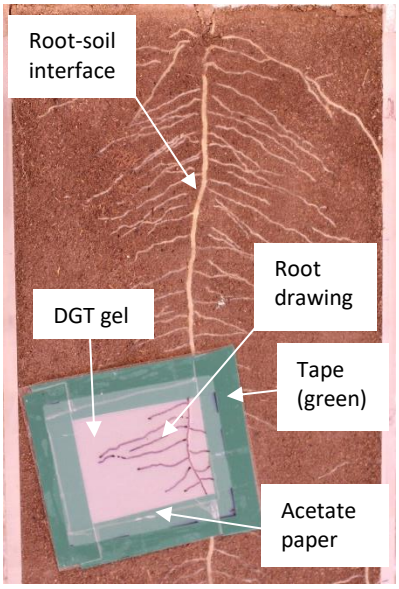
** and * indicate the level of significance (**$p < 0.01$ and *$p < 0.05$), n.s. not significant, n.a. not applicable, TOAs total organic anion

Appendix D

Supporting Information for Chapter 5

Preparation steps for DGT assembly used for labile P imaging sampling in our study:

- The DGT binding gel was cut on a clean and acid washed chopping board with a razor blade to the size of region of interest (ROI).
- Cut a 10 µm-thin nucleopore membrane (0.2 µm pore size) to a size that extends the gel size by ≥ 0.5 cm at each side and place it on the chopping board beside the binding gel,
- Grab the gel from the two upper corners with the forceps and place it on the nucleopore membrane, apply some DI water to facilitate the handling of the gel on the top of the membrane,
- Eliminate excess water with tissue paper.
- Prepare the acetate plastic paper (acid washed), its size should be larger (by 1 cm at each side) than the nucleopore membrane,
- Grab the membrane and gel together from the edges and place them on the acetate plastic paper.
- Carefully fix the membrane along all four edges on the acetate paper using vinyl electrical tape, this step is critical as it is irreversible.



An example of DGT assembly deployed on root-soil interface. The roots were drawn on the top layer (acetate plastic paper) to facilitate the determination of their location on the gel after deployment.

Recommendations :

- While taping, avoid creating air bubbles between the gel and the membrane, the bubbles can be chassed to the edges which were not taped yet.

- It is preferable to place a piece of tape in one of the corners (upper left corner for example), this will help in identifying easily in which position and direction the gel was deployed.

- The DGT gels are generally deployed for 20-24 hours. It is recommended to place a piece of tissue paper on the gel once deployed to chasse air bubbles which might be created between the soil-root interface and the nucleopore membrane. Air bubbles must be minimized as they affect the diffusion and therefore the labile P extracted and its distribution on the gel.

- Once the deployment time is over, take a picture of your DGT gel while it is still on the soil-root interface, then harvest your DGT gel and wash off gently the soil with distilled water. The final step depends on which method you are intending to use to analyze your gels, if you are using the staining method (our case) you will have to recuperate your gel by cutting off the tap using a razor blade and then proceed for coloration according to Ding et al. (2013) If you are using the LA-ICP-MS method, then you will have to follow another procedure described by Hummel et al. (2021); Santner et al. (2012).

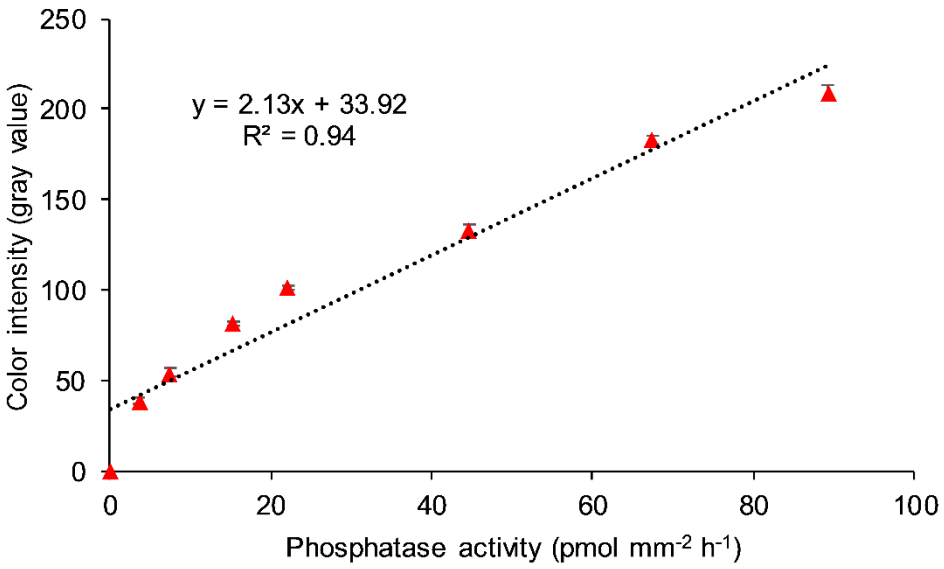


Figure D.1 Zymography calibration line, red triangles are the average of three replicates (n = 3) and error bars represent the standard error.

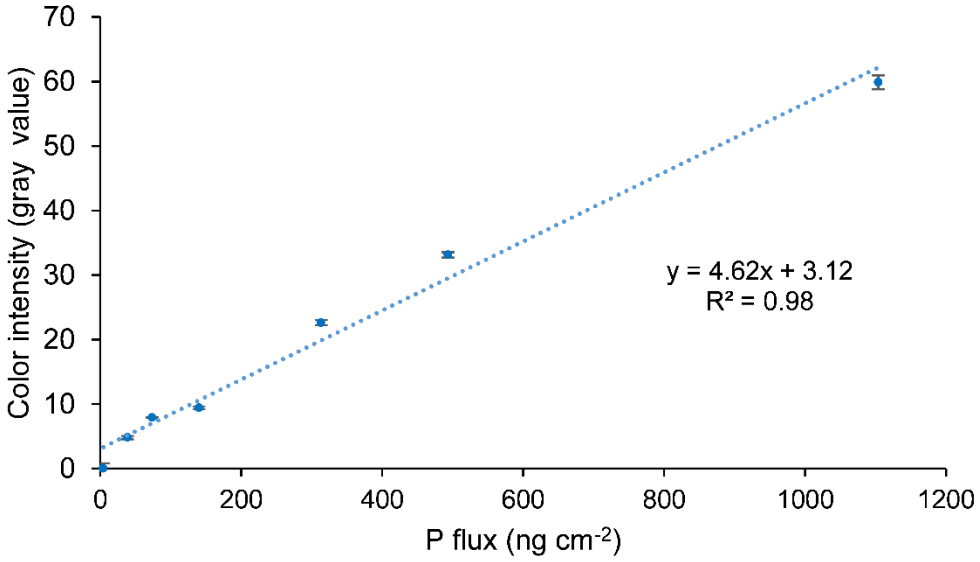


Figure D.2 Phosphorus calibration line, the blue dots are the average of three replicates (n = 3) and error bars represent the standard deviation.

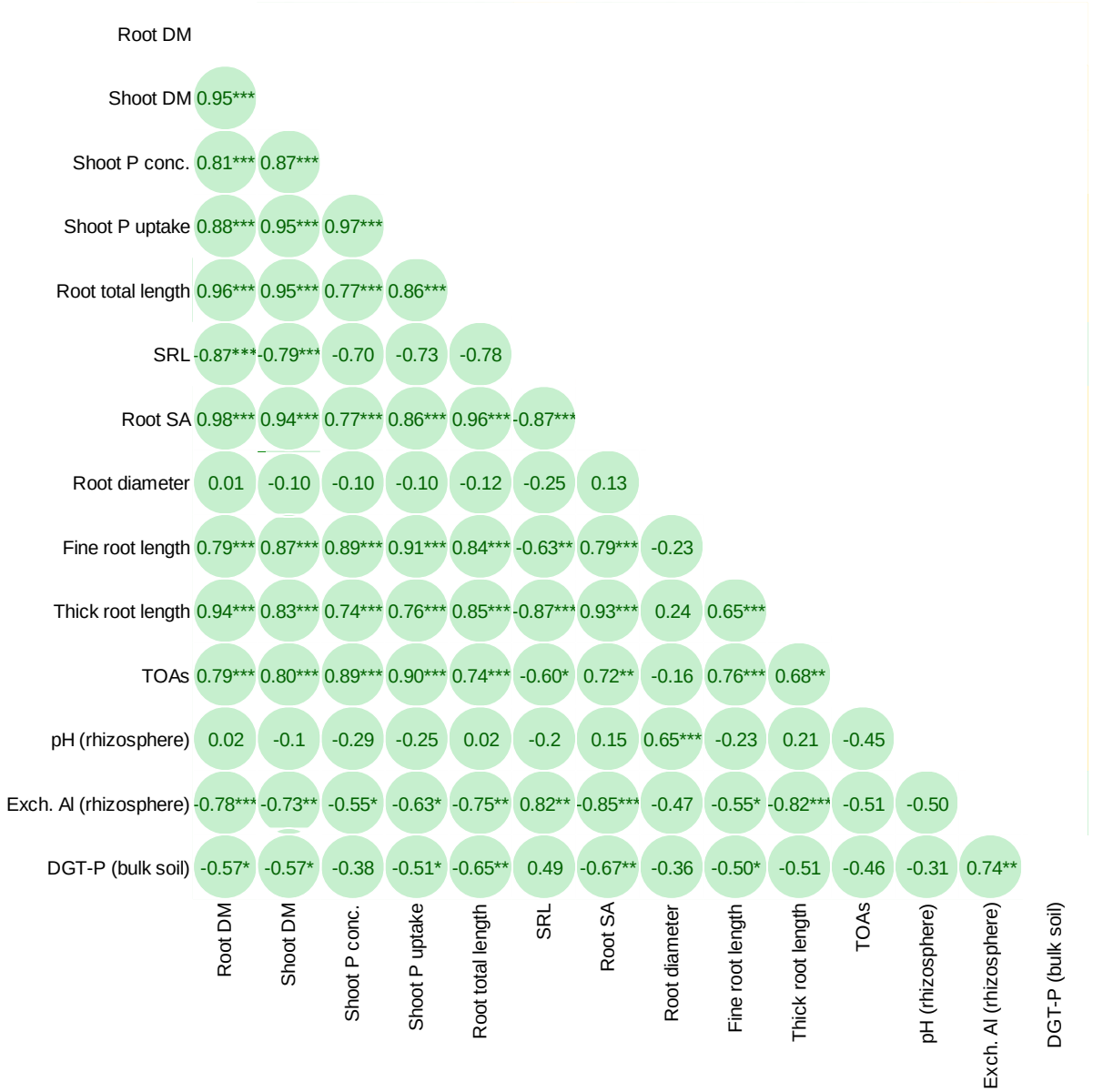


Figure D.3 Pearson's correlation between root morphological traits, total organic anions (TOAs), rhizosphere acidity (pH and exchangeable Al), shoot P uptake/P concentration, and available P in the bulk soil (DGT-P). Asterisks indicate the statistical significance (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$).

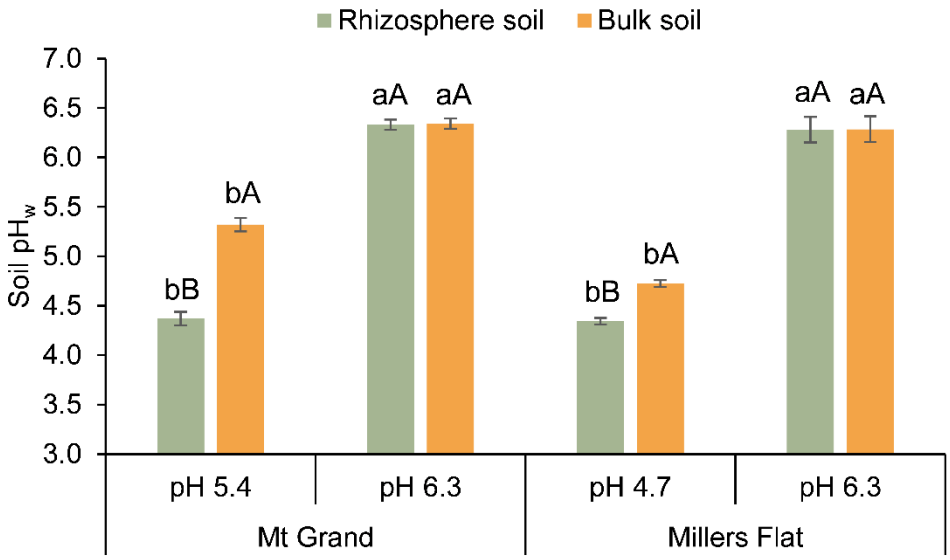


Figure D.4 Bulk and rhizosphere pH_{water} of two contrasting soils (different acidities and P fertilities: Mt Grand and Millers Flat) after 5 weeks growth of *Lupinus angustifolius*. Bars show the mean (± SE) of 4 replicates. Lower-case letters indicate significant differences ($p < 0.05$ after two-sample t-test) between the two pHs within each soil (Mt Grand: pH 5.3 and 6.3, Millers Flat: pH 4.7 and 6.3) forbulk and rhizosphere soils separately. Capital letters indicate significant differences ($p < 0.05$ after two-sample t-test) between bulk and rhizosphere soils per pH condition.

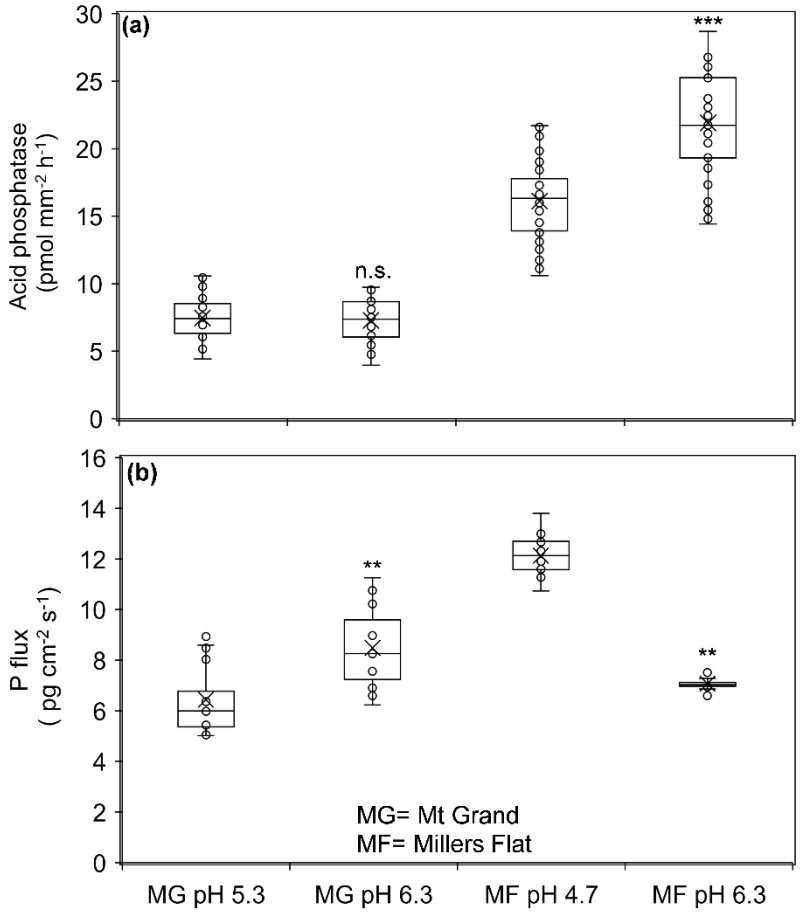


Figure D.5 Bulk soil acid phosphatase activities (a) and P fluxes (b) in Mt Grand and Millers Flat soils under two different pHs per soil. Marks (×) inside boxes: means of n= 30–40 squares (3×3 mm) for acid phosphatase and n = 15–20 squares (3×3 mm) for P flux. Circles: individual measurements. Asterisks: significant differences (*p < 0.001 after two-sample t-test) between the two soil pHs , n.s.: not significant.**

Table D.1 Nutrient concentration in *Lupinus angustifolius* shoots as affected by soil pH increase. Asterisks indicate the statistical significance (p* < 0.05, ***p* < 0.01, ****p* < 0.001), n.s. not significant.**

	Mt Grand soil			Millers Flat soil		
	pH 5.3	pH 6.3	*p*-value	pH 4.7	pH 6.3	*p*-value
g kg^{-1}						
N	0.29±0.01	0.48±0.01	***	0.44±0.02	0.59±0.04	*
K	15.73±0.30	11.44±0.05	***	16.98±0.86	12.33±0.37	**
Ca	13.54±o.41	23.89±1.08	***	3.06±0.39	15.40±0.97	***
Mg	5.84±0.19	4.08±0.06	***	3.81±0.28	3.25±0.14	n.s.
Na	0.43±0.11	0.31±0.11	n.s.	0.57±0.06	0. 59±0.09	n.s.
S	2.32±0.11	2.25±0.06	n.s.	2.10±0.22	2.40±0.46	**
mg kg^{-1}						
Mn	1867.31±76.00	948.71±54.00	***	373.79±28.00	33.78±5.60	***
Zn	115.49±10.00	36.25±3.00	***	110.72±4.00	33.07±2.20	***
Cu	10.33±0.46	5.30±0.31	***	5.50±0.16	5.35±0.29	n.s.
Mo	0.90±0.08	0.80±0.09	n.s.	0.56±0.07	0.61±0.03	n.s.
Fe	209.66±16	204.63±16	n.s.	450.92±29.00	355.19±22.00	*
B	54.94±7.00	36.71±9.80	n.s.	75.46±5.00	41.79±7.70	**
Al	54.83±6.50	40.79±7.70	n.s.	116.05±40.00	80.55±17.00	n.s.

Table D.2 Multiple regression results: standardized coefficients.

Term	Coef	SE Coef	*t*-value	*p*-value	VIF
Constant	1.3373	0.0622	21.50	0.000	
Rhizosphere pH	-0.585	0.114	-5.12	0.000	3.13
Rhizosphere exchangeable Al	-0.742	0.132	-5.61	0.000	4.19
Fine root (Ø ≤ 0.5 mm) length	0.480	0.119	4.05	0.002	3.38

VIF variance inflation factor, SE standard error

Multiple regression equation in standardized coefficient

Shoot P uptake = 1.34 – 0.585 (rhizosphere pH)- 0.742 (rhizosphere exchangeable Al) + 0.480 (fine root length) (**D.1**)
N =16, adj r^2 = 95.8%, $p < 0.001$

Table D.3 Normalized P mobilization extent (extent of P mobilization divided by root radius) in the rhizosphere of *Lupinus angustifolius* grown in Mt Grand soil at two different pHs (pH 5.3 and pH 6.3), the difference in the normalized P mobilization extent between the two pHs was not significant ($p > 0.05$) according to a two sample t-test (indicated by lower case letter "a").

Root Nb	Mt Grand, pH 5.3			Mt Grand, pH 6.3		
	P-mobilization extent (mm)	Root radius (mm)	Normalized P-mobilization extent	P -mobilization extent (mm)	Root radius (mm)	Normalized P-mobilization extent
1	0.38	0.34	1.12	0.59	0.45	1.31
2	0.26	0.30	0.87	0.59	0.40	1.47
3	0.30	0.30	1.00	0.38	0.43	0.89
Average	0.31	0.31	**1.00** a	0.52	0.43	**1.21** a

Appendix E Statistical Outcomes

Chapter 2

Table E.1 Three way-ANOVA analysis showing the statstical significance of the main effects of three factors: soil type, PG rate and lime rate, and their interactions on soil pH, Olsen P, echangeable Al and plant P and S uptakes.

	pH_{CaCl2}	pH_{water}	Olsen P	Exch. Al	P uptake	S uptake
Soil (factor 1)	n.s.	n.s.	***	***	***	***
PG rate (factor 2)	n.s.	***	***	***	n.s.	***
Lime rate (factor 3)	***	***	***	n.s.	n.a.	n.a.
SoilxPG rate	n.s.	*	n.s.	n.s.	n.s.	n.s.
SoilxLime rate	n.s.	n.s.	*	n.s.	n.a.	n.a
PG ratexLime rate	n.s.	n.s.	n.s.	n.s.	n.a.	n.a
SoilxPG ratexLime rate	*	n.s.	n.s.	n.s.	n.a.	n.a

Asterisks indicate the level of statistical significance (*p <0.05, **p < 0.01, ***p< 0.001); n.s. = not significant; n.a. = not applicable; PG = phosphogypsum; Exch. Al = exchangeable aluminium

N.B. For P and S uptakes, only two factors (1 and 2) and their interactions were considered (a two-way ANOVA was carried out in this case). Lime rate (factor 3) and its interactions with other factors were eliminated in this case because the shoot DM yields accumulated in the absence of lime (0 t ha^{-1}) were very small (less than the required quantity for ICP analysis specifically in Molesworth soil) hence the use of ''n.a.'' abbreviation.

Table E.2 Three way-ANOVA analysis showing the statstical significance of the main effects of three factors: soil type, PS rate and lime rate, and their interactions on soil pH, Olsen P, echangeable Al and plant P and S uptakes.

	pH_{CaCl2}	pH_{water}	Olsen P	Exch. Al	P uptake	S uptake
Soil (factor 1)	n.s.	n.s.	***	**	***	***
PS rate (factor 2)	**	n.s.	***	n.s	n.s.	***
Lime rate (factor 3)	***	***	***	**	n.a.	n.a.
SoilxPG rate	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
SoilxLime rate	*	n.s.	*	n.s.	n.a.	n.a
PG ratexLime rate	n.s.	n.s.	n.s.	n.s.	n.a.	n.a
SoilxPG ratexLime rate	n.s.	n.s.	n.s.	n.s.	n.a.	n.a

Asterisks indicate the level of statistical significance (*p<0.05, **p< 0.01, ***p< 0.001); n.s. = not significant; n.a. = not applicable; PS = P and S soluble fertilizer; Exch. Al = exchangeable aluminium

Table E.3 One-way ANOVA analysis showing the statistical significance of the effect of phosphogypsum application rate on the attributes of four soils separately.

	Molesworth (planted)	Molesworth (incubated)	Glenmore (planted)	Lindis Peaks (incubated)
Soil solution attributes				
pH	**	n.s.	**	**
Ionic strength	n.s.	***	***	***
DOC	n.s.	n.s.	*	n.s.
Ca^{2+}	***	**	***	***
Mg^{2+}	n.s.	**	***	***
K^{+}	n.s.	*	n.s.	***
Na^{+}	n.s.	**	**	***
Zn^{2+}	n.s.	n.s.	***	***
Al^{3+}	**	***	*	***
NO_3^{-}	**	***	n.s.	n.s.
SO_4^{2-}	***	***	***	***
PO_4^{3-}	***	n.s.	**	***
F^{-}	***	**	**	***
Cl^{-}	n.s.	n.s.	n.s.	n.s.
Soil solid phase attributes				
pH_{water}	n.s.	***	*	***
pH_{CaCl2}	**	***	n.s.	*
Al_{KCl}	**	***	n.s.	**
Al_{CaCl2}	n.s.	***	n.s.	n.s.
Total N	n.s.	n.s.	n.s.	n.s.
Total C	n.s.	n.s.	n.s.	n.s.
Exchangeable H^{+}	**	**	n.s.	n.s.
CEC	***	***	***	***
BS (%)	***	***	***	***
Aluminum saturation	***	n.s.	n.s.	**
Olsen P	*	***	n.s.	***
Aluminium species in the soil solution				
Al^{3+}	*	***	**	***
Al-OH	*	***	n.s.	**
Al-SO_4	**	***	***	***
Al-F	**	***	**	***
Al-PO_4	*	n.s.	n.s.	**
Al-DOM	n.s.	**	**	*
Total dissolved Al	**	***	n.s.	***

Asterisks indicate the level of statistical significance (*p<0.05, **p< 0.01, ***p< 0.001); n.s. = not significant

Chapter 4

Table E.4 Two-way ANOVA analysis showing the statistical significance of the main effects of two fators (species and soil pH level) and their interaction on plant and soil properties

	Species	pH	Species×pH
Shoot yield	***	n.s.	n.s.
Root yield	**	n.s.	n.s.
Root:shoot ratio	n.s.	n.s.	n.s.
Shoot P uptake	***	n.s.	n.s.
Rhizosphere soil			
Exchangeable Al (KCl)	**	***	n.s.
AcPME	***	**	n.s.
AlPME	***	*	n.s.
PDE	*	**	n.s.
TOAs	***	n.s.	n.s.
Microbial P	***	***	n.s.
Labile P_i	***	***	n.s.
Labile P_o	***	***	n.s.
Moderately Labile P_i	n.s.	n.s.	n.s.
Moderately Labile P_o	*	n.s.	n.s.
Stable P_i	n.s.	*	n.s.
Stable P_o	***	***	n.s.
Residual P	n.s.	n.s.	*
Bulk soil			
Exchangeable Al (KCl)	n.s.	***	n.s.
Labile P_i	**	**	n.s.
Labile P_o	***	***	n.s.
Moderately Labile P_i	***	n.s.	n.s.
Moderately Labile P_o	n.s.	n.s.	n.s.
Stable P_i	n.s.	n.s.	n.s.
Stable P_o	*	n.s.	n.s.
Residual P	n.s.	n.s.	n.s.

Asterisks indicate the level of statistical significance (*p< 0.05, **p< 0.01, ***p< 0.001); n.s. = not significant; TOAs = Total organic anions; AcPME = Acid phosphomonoesterase; AlPME = Alkaline phosphomonoesterase; PDE = Phosphodiesterase

Chapter 5

Table E.5 Two-way ANOVA analysis showing the statistical significance of the main effects of two fators (soil and pH level) on plant and soil properties

	Soil	pH	Soil×pH
Shoot yield	***	*	n.a.
Shoot P concentration	***	*	n.a.
Shoot P uptake	***	*	n.a.
Root yield	***	n.s.	n.a.
Root morphological traits			
Total root length	***	n.s.	n.a.
Root surface area	***	**	n.a.
Average root diameter	*	**	n.a.
Specific root length	*	n.s.	n.a.
Fine root length	**	*	n.a.
Thick root length	***	**	n.a.
Total organic anions	**	**	n.a.
Exchangeable Al			n.a.
Bulk soil	***	***	n.a.
Rhizosphere soil	***	***	n.a.
pH			n.a.
Bulk soil	n.s.	***	n.a.
Rhizosphere soil	n.s.	***	n.a.

n.a. = not applicable because the interaction cannot be estimated in this case providing that the two soils have different initial pH values (pH 5.3 versus pH 4.7).
Asterisks indicate the level of statistical significance (*p<0.05, **p< 0.01, p< 0.001); n.s. = not significant

Chapter 6

Table E.6 One-way ANOVA analysis showing the effect of lime rate (0, 2, 5 and 10 t ha^{-1}) on soil properties at 0-3 cm depth

	Lime treatment
Soil pH	***
Olsen P	n.s.
Resin P	n.s.
AMN	n.s.
MBP	n.s.
Exchangeable Al (KCl)	*
Exchangeable Al ($CaCl_2$)	n.s.
Total C	n.s.
Absolute activity	
AcPME	n.s.
AlPME	n.s.
PDE	n.s.
Specific activity	
AcPME	*
AlPME	*
PDE	n.s.

Asterisks indicate the level of statistical significance (*p< 0.05, **p< 0.01, ***p< 0.001); n.s. = not significant; AcPME = Acid phosphomonoesterase; AlPME = Alkaline phosphomonoesterase; PDE = Phosphodiesterase; MBP = Microbial biomass phosphorus; ANM = Anaerobic mineralizable nitrogen

References

Abel, S., Nürnberger, T., Ahnert, V., Krauss, G.-J., and Glund, K. (2000). Induction of an extracellular cyclic nucleotide phosphodiesterase as an accessory ribonucleolytic activity during phosphate starvation of cultured tomato cells. *Plant Physiology* 122, 543-552.

Abril, J.-M., García-Tenorio, R., Enamorado, S. M., Hurtado, M. D., Andreu, L., and Delgado, A. (2008). The cumulative effect of three decades of phosphogypsum amendments in reclaimed marsh soils from SW Spain: 226Ra, 238U and Cd contents in soils and tomato fruit. *Science of the Total Environment* 403, 80-88.

Achat, D. L., Augusto, L., Bakker, M. R., Gallet-Budynek, A., and Morel, C. (2012). Microbial processes controlling P availability in forest spodosols as affected by soil depth and soil properties. *Soil Biology and Biochemistry* 44, 39-48.

Achat, D. L., Morel, C., Bakker, M. R., Augusto, L., Pellerin, S., Gallet-Budynek, A., and Gonzalez, M. (2010). Assessing turnover of microbial biomass phosphorus: combination of an isotopic dilution method with a mass balance model. *Soil Biology and Biochemistry* 42, 2231-2240.

Acosta-Martinez, V., and Tabatabai, M. (2000). Enzyme activities in a limed agricultural soil. *Biology and fertility of soils* 31, 85-91.

Adams, F., and Rawajfih, Z. (1977). Basaluminite and alunite: A possible cause of sulfate retention by acid soils. *Soil Science Society of America Journal* 41, 686-692.

Adams, M., Hawke, D., Nilsson, N., and Powell, K. (2000). The relationship between soil solution pH and Al3+ concentrations in a range of South Island (New Zealand) soils. *Soil Research* 38, 141-154.

AĞAR, A. İ. (2011). Reclamation of saline and sodic soil by using divided doses of phosphogypsum in cultivated condition. *African Journal of Agricultural Research* 6, 4243-4252.

Aitken, R. (1992). Relationships between extractable Al, selected soil properties, pH buffer capacity and lime requirement in some acidic Queensland soils. *Soil Research* 30, 119-130.

Al-Enazy, A.-A., Al-Barakah, F., Al-Oud, S., and Usman, A. (2018). Effect of phosphogypsum application and bacteria co-inoculation on biochemical properties and nutrient availability to maize plants in a saline soil. *Archives of Agronomy and Soil Science* 64, 1394-1406.

Alcordo, I. S., and Rechcigl, J. E. (1993). Phosphogypsum in agriculture: A review. *Advances in Agronomy* 49, 55-118.

Alexander, M. (1977). "Introduction to soil microbiology," 2nd/Ed. John Wiley and Sons, New York, USA.

Alleoni, L. R., Cambri, M. A., Caires, E. F., and Garbuio, F. J. (2010). Acidity and aluminum speciation as affected by surface liming in tropical no-till soils. *Soil Science Society of America Journal* 74, 1010-1017.

Allison, S. D. (2006). Soil minerals and humic acids alter enzyme stability: implications for ecosystem processes. *Biogeochemistry* 81, 361-373.

Alva, A., Sumner, M., and Miller, W. (1990). Reactions of gypsum or phosphogypsum in highly weathered acid subsoils. *Soil Science Society of America Journal* 54, 993-998.

Alva, A., Sumner, M., and Noble, A. (1988). Alleviation of aluminum toxicity by phosphogypsum. *Communications in soil science and plant analysis* 19, 385-403.

Álvarez, E., Viadé, A., and Fernández-Marcos, M. (2009). Effect of liming with different sized limestone on the forms of aluminium in a Galician soil (NW Spain). *Geoderma* 152, 1-8.

Alves, L. A., Tiecher, T. L., Flores, J. P. M., Filippi, D., Gatiboni, L. C., Bayer, C., de Castro Pias, O. H., Marquez, A. A., Bordignon, V., and Goulart, R. Z. (2021). Soil chemical properties and crop response to gypsum and limestone on a coarse-textured Ultisol under no-till in the Brazilian Pampa biome. *Geoderma Regional* 25, e00372.

Anderson, J. M., and Ingram, J. (1993). "Tropical soil biology and fertility: a handbook of methods," 2nd/Ed. C.A.B International, Wallingford, UK.

Armstrong, A., and Tanton, T. (1992). Gypsum applications to aggregated saline—sodic clay topsoils. *Journal of Soil Science* 43, 249-260.

Arnold, W., and Hurst, F. (1980). Uranium Control in Phosphogypsum. *In* "International Symposium on Phosphogypsum", Vol. 2, pp. 367-382.

Asmar, F., and Gissel-Nielsen, G. (1997). Extracellular phosphomono-and phosphodiesterase associated with and released by the roots of barley genotypes: a non-destructive method for the measurement of the extracellular enzymes of roots. *Biology and fertility of soils* 25, 117-122.

Asomaning, S. K. (2020). Processes and Factors Affecting Phosphorus Sorption in Soils. *In* "Sorption in 2020s" (G. Kyzas and N. Lazaridis, eds.), pp. 45. IntechOpen, London, UK.

Auler, A. C., Caires, E. F., Pires, L. F., Galetto, S. L., Romaniw, J., and Charnobay, A. C. (2019). Lime effects in a no-tillage system on Inceptisols in Southern Brazil. *Geoderma Regional* 16, e00206.

Azeez, M. O., Christensen, J. T., Ravnskov, S., Heckrath, G. J., Labouriau, R., Christensen, B. T., and Rubæk, G. H. (2020). Phosphorus in an arable coarse sandy soil profile after 74 years with different lime and P fertilizer applications. *Geoderma* 376, 114555.

Baes, C., and Mesmer, R. (1986). "The Hydrolysis of Cations, Reprint edition, original edition 1976 ed," Krieger Publishing Company, Malabar, FLorida, USA.

Bakker, M., Kerisit, R., Verbist, K., and Nys, C. (2000). Effects of liming on rhizosphere chemistry and growth of fine roots and of shoots of sessile oak (Quercus petraea). *In* "The Supporting Roots of Trees and Woody Plants: Form, Function and Physiology", pp. 405-417. Springer.

Barman, M., Shukla, L. M., Datta, S. P., and Rattan, R. K. (2014). Effect of applied lime and boron on the availability of nutrients in an acid soil. *Journal of Plant Nutrition* 37, 357-373.

Barrow, N. (1984). Modelling the effects of pH on phosphate sorption by soils. *Journal of Soil Science* 35, 283-297.

Barrow, N. (2017). The effects of pH on phosphate uptake from the soil. *Plant and soil* 410, 401-410.

Barrow, N. (2020). Comparing two theories about the nature of soil phosphate. *European Journal of Soil Science*.

Barrow, N., Debnath, A., and Sen, A. (2020a). Effect of pH and prior treatment with phosphate on the rate and amount of reaction of soils with phosphate. *European Journal of Soil Science* 72, 243-253.

Barrow, N., Debnath, A., and Sen, A. (2020b). Measurement of the effects of pH on phosphate availability. *Plant and Soil* 454, 217-224.

Barrow, N., Debnath, A., and Sen, A. (2021). Effect of pH and prior treatment with phosphate on the rate and amount of reaction of soils with phosphate. *European Journal of Soil Science* 72, 243-253.

Barrow, N. J. (2021). Comparing two theories about the nature of soil phosphate. *European Journal of Soil Science* 72, 679-685.

Basak, B., and Biswas, D. (2016). Potentiality of Indian rock phosphate as liming material in acid soil. *Geoderma* 263, 104-109.

Beltayef, H., Melki, M., Saidi, W., Hajri, R., Cruz, C., Muscolo, A., and ben Youness, M. (2021). Potential Piriformospora indica effect on growth and mineral nutrition of Phaseolus vulgaris crop under low phosphorus intake. *Journal of Plant Nutrition* 44, 498-507.

Berenji, S., Moot, D., Moir, J., Ridgway, H., and Rafat, A. (2017). Dry matter yield, root traits, and nodule occupancy of lucerne and Caucasian clover when grown in acidic soil with high aluminium concentrations. *Plant and Soil* 416, 227-241.

Bi, S.-P., Zhang, F.-p., Wang, X.-l., and Zou, G.-w. (2001). Analytical methodologies for aluminium speciation in environmental and biological samples–a review. *Fresenius' journal of analytical chemistry* 370, 984-996.

Black, A., and Cameron, K. (1984). Effect of leaching on soil properties and lucerne growth following lime and gypsum amendments to a soil with an acid subsoil. *New Zealand journal of agricultural research* 27, 195-200.

Black, A., Loxton, G., Ryan-Salter, T. P., and Moot, D. J. (2014). Sheep performance on perennial lupins over three years at Sawdon Station, Lake Tekapo. *In* "Proceedings of the New Zealand Grassland Association", pp. 35-40.

Blackmore, L., Searle, P., and Daly, B. (1987). Methods for Chemical Analysis of Soils. New Zealand Soil Bureau Scientific Report No. 80. Wellington, New Zealand: Department of Scientific and Industrial Research.

Blackwell, M., Darch, T., and Haslam, R. (2019). Phosphorus use efficiency and fertilizers: future opportunities for improvements. *Frontiers of Agricultural Science and Engineering* 6, 332-340.

Blakemore, L., Searle, P., and Daly, B. (1987). Methods for chemical analysis of soils. New Zealand Soil Bureau Scientific Report No. 80. pp. 103, Wellington, New Zealand.

Boitt, G., Simpson, Z. P., Tian, J. H., Black, A., Wakelin, S. A., and Condron, L. M. (2018a). Plant biomass management impacts on short-term soil phosphorus dynamics in a temperate grassland. *Biology and Fertility of Soils* 54, 397-409.

Boitt, G., Tian, J., Black, A., Wakelin, S., and Condron, L. (2018b). Effects of long-term irrigation on soil phosphorus under temperate grazed pasture. *European Journal of Soil Science* 69, 95-102.

Bolan, N., Hedley, M., and White, R. (1991a). Processes of soil acidification during nitrogen cycling with emphasis on legume based pastures. *Plant and soil* 134, 53-63.

Bolan, N. S., Adriano, D. C., and Curtin, D. (2003). Soil acidification and liming interactions with nutrient and heavy metal transformation and bioavailability. *Advances in agronomy* 78, 5-272.

Bolan, N. S., and Hedley, M. J. (2003). Role of carbon, nitrogen, and sulfur cycles in soil acidification. *In* "Handbook of soil acidity" (Z. Rengel, ed.), pp. 29-56. Marcel Dekker, Inc., New York, USA.

Bolan, N. S., Hedley, M. J., and White, R. E. (1991b). Processes of soil acidification during nitrogen cycling with emphasis on legume based pastures. *Plant and Soil* 134, 53-63.

Bouray, M., Moir, J., Condron, L., and Lehto, N. (2020). Impacts of phosphogypsum, soluble fertilizer and lime amendment of acid soils on the bioavailability of phosphorus and sulphur under lucerne (*Medicago sativa*). *Plants* 9, 1-17.

Bowatte, S., Tillman, R., Carran, A., and Gillingham, A. (2006). Can phosphorus fertilisers alone increase levels of soil nitrogen in New Zealand hill country pastures? *Nutrient Cycling in Agroecosystems* 75, 57-66.

Bowden, J., Nagarajah, S., Barrow, N., Posner, A., and Quirk, J. (1980). Describing the adsorption of phosphate, citrate and selenite on a variable-charge mineral surface. *Soil Research* 18, 49-60.

Bowman, R., and Moir, J. (1993). Basic EDTA as an extractant for soil organic phosphorus. *Soil Science Society of America Journal* 57, 1516-1518.

Brady, D., Edwards, D., Asher, C., and Blamey, F. (1993). Calcium amelioration of aluminium toxicity effects on root hair development in soybean [Glycine max (L.) Merr.]. *New phytologist* 123, 531-538.

Brautigan, D., Rengasamy, P., and Chittleborough, D. (2012). Aluminium speciation and phytotoxicity in alkaline soils. *Plant and Soil* 360, 187-196.

Brookes, P. C., Powlson, D., and Jenkinson, D. (1984). Phosphorus in the soil microbial biomass. *Soil Biology and Biochemistry* 16, 169-175.

Brookes, P. C., Powlson, D. S., and Jenkinson, D. S. (1982). Measurement of microbial biomass phosphorus in soil. *Soil Biology and Biochemistry* 14, 319-329.

Browman, M., and Tabatabai, M. (1978). Phosphodiesterase activity of soils. *Soil Science Society of America Journal* 42, 284-290.

Brown, I. C. (1943). A rapid method of determining exchangeable hydrogen and total exchangeable bases of soils. *Soil Science* 56, 353-358.

Brown, T. T., Koenig, R. T., Huggins, D. R., Harsh, J. B., and Rossi, R. E. (2008). Lime effects on soil acidity, crop yield, and aluminum chemistry in direct-seeded cropping systems. *Soil Science Society of America Journal* 72, 634-640.

Brownlie, W. J., Sutton, M. A., Reay, D. S., Heal, K. V., Hermann, L., Kabbe, C., and Spears, B. M. (2021). Global actions for a sustainable phosphorus future. *Nature Food* 2, 71-74.

Caires, E., Garbuio, F., Churka, S., Barth, G., and Corrêa, J. (2008). Effects of soil acidity amelioration by surface liming on no-till corn, soybean, and wheat root growth and yield. *European Journal of Agronomy* 28, 57-64.

Caires, E. F., Alleoni, L. R., Cambri, M. A., and Barth, G. (2005). Surface application of lime for crop grain production under a no-till system. *Agronomy Journal* 97, 791-798.

Caires, E. F., Garbuio, F. J., Churka, S., and Joris, H. A. (2011). Use of Gypsum for Crop Grain Production under a Subtropical No-Till Cropping System. *Agronomy Journal* 103, 1804-1814.

Caires, E. F., and Guimarães, A. M. (2018). A Novel Phosphogypsum Application Recommendation Method under Continuous No-Till Management in Brazil. *Agronomy Journal* 110, 1987-1995.

Calba, H., Cazevieille, P., Thée, C., and Poss, R. (2004). The dynamics of protons, aluminium, and calcium in the rhizosphere of maize cultivated in tropical acid soils: experimental study and modelling. *Plant and soil* 260, 33-46.

Cao, X., Hu, P., Tan, C., Wu, L., Peng, B., Christie, P., and Luo, Y. (2018). Effects of a natural sepiolite bearing material and lime on the immobilization and persistence of cadmium in a contaminated acid agricultural soil. *Environmental Science and Pollution Research* 25, 22075-22084.

Carey, P., and Metherell, A. (2003). Rates of release of non-exchangeable potassium in New Zealand soils measured by a modified sodium tetraphenyl-boron method. *New Zealand Journal of Agricultural Research* 46, 185-197.

Carmeis Filho, A. C., Penn, C. J., Crusciol, C. A., and Calonego, J. C. (2017). Lime and phosphogypsum impacts on soil organic matter pools in a tropical Oxisol under long-term no-till conditions. *Agriculture, Ecosystems & Environment* 241, 11-23.

Carvalho, M. C. S., and van Raij, B. (1997). Calcium sulphate, phosphogypsum and calcium carbonate in the amelioration of acid subsoils for root growth. *Plant and Soil* 192, 37-48.

Chamier, J., Wicht, M., Cyster, L., and Ndindi, N. P. (2015). Aluminium (Al) fractionation and speciation; getting closer to describing the factors influencing Al3+ in water impacted by acid mine drainage. *Chemosphere* 130, 17-23.

Chen, C., Condron, L., Davis, M., and Sherlock, R. (2002). Phosphorus dynamics in the rhizosphere of perennial ryegrass (Lolium perenne L.) and radiata pine (Pinus radiata D. Don.). *Soil Biology and Biochemistry* 34, 487-499.

Chen, C. R., Sinaj, S., Condron, L. M., Frossard, E., Sherlock, R. R., and Davis, M. R. (2003). Characterization of phosphorus availability in selected New Zealand grassland soils. *Nutrient Cycling in Agroecosystems* 65, 89-100.

Chen, M., and Graedel, T. (2016). A half-century of global phosphorus flows, stocks, production, consumption, recycling, and environmental impacts. *Global Environmental Change* 36, 139-152.

Chen, Y. L., Dunbabin, V. M., Diggle, A. J., Siddique, K. H., and Rengel, Z. (2013). Phosphorus starvation boosts carboxylate secretion in P-deficient genotypes of *Lupinus angustifolius* with contrasting root structure. *Crop and Pasture Science* 64, 588-599.

Chen, Z. C., and Liao, H. (2016). Organic acid anions: an effective defensive weapon for plants against aluminum toxicity and phosphorus deficiency in acidic soils. *Journal of Genetics and Genomics* 43, 631-638.

Cheng, L., Bucciarelli, B., Shen, J., Allan, D., and Vance, C. (2011). Update on White Lupin Cluster Roots: Acclimation to Phosphorus Deficiency. *Plant physiology*, 1025-1032.

Cheng, Y., Howieson, J. G., O'Hara, G. W., Watkin, E. L. J., Souche, G., Jaillard, B., and Hinsinger, P. (2004). Proton release by roots of *Medicago murex* and *Medicago sativa* growing in acidic conditions, and implications for rhizosphere pH changes and nodulation at low pH. *Soil Biology and Biochemistry* 36, 1357-1365.

Chernysh, Y., Yakhnenko, O., Chubur, V., and Roubík, H. (2021). Phosphogypsum Recycling: A Review of Environmental Issues, Current Trends, and Prospects. *Applied Sciences* 11, 1575.

Cioccio, S., Gopalapillai, Y., Dan, T., and Hale, B. (2017). Effect of liming on nickel bioavailability and toxicity to oat and soybean grown in field soils containing aged emissions from a nickel refinery. *Environmental toxicology and chemistry* 36, 1110-1119.

Clarkson, D. T. (1985). Factors affecting mineral nutrient acquisition by plants. *Annual review of plant physiology* 36, 77-115.

Clements, J., White, P., and Buirchell, B. (1993). The root morphology of *Lupinus angustifolius* in relation to other *Lupinus* species. *Australian Journal of Agricultural Research* 44, 1367-1375.

Cochrane, B., Reichert, J., Eltz, F., and Norton, L. (2005). Controlling soil erosion and runoff with polyacrylamide and phosphogypsum on subtropical soil. *Transactions of the Asae* 48, 149-154.

Condron, L., Cornforth, I., Davis, M., and Newman, R. (1996). Influence of conifers on the forms of phosphorus in selected New Zealand grassland soils. *Biology and fertility of soils* 21, 37-42.

Condron, L., and Goh, K. (1989). Effects of long-term phosphatic fertilizer applications on amounts and forms of phosphorus in soils under irrigated pasture in New Zealand. *Journal of soil science* 40, 383-395.

Condron, L., and Goh, K. (1990). Nature and availability of residual phosphorus in longterm fertilized pasture soils in New Zealand. *The Journal of Agricultural Science* 114, 1-9.

Condron, L., Tiessen, H., Trasar-Cepeda, C., Moir, J., and Stewart, J. (1993). Effects of liming on organic matter decomposition and phosphorus extractability in an acid humic Ranker soil from northwest Spain. *Biology and Fertility of Soils* 15, 279-284.

Condron, L. M., and Newman, S. (2011). Revisiting the fundamentals of phosphorus fractionation of sediments and soils. *Journal of Soils and Sediments* 11, 830-840.

Condron, L. M., Turner, B. L., and Cade-Menun, B. J. (2005). Chemistry and dynamics of soil organic phosphorus. *In* "Phosphorus: agriculture and the environment" (J. T. Sims and A. N. Sharpley, eds.), Vol. 46, pp. 87-121. American Society of Agronomy, USA.

Conyers, M., Scott, B., Fisher, R., and Lill, W. (1995). Predicting the field performance of twelve commercial liming materials from southern Australia. *Fertilizer research* 44, 151-161.

Craighead, M. (2005). The effect of two lime sources on short-term changes in soil pH under Marlborough hill country pasture. *In* "Proceedings of the New Zealand Grassland Association", pp. 155-162.

Craighead, M., and Metherell, A. (2006). The impact of the form and frequency of sulphur on pasture yield and composition in South Island high country. *In* "New Zealand Grassland Association Conference", Vol. 68, pp. 361-367. New Zealand Grassland Association, Dunedin, NZ.

Cross, A. F., and Schlesinger, W. H. (1995). A literature review and evaluation of the. Hedley fractionation: Applications to the biogeochemical cycle of soil phosphorus in natural ecosystems. *Geoderma* 64, 197-214.

Crusciol, C. A., Artigiani, A. C., Arf, O., Carmeis Filho, A. C., Soratto, R. P., Nascente, A. S., and Alvarez, R. C. (2016). Surface application of lime–silicate–phosphogypsum mixtures for improving tropical soil properties and irrigated common bean yield. *Soil Science Society of America Journal* 80, 930-942.

Cunha, G. O. d. M., Almeida, J. A. d., Ernani, P. R., Pereira, É. R., Skoronski, É., Lourenço, L. S., and Brunetto, G. (2018). Chemical species and aluminum concentration in the solution of acid soils cultivated with soybean and corn under liming. *Revista Brasileira de Ciência do Solo* 42, 1-16.

Curtin, D., and Syers, J. (2001). Lime-induced changes in indices of soil phosphate availability. *Soil Science Society of America Journal* 65, 147-152.

Curtin, D., and Trolove, S. (2013). Predicting pH buffering capacity of New Zealand soils from organic matter content and mineral characteristics. *Soil Research* 51, 494-502.

Da Conceicao, F. T., and Bonotto, D. M. (2006). Radionuclides, heavy metals and fluorine incidence at Tapira phosphate rocks, Brazil, and their industrial (by) products. *Environmental Pollution* 139, 232-243.

Dai, Y., Zheng, H., Jiang, Z., and Xing, B. (2020). Combined effects of biochar properties and soil conditions on plant growth: A meta-analysis. *Science of the Total Environment* 713, 136635.

Davis, M. (1991). The comparative phosphorus requirements of some temperate perennial legumes. *Plant and soil* 133, 17-30.

Davison, W., and Zhang, H. (2016). Introduction to DGT. *In* "Diffusive Gradients in Thin-Films for Environmental Measurements" (W. Davison, ed.), pp. 1-9. Cambridge University Press, Cambridge.

de la Luz Mora, M., Pinilla, L., Rosas, A., and Cartes, P. (2008). Selenium uptake and its influence on the antioxidative system of white clover as affected by lime and phosphorus fertilization. *Plant and Soil* 303, 139-149.

de Medeiros, E. V., de Alcantara Notaro, K., de Barros, J. A., da Silva Moraes, W., Silva, A. O., and Moreira, K. A. (2015). Absolute and specific enzymatic activities of sandy entisol from tropical dry forest, monoculture and intercropping areas. *Soil and Tillage Research* 145, 208-215.

de Souza Cardoso, A. A., Santos, J. Z. L., Oka, J. M., Ferreira, M. d. S., Barbosa, T. M. B., and Tucci, C. A. F. (2020). Ammonium supply enhances growth and phosphorus uptake of mahogany (Swietenia macrophylla) seedlings compared to nitrate. *Journal of Plant Nutrition*, 1-16.

Degirmenci, N., Okucu, A., and Turabi, A. (2007). Application of phosphogypsum in soil stabilization. *Building and environment* 42, 3393-3398.

Degryse, F., and McLaughlin, M. J. (2014). Phosphorus diffusion from fertilizer: visualization, chemical measurements, and modeling. *Soil Science Society of America Journal* 78, 832-842.

Degryse, F., Smolders, E., and Parker, D. (2009). Partitioning of metals (Cd, Co, Cu, Ni, Pb, Zn) in soils: concepts, methodologies, prediction and applications–a review. *European Journal of Soil Science* 60, 590-612.

Delhaize, E., and Ryan, P. R. (1995). Aluminum toxicity and tolerance in plants. *Plant physiology* 107, 315.

Dick, W., and Tabatabai, M. (1977a). Determination of orthophosphate in aqueous solutions containing labile organic and inorganic phosphorus compounds. *Journal of Environmental Quality* 6, 82-85.

Dick, W., and Tabatabai, M. (1977b). Determination of Orthophosphate in Aqueous Solutions Containing Labile Organic and Inorganic Phosphorus Compounds 1. *Journal of Environmental Quality* 6, 82-85.

Ding, S., Wang, Y., Xu, D., Zhu, C., and Zhang, C. (2013). Gel-based coloration technique for the submillimeter-scale imaging of labile phosphorus in sediments and soils with diffusive gradients in thin films. *Environmental science & technology* 47, 7821-7829.

Ding, S., Xu, D., Sun, Q., Yin, H., and Zhang, C. (2010). Measurement of dissolved reactive phosphorus using the diffusive gradients in thin films technique with a high-capacity binding phase. *Environmental science & technology* 44, 8169-8174.

Dissanayaka, D., Wickramasinghe, W., Marambe, B., and Wasaki, J. (2017). Phosphorus-mobilization strategy based on carboxylate exudation in lupins (*Lupinus*, Fabaceae): a mechanism facilitating the growth and phosphorus acquisition of neighbouring plants under phosphorus-limited conditions. *Experimental Agriculture* 53, 308-319.

Dodd, M., and Ledgard, S. (1999). Long term (?) effects of withholding superphosphate application on North Island hill country: a 10-year update. *In* "Proceedings of the New Zealand Grassland Association", pp. 63-68.

Duputel, M., Van Hoye, F., Toucet, J., and Gérard, F. (2013). Citrate adsorption can decrease soluble phosphate concentration in soil: Experimental and modeling evidence. *Applied geochemistry* 39, 85-92.

During, C., Jackson, B., and Dyson, C. (1984). Lime effects on hill country: 2. Relation of soil moisture, pH, and aluminium values with pasture yield. *New Zealand journal of agricultural research* 27, 531-535.

Edmeades, D., Pringle, R., Mansell, G., Shannon, P., Ritchie, J., and Stewart, K. (1985). Effects of lime on pasture production on soils in the North Island of New Zealand 5. Description of a lime recommendation scheme. *New Zealand journal of experimental agriculture* 13, 47-58.

Edmeades, D., Pringle, R., Shannon, P., and Mansell, G. (1984). Effects of lime on pasture production on soils in the North Island of New Zealand: 4. Predicting lime responses. *New Zealand journal of agricultural research* 27, 371-382.

Edmeades, D., Smart, C., and Wheeler, D. (1983). Aluminium toxicity in New Zealand soils: preliminary results on the development of diagnostic criteria. *New Zealand journal of agricultural research* 26, 493-501.

Edmeades, D. C., McBride, R. M., and Gray, M. (2016). An assessment of current fertiliser practices in New Zealand hill country. *In* "Hill Coutry Symposium" (E. R. Thom, ed.), Vol. 16, pp. 173-178. New Zealand Grassland Association Rotorua, New Zealand.

Eivazi, F., and Tabatabai, M. (1977). Phosphatases in soils. *Soil biology and biochemistry* 9, 167-172.

Ekenler, M., and Tabatabai, M. (2003). Responses of phosphatases and arylsulfatase in soils to liming and tillage systems. *Journal of Plant Nutrition and Soil Science* 166, 281-290.

El Zrelli, R., Rabaoui, L., Daghbouj, N., Abda, H., Castet, S., Josse, C., van Beek, P., Souhaut, M., Michel, S., and Bejaoui, N. (2018). Characterization of phosphate rock and phosphogypsum from Gabes

phosphate fertilizer factories (SE Tunisia): high mining potential and implications for environmental protection. *Environmental Science and Pollution Research* 25, 14690-14702.

Elser, J., and Haygarth, P. (2020). Phosphorus living. *In* "Phosphorus: Past and Future". Oxford University Press, Madison, USA.

Eslamian, F., Qi, Z., and Qian, C. (2021). Lime Amendments to Enhance Soil Phosphorus Adsorption Capacity and to Reduce Phosphate Desorption. *Water, Air, & Soil Pollution* 232, 1-14.

Eslamian, F., Qi, Z., Tate, M. J., and Romaniuk, N. (2020). Lime application to reduce phosphorus release in different textured intact and small repacked soil columns. *Journal of Soils and Sediments*, 1-14.

Eswaran, H., Reich, P., and Beinroth, F. (1997). Global distribution of soils with acidity. *In* "Plant-Soil interactions at low pH" (A. Moniz, A. Furlani, R. Schaffert, N. Fageria, C. Rosolem and H. Cantarella, eds.), pp. 159-164. Brazilian Soil Science Society, Campinas, Brazil.

Evans Jr, A., Zelazny, L., and Zipper, C. (1988). Solution parameters influencing dissolved organic carbon levels in three forest soils. *Soil Science Society of America Journal* 52, 1789-1792.

Fageria, N., and Baligar, V. (2008). Ameliorating soil acidity of tropical Oxisols by liming for sustainable crop production. *Advances in agronomy* 99, 345-399.

Fairhust, T. (1999). The importance, distribution and causes of phosphorus deficiency as a constraint to crop production in the tropics. *In* "Agroforestry Forum", Vol. 9, pp. 2-8.

FAO, and ITPS (2015). "Status of the world's soil resources (SWSR)–main report." Food and agriculture organization of the United Nations and intergovernmental technical panel on soils, Rome, Italy.

Fierer, N., Schimel, J. P., and Holden, P. A. (2003). Variations in microbial community composition through two soil depth profiles. *Soil Biology and Biochemistry* 35, 167-176.

Fox, T., and Comerford, N. (1992). Rhizosphere phosphatase activity and phosphatase hydrolyzable organic phosphorus in two forested spodosols. *Soil Biology and Biochemistry* 24, 579-583.

Fuentes, J. P., Bezdicek, D. F., Flury, M., Albrecht, S., and Smith, J. L. (2006). Microbial activity affected by lime in a long-term no-till soil. *Soil and Tillage Research* 88, 123-131.

Gahoonia, T. S., and Nielsen, N. E. (1992). The effects of root-induced pH changes on the depletion of inorganic and organic phosphorus in the rhizosphere. *Plant and Soil* 143, 185-191.

Garcia, G. V., Campos, M. E., Wyngaard, N., Reussi-Calvo, N. I., San Martino, S., Covacevich, F., and Studdert, G. A. (2021). Anaerobically mineralized nitrogen within macroaggregates as a soil health indicator. *Catena* 198, 105034.

Garcia, G. V., Wyngaard, N., Calvo, N. I. R., San Martino, S., Covacevich, F., and Studdert, G. A. (2020). Soil survey reveals a positive relationship between aggregate stability and anaerobically mineralizable nitrogen. *Ecological Indicators* 117, 106640.

Gatiboni, L. C., Schmitt, D. E., Tiecher, T., Veloso, M. G., dos Santos, D. R., Kaminski, J., and Brunetto, G. (2021). Plant uptake of legacy phosphorus from soils without P fertilization. *Nutrient Cycling in Agroecosystems* 119, 139-151.

George, T., Turner, B. L., Gregory, P., Cade-Menun, B., and Richardson, A. (2006). Depletion of organic phosphorus from Oxisols in relation to phosphatase activities in the rhizosphere. *European Journal of Soil Science* 57, 47-57.

George, T. S., Fransson, A.-M., Hammond, J. P., and White, P. J. (2011). Phosphorus nutrition: rhizosphere processes, plant response and adaptations. *In* "Phosphorus in action" (E. Bünemann, A. Oberson and E. Frossard, eds.), pp. 245-271. Soil Biology book series, Springer, Berlin, Heidelberg.

Gerke, J. (1994). Aluminum complexation by humic substances and aluminum species in the soil solution. *Geoderma* 63, 165-175.

Gerke, J., Römer, W., and Jungk, A. (1994). The excretion of citric and malic acid by proteoid roots of *Lupinus albus L.*; effects on soil solution concentrations of phosphate, iron, and aluminum in the proteoid rhizosphere in samples of an oxisol and a luvisol. *Zeitschrift für Pflanzenernährung und Bodenkunde* 157, 289-294.

Gessa, C. E., Mimmo, T., Deiana, S., and Marzadori, C. (2005). Effect of aluminium and pH on the mobility of phosphate through a soil-root interface model. *Plant and soil* 272, 301-311.

Giesler, R., Moldan, F., Lundström, U., and Hultberg, H. (1996). Reversing acidification in a forested catchment in southwestern Sweden: Effects on soil solution chemistry. *Journal of environmental quality* 25, 110-119.

Giles, C., Dupuy, L., Boitt, G., Brown, L., Condron, L., Darch, T., Blackwell, M., Menezes-Blackburn, D., Shand, C., and Stutter, M. (2018). Root development impacts on the distribution of phosphatase activity: Improvements in quantification using soil zymography. *Soil Biology and Biochemistry* 116, 158-166.

Gillingham, A., Gray, M., and Smith, D. (1998). Pasture responses to phosphorus and nitrogen fertilisers on dry hill country. *In* "Proceedings of the New Zealand Grassland Association", pp. 135-140.

Gillingham, A., Maber, J., Morton, J., and Tuohy, M. (1999). Precise aerial fertiliser application on hill country. *In* "Proceedings of the New Zealand Grassland Association", pp. 221-226.

Griffin, G. (1971). Effect of Liming on the Soil Test Level of Phosphorous as Determined By Three Methods. *Soil Science Society of America Journal* 35, 540-542.

Guan, D.-X., Williams, P. N., Luo, J., Zheng, J.-L., Xu, H.-C., Cai, C., and Ma, L. Q. (2015). Novel precipitated zirconia-based DGT technique for high-resolution imaging of oxyanions in waters and sediments. *Environmental science & technology* 49, 3653-3661.

Guo, J., Vogt, R. D., Zhang, X., Zhang, Y., Seip, H. M., and Tang, H. (2004). Ca-H-Al exchanges and aluminium mobility in two Chinese acidic forest soils: a batch experiment. *Environmental Geology* 45, 1148-1153.

Guo, J., Zhang, X., Vogt, R. D., Xiao, J., Zhao, D., Xiang, R., and Luo, J. (2007). Evaluating main factors controlling aluminum solubility in acid forest soils, southern and southwestern China. *Applied Geochemistry* 22, 388-396.

Gupta, N., Gaurav, S. S., and Kumar, A. (2013). Molecular basis of aluminium toxicity in plants: a review. *American Journal of Plant Sciences* 2013.

Gustafsson, J. P. (2001). Modelling competitive anion adsorption on oxide minerals and an allophane-containing soil. *European Journal of Soil Science* 52, 639-653.

Gustafsson, J. P. (2020). Visual MINTEQ version 3.1. https://vminteq.lwr.kth.se/download/KTH (accessed 10 August 2020). Royal Institute of Technology, Stockholm, Sweden.

Hafner, H., Ndunguru, B., Bationo, A., and Marschner, H. (1992). Effect of nitrogen, phosphorus and molybdenum application on growth and symbiotic N 2-fixation of groundnut in an acid sandy soil in Niger. *Fertilizer Research* 31, 69-77.

Hale, B., Evans, L., and Lambert, R. (2012). Effects of cement or lime on Cd, Co, Cu, Ni, Pb, Sb and Zn mobility in field-contaminated and aged soils. *Journal of hazardous materials* 199, 119-127.

Haling, R. E., Brown, L. K., Stefanski, A., Kidd, D. R., Ryan, M. H., Sandral, G. A., George, T. S., Lambers, H., and Simpson, R. J. (2018). Differences in nutrient foraging among *Trifolium subterraneum* cultivars deliver improved P-acquisition efficiency. *Plant and Soil* 424, 539-554.

Halstead, R., Lapensee, J., and Ivarson, K. (1963). Mineralization of soil organic phosphorus with particular reference to the effect of lime. *Canadian Journal of Soil Science* 43, 97-106.

Hamilton, E. J., Miles, R. J., Lukaszewska, K., Remley, M., Massie, M., and Blevins, D. G. (2012). Liming of two acidic soils improved grass tetany ratio of stockpiled tall fescue without increasing plant available phosphorus. *Journal of plant nutrition* 35, 497-510.

Hammond, J. P., Broadley, M. R., and White, P. J. (2004). Genetic responses to phosphorus deficiency. *Annals of botany* 94, 323-332.

Han, T., Cai, A., Liu, K., Huang, J., Wang, B., Li, D., Qaswar, M., Feng, G., and Zhang, H. (2019). The links between potassium availability and soil exchangeable calcium, magnesium, and aluminum are mediated by lime in acidic soil. *Journal of Soils and Sediments* 19, 1382-1392.

Harrison, A. (1982). Labile organic phosphorus mineralization in relationship to soil properties. *Soil Biology and Biochemistry* 14, 343-351.

Harrison, A. (1983). Relationship between intensity of phosphatase activity and physico-chemical properties in woodland soils. *Soil Biology and Biochemistry* 15, 93-99.

Hawke, D. J., Powell, K. J., and Gregor, J. E. (1996). Determination of the aluminium complexing capacity of fulvic acids and natural waters, with examples from five New Zealand rivers. *Marine and freshwater research* 47, 11-17.

Hayano, K. (1977). Extraction and properties of phosphodiesterase from a forest soil. *Soil Biology and Biochemistry* 9, 221-223.

Hayes, R. C., Li, G. D., Conyers, M. K., Virgona, J. M., and Dear, B. S. (2016). Lime increases productivity and the capacity of lucerne (*Medicago sativa L.*) and phalaris (Phalaris aquatica L.) to utilise stored soil water on an acidic soil in south-eastern Australia. *Plant and Soil* 400, 29-43.

Haynes, R., and Ludecke, T. (1981). Effect of lime and phosphorus applications on concentrations of available nutrients and on P, Al and Mn uptake by two pasture legumes in an acid soil. *Plant and soil* 62, 117-128.

Haynes, R., and Williams, P. (1993). Nutrient cycling and soil fertility in the grazed pasture ecosystem. *Advances in agronomy* 49, 119-199.

Haynes, R. J. (1982). Effects of liming on phosphate availability in acid soils - a critical-review. *Plant and Soil* 68, 289-308.

He, Z., and Honeycutt, C. W. (2005). A modified molybdenum blue method for orthophosphate determination suitable for investigating enzymatic hydrolysis of organic phosphates. *Communications in Soil Science and Plant Analysis* 36, 1373-1383.

Hedley, M. J., Stewart, J., and Chauhan, B. (1982). Changes in inorganic and organic soil phosphorus fractions induced by cultivation practices and by laboratory incubations 1. *Soil Science Society of America Journal* 46, 970-976.

Hedley, M. J., and Stewart, J. W. B. (1982). Method to measure microbial phosphate in soils. *Soil Biology and Biochemistry* 14, 377-385.

Helal, H. M., and Sauerbeck, D. (1989). Carbon turnover in the rhizosphere. *Zeitschrift für Pflanzenernährung und Bodenkunde* 152, 211-216.

Hendrie, D. L., Moir, J. L., Boitt, G., Simpson, Z. P., and Condron, L. M. (2021). Investigating the relationships between soil acidity and phosphorus fractions in high country farmland of New Zealand's South Island. *Soil Research*.

Hendrie, D. L., Moir, J. L., Stevens, E. J., Black, A. D., and Moot, D. J. (2018). Soil pH, exchangeable aluminium and legume yield responses to deep-placed lime at Omarama Station. *Journal of New Zealand Grasslands*, 137-144.

Hentati, O., Abrantes, N., Caetano, A. L., Bouguerra, S., Gonçalves, F., Römbke, J., and Pereira, R. (2015). Phosphogypsum as a soil fertilizer: Ecotoxicity of amended soil and elutriates to bacteria, invertebrates, algae and plants. *Journal of hazardous materials* 294, 80-89.

Herbien, S., and Neal, J. (1990). Soil pH and phosphatase activity. *Communications in soil science and plant analysis* 21, 439-456.

Hermans, C., Hammond, J. P., White, P. J., and Verbruggen, N. (2006). How do plants respond to nutrient shortage by biomass allocation? *Trends in plant science* 11, 610-617.

Hewitt, A. E. (2010). "New Zealand soil classification," Rep. No. 1172-269X. Manaaki Whenua Press, Landcare Research Lincoln, NZ.

Hiemstra, T., and Van Riemsdijk, W. (1999). Surface structural ion adsorption modeling of competitive binding of oxyanions by metal (hydr) oxides. *Journal of colloid and interface science* 210, 182-193.

Hill, J., Simpson, R., Moore, A., and Chapman, D. (2006). Morphology and response of roots of pasture species to phosphorus and nitrogen nutrition. *Plant and Soil* 286, 7-19.

Hinsinger, P. (2001). Bioavailability of soil inorganic P in the rhizosphere as affected by root-induced chemical changes: a review. *Plant and soil* 237, 173-195.

Hinsinger, P., Brauman, A., Devau, N., Gérard, F., Jourdan, C., Laclau, J.-P., Le Cadre, E., Jaillard, B., and Plassard, C. (2011). Acquisition of phosphorus and other poorly mobile nutrients by roots. Where do plant nutrition models fail? *Plant and Soil* 348, 29.

Hinsinger, P., Herrmann, L., Lesueur, D., Robin, A., Trap, J., Waithaisong, K., and Plassard, C. (2018). Impact of roots, microorganisms and microfauna on the fate of soil phosphorus in the rhizosphere. *Annual Plant Reviews online*, 377-407.

Hinsinger, P., Plassard, C., Tang, C., and Jaillard, B. (2003). Origins of root-mediated pH changes in the rhizosphere and their responses to environmental constraints: a review. *Plant and soil* 248, 43-59.

Hocking, P., and Jeffery, S. (2004). Cluster-root production and organic anion exudation in a group of old-world lupins and a new-world lupin. *Plant and Soil* 258, 135-150.

Holland, J., Bennett, A., Newton, A., White, P., McKenzie, B., George, T., Pakeman, R., Bailey, J., Fornara, D., and Hayes, R. (2018). Liming impacts on soils, crops and biodiversity in the UK: A review. *Science of the Total Environment* 610, 316-332.

Holland, J., White, P., Glendining, M., Goulding, K., and McGrath, S. (2019). Yield responses of arable crops to liming–An evaluation of relationships between yields and soil pH from a long-term liming experiment. *European Journal of Agronomy* 105, 176-188.

Honvault, N., Houben, D., Nobile, C., Firmin, S., Lambers, H., and Faucon, M.-P. (2020). Tradeoffs among phosphorus-acquisition root traits of crop species for agroecological intensification. *Plant and Soil*, 1-14.

Horsnell, L. (1985). The growth of improved pastures on acid soils. 3. Response of lucerne to phosphate as affected by calcium and potassium sulfates and soil aluminium levels. *Australian journal of experimental agriculture* 25, 557-561.

Hou, E., Luo, Y., Kuang, Y., Chen, C., Lu, X., Jiang, L., Luo, X., and Wen, D. (2020). Global meta-analysis shows pervasive phosphorus limitation of aboveground plant production in natural terrestrial ecosystems. *Nature Communications* 11, 637.

Houben, D., Pircar, J., and Sonnet, P. (2012). Heavy metal immobilization by cost-effective amendments in a contaminated soil: effects on metal leaching and phytoavailability. *Journal of Geochemical Exploration* 123, 87-94.

Hoyt, P., and Nyborg, M. (1972). Use of dilute calcium chloride for the extraction of plant-available aluminum and manganese from acid soil. *Canadian journal of soil science* 52, 163-167.

Hue, N., Adams, F., and Evans, C. (1985). Sulfate Retention by an Acid BE Horizon of an Ultisol. *Soil Science Society of America Journal* 49, 1196-1200.

Hui, D., Mayes, M. A., and Wang, G. (2013). Kinetic parameters of phosphatase: a quantitative synthesis. *Soil Biology and Biochemistry* 65, 105-113.

Hummel, C., Boitt, G., Santner, J., Lehto, N. J., Condron, L., and Wenzel, W. W. (2021). Co-occurring increased phosphatase activity and labile P depletion in the rhizosphere of *Lupinus angustifolius* assessed with a novel, combined 2D-imaging approach. *Soil Biology and Biochemistry* 153, 107963.

Hütsch, B. W., Augustin, J., and Merbach, W. (2002). Plant rhizodeposition—an important source for carbon turnover in soils. *Journal of plant nutrition and soil science* 165, 397-407.

IAEA (2013). "Radiation Protection and Management of NORM Residues in the Phosphate Industry." International Atomic Energy Agency, Vienna, Austria.

Illera, V., Garrido, F., Vizcayno, C., and García-González, M. (2004). Field application of industrial by-products as Al toxicity amendments: chemical and mineralogical implications. *European journal of soil science* 55, 681-692.

Inagaki, T. M., de Moraes Sá, J. C., Caires, E. F., and Gonçalves, D. R. P. (2016). Lime and gypsum application increases biological activity, carbon pools, and agronomic productivity in highly weathered soil. *Agriculture, Ecosystems & Environment* 231, 156-165.

Iversen, C. M., McCormack, M. L., Powell, A. S., Blackwood, C. B., Freschet, G. T., Kattge, J., Roumet, C., Stover, D. B., Soudzilovskaia, N. A., and Valverde-Barrantes, O. J. (2017). A global Fine-Root Ecology Database to address below-ground challenges in plant ecology. *New Phytologist* 215, 15-26.

Jaillard, B., Plassard, C., and Hinsinger, P. (2003). Measurements of H^+ fluxes and concentrations in the rhizosphere. *In* "Handbook of Soil Acidity" (Z. Rengel, ed.), pp. 231-266.

Jarak, M., Belic, M., Govedarica, M., Milosevic, N., and Djuric, S. (2003). Effect of phosphogypsum and peat on microbiological and chemical properties of arenosol. *Acta Biologica Iugoslavica. Serija A*.

Jarrell, W., and Beverly, R. (1981). The dilution effect in plant nutrition studies. *Advances in agronomy* 34, 197-224.

Jeffery, R. P., Simpson, R. J., Lambers, H., Kidd, D. R., and Ryan, M. H. (2016). Root morphology acclimation to phosphorus supply by six cultivars of *Trifolium subterraneum L*. *Plant and Soil* 412, 21-34.

Jeffery, R. P., Simpson, R. J., Lambers, H., Kidd, D. R., and Ryan, M. H. (2017). Plants in constrained canopy micro-swards compensate for decreased root biomass and soil exploration with increased amounts of rhizosphere carboxylates. *Functional Plant Biology* 44, 552-562.

Jessop, R., and Mahoney, J. (1982). Effects of lime on the growth and nodulation of four grain legumes. *Soil Research* 20, 265-268.

Johnston, A., Goulding, K., and Poulton, P. (1986). Soil acidification during more than 100 years under permanent grassland and woodland at Rothamsted. *Soil use and management* 2, 3-10.

Jones, A. M., Collins, R. N., and Waite, T. D. (2011). Mineral species control of aluminum solubility in sulfate-rich acidic waters. *Geochimica et Cosmochimica Acta* 75, 965-977.

Jones, D., and Brassington, D. (1998). Sorption of organic acids in acid soils and its implications in the rhizosphere. *European Journal of Soil Science* 49, 447-455.

Jones, D. L. (1998). Organic acids in the rhizosphere–a critical review. *Plant and soil* 205, 25-44.

Juma, N., and Tabatabai, M. (1978). Distribution of phosphomonoesterases in soils. *Soil Science* 126, 101-108.

Kacimi, L., Simon-Masseron, A., Ghomari, A., and Derriche, Z. (2006). Reduction of clinkerization temperature by using phosphogypsum. *Journal of hazardous materials* 137, 129-137.

Kalkhoran, S. S., Pannell, D., Thamo, T., Polyakov, M., and White, B. (2020). Optimal lime rates for soil acidity mitigation: impacts of crop choice and nitrogen fertiliser in Western Australia. *Crop and Pasture Science* 71, 36-46.

Kaninga, B., Lark, R. M., Chishala, B. H., Maseka, K. K., Sakala, G. M., Young, S. D., Tye, A., Hamilton, E. M., and Watts, M. J. (2021). Crop uptake of heavy metals in response to the environment and agronomic practices on land near mine tailings in the Zambian Copperbelt Province. *Environmental Geochemistry and Health*, 1-15.

Kearney, J., Moot, D. J., and Pollock, K. M. (2010). On-farm comparision of pasture production in relation to rainfall in central Otago. *In* "Proceedings of the New Zealand Grassland Association", Vol. 72, pp. 121-126.

Keeney, D., and Bremner, J. (1966a). Comparison and Evaluation of Laboratory Methods of Obtaining an Index of Soil Nitrogen Availability 1. *Agronomy journal* 58, 498-503.

Keeney, D. R., and Bremner, J. (1966b). Comparison and evaluation of laboratory methods of obtaining an index of soil nitrogen availability. *Agronomy journal* 58, 498-503.

Kemmitt, S. J., Wright, D., Goulding, K. W., and Jones, D. L. (2006). pH regulation of carbon and nitrogen dynamics in two agricultural soils. *Soil Biology and Biochemistry* 38, 898-911.

Kemp, P. D., Kenyon, P. R., and Morris, S. T. (2010). The use of legume and herb forage species to create high performance pastures for sheep and cattle grazing systems. *Revista Brasileira de Zootecnia* 39, 169-174.

Kidd, P. S., and Proctor, J. (2001). Why plants grow poorly on very acid soils: are ecologists missing the obvious? *Journal of Experimental Botany* 52, 791-799.

Kiflu, A., Beyene, S., and Jeff, S. (2017). Fractionation and availability of phosphorus in acid soils of Hagereselam, Southern Ethiopia under different rates of lime. *Chemical and Biological Technologies in Agriculture* 4, 1-7.

Kinniburgh, D. G., van Riemsdijk, W. H., Koopal, L. K., Borkovec, M., Benedetti, M. F., and Avena, M. J. (1999). Ion binding to natural organic matter: competition, heterogeneity, stoichiometry and thermodynamic consistency. *Colloids and Surfaces A: Physicochemical and Engineering Aspects* 151, 147-166.

Kinraide, T. B. (1991). Identity of the rhizotoxic aluminium species. *In* "Plant-soil interactions at low pH. Developments in Plant and Soil Sciences" (R. J. Wright, V. C. Baligar and R. P. Murrmann, eds.), pp. 717-728. Springer, Dordrecht, Netherlands.

Kinraide, T. B. (1997). Reconsidering the rhizotoxicity of hydroxyl, sulphate, and fluoride complexes of aluminium. *Journal of Experimental Botany* 48, 1115-1124.

Kirchhof, G., Jayawardane, N., Blackwell, J., and Murray, E. (1995). Lime-slotting technique to ameliorate subsoil acidity in a clay soil. 1. Effects on soil-pH and physical characteristics. *Soil Research* 33, 425-441.

Kiss, S., Drăgan-Bularda, M., and Rădulescu, D. (1975). Biological Significance of Enzymes Accumulated in Soil. *In* "Advances in Agronomy" (N. C. Brady, ed.), Vol. 27, pp. 25-87. Academic Press.

Korentajer, L., Byrnes, B. H., and Hellums, D. T. (1983). The Effect of Liming and Leaching on the Sulfur-Supplying Capacity of Soils 1. *Soil Science Society of America Journal* 47, 525-530.

Kreuzeder, A., Santner, J., Scharsching, V., Oburger, E., Hoefer, C., Hann, S., and Wenzel, W. W. (2018). In situ observation of localized, sub-mm scale changes of phosphorus biogeochemistry in the rhizosphere. *Plant and soil* 424, 573-589.

Kunhikrishnan, A., Thangarajan, R., Bolan, N., Xu, Y., Mandal, S., Gleeson, D., Seshadri, B., Zaman, M., Barton, L., and Tang, C. (2016). Functional relationships of soil acidification, liming, and greenhouse gas flux. *Advances in agronomy* 139, 1-71.

Lacey, M., and Wilson, C. (2001). Relationship of common scab incidence of potatoes grown in Tasmanian ferrosol soils with pH, exchangeable cations and other chemical properties of those soils. *Journal of Phytopathology* 149, 679-683.

Lambers, H., Clements, J. C., and Nelson, M. N. (2013). How a phosphorus-acquisition strategy based on carboxylate exudation powers the success and agronomic potential of lupines (*Lupinus*, Fabaceae). *American Journal of Botany* 100, 263-288.

Lambers, H., Hayes, P. E., Laliberté, E., Oliveira, R. S., and Turner, B. L. (2015). Leaf manganese accumulation and phosphorus-acquisition efficiency. *Trends in plant science* 20, 83-90.

Lambers, H., Shane, M. W., Cramer, M. D., Pearse, S. J., and Veneklaas, E. J. (2006). Root structure and functioning for efficient acquisition of phosphorus: matching morphological and physiological traits. *Annals of botany* 98, 693-713.

Lambers, H., and Teste, F. P. (2013). Interactions between arbuscular mycorrhizal and non-mycorrhizal plants: do non-mycorrhizal species at both extremes of nutrient availability play the same game. *Plant Cell Environ* 36, 1911-1915.

Lambert, M., Mackay, A., Devantier, B., McDougall, D., Barker, D., and Park-Ng, Z. (2003). Redefining the production potential of hill pastures using fertiliser nitrogen. *In* "Proceedings of the New Zealand Grassland Association", pp. 35-40.

Lauricella, D., Butterly, C. R., Weng, Z. H., Clark, G. J., Sale, P. W., Li, G., and Tang, C. (2021). Impact of novel materials on alkalinity movement down acid soil profiles when combined with lime. *Journal of Soils and Sediments* 21, 52-62.

Le Mare, P., and Leon, L. (1989). The effects of lime on adsorption and desorption of phosphate in five Colombian soils. *Journal of Soil Science* 40, 59-69.

Lee, C. H., Ha, B. Y., Lee, Y. B., and Kim, P. J. (2009). Effect of alkalized phosphogypsum on soil chemical and biological properties. *Communications in soil science and plant analysis* 40, 2072-2086.

Lemire, E., Taillon, K. M., and Hendershot, W. H. (2006). Using pH-dependent CEC to determine lime requirement. *Canadian journal of soil science* 86, 133-139.

Leprince, F., and Quiquampoix, H. (1996). Extracellular enzyme activity in soil: effect of pH and ionic strength on the interaction with montmorillonite of two acid phosphatases secreted by the ectomycorrhizal fungus Hebeloma cylindrosporum. *European Journal of Soil Science* 47, 511-522.

Li, G., Li, H., Leffelaar, P. A., Shen, J., and Zhang, F. (2015). Dynamics of phosphorus fractions in the rhizosphere of fababean (Vicia faba L.) and maize (Zea mays L.) grown in calcareous and acid soils. *Crop and Pasture Science* 66, 1151-1160.

Li, G. D., Conyers, M. K., Helyar, K. R., Lisle, C. J., Poile, G. J., and Cullis, B. R. (2019a). Long-term surface application of lime ameliorates subsurface soil acidity in the mixed farming zone of south-eastern Australia. *Geoderma* 338, 236-246.

Li, H., Shen, J., Zhang, F., Tang, C., and Lambers, H. (2008). Is there a critical level of shoot phosphorus concentration for cluster-root formation in *Lupinus albus*? *Functional Plant Biology* 35, 328-336.

Li, L., Li, S.-M., Sun, J.-H., Zhou, L.-L., Bao, X.-G., Zhang, H.-G., and Zhang, F.-S. (2007). Diversity enhances agricultural productivity via rhizosphere phosphorus facilitation on phosphorus-deficient soils. *Proceedings of the National Academy of Sciences* 104, 11192-11196.

Li, Y., Cui, S., Chang, S. X., and Zhang, Q. (2019b). Liming effects on soil pH and crop yield depend on lime material type, application method and rate, and crop species: a global meta-analysis. *Journal of Soils and Sediments* 19, 1393-1406.

Ligaba, A., Yamaguchi, M., Shen, H., Sasaki, T., Yamamoto, Y., and Matsumoto, H. (2004). Phosphorus deficiency enhances plasma membrane H+-ATPase activity and citrate exudation in greater purple lupin (*Lupinus pilosus*). *Functional Plant Biology* 31, 1075-1083.

Liyuan, H., Zhang, X., Gu, F., Li, Z., Zhang, Y., and Ning, C. (2021). Arbuscular mycorrhizal enhancement of phosphorus uptake and yields of maize under high planting density in the black soil region of China. *Scientific Reports (Nature Publisher Group)* 11.

Lombi, E., Hamon, R. E., McGrath, S. P., and McLaughlin, M. J. (2003). Lability of Cd, Cu, and Zn in polluted soils treated with lime, beringite, and red mud and identification of a non-labile colloidal fraction of metals using isotopic techniques. *Environmental Science & Technology* 37, 979-984.

López, I., Lambert, M., Mackay, A., and Valentine, I. (2003). The influence of topography and pasture management on soil characteristics and herbage accumulation in hill pasture in the North Island of New Zealand. *Plant and Soil* 255, 421-434.

Luther, S., Dudas, M., and Rutherford, P. (1993). Radioactivity and chemical characteristics of Alberta phosphogypsum. *Water, Air, and Soil Pollution* 69, 277-290.

Lynch, J. P. (2007). Roots of the second green revolution. *Australian Journal of Botany* 55, 493-512.

Lynch, J. P., and Brown, K. M. (2008). Root strategies for phosphorus acquisition. *In* "The ecophysiology of plant-phosphorus interactions" (P. J. White and J. P. Hammond, eds.), Vol. 7, pp. 83-116. Springer, Dordrecht.

Ma, J., Wang, Z.-Y., Stevenson, B. A., Zheng, X.-J., and Li, Y. (2013). An inorganic CO 2 diffusion and dissolution process explains negative CO 2 fluxes in saline/alkaline soils. *Scientific reports* 3, 1-7.

Ma, J. F. (2000). Role of organic acids in detoxification of aluminum in higher plants. *Plant Cell Physiology* 41.

Ma, J. F., Ryan, P. R., and Delhaize, E. (2001). Aluminium tolerance in plants and the complexing role of organic acids. *Trends in plant science* 6, 273-278.

Ma, X., Liu, Y., Zarebanadkouki, M., Razavi, B. S., Blagodatskaya, E., and Kuzyakov, Y. (2018a). Spatiotemporal patterns of enzyme activities in the rhizosphere: effects of plant growth and root morphology. *Biology and Fertility of Soils* 54, 819-828.

Ma, X., Mason-Jones, K., Liu, Y., Blagodatskaya, E., Kuzyakov, Y., Guber, A., Dippold, M. A., and Razavi, B. S. (2019). Coupling zymography with pH mapping reveals a shift in lupine phosphorus acquisition strategy driven by cluster roots. *Soil Biology and Biochemistry* 135, 420-428.

Ma, X., Zarebanadkouki, M., Kuzyakov, Y., Blagodatskaya, E., Pausch, J., and Razavi, B. S. (2018b). Spatial patterns of enzyme activities in the rhizosphere: effects of root hairs and root radius. *Soil Biology and Biochemistry* 118, 69-78.

Mackay, A., Rhodes, A., Power, I., and Wedderburn, M. (2012). Has the eco-efficiency of sheep and beef farms changed in the last 20 years? *In* "Proceedings of the New Zealand Grassland Association", pp. 11-16.

Mackay, A. D. (2008). Impacts of intensification of pastoral agriculture on soils: Current and emerging challenges and implications for future land uses. *New Zealand Veterinary Journal* 56, 281-288.

Mackay, A. D., Vibart, R., McKenzie, C., Costall, D., Bilotto, F., and Kelliher, F. M. (2021). Soil organic carbon stocks in hill country pastures under contrasting phosphorus fertiliser and sheep stocking regimes, and topographical features. *Agricultural Systems* 186, 102980.

MacLean, D., Hansen, K., and Schneider, R. (1992). Amelioration of aluminium toxicity in wheat by fluoride. *New phytologist* 121, 81-88.

MacLeod, C. J., and Moller, H. (2006). Intensification and diversification of New Zealand agriculture since 1960: An evaluation of current indicators of land use change. *Agriculture, ecosystems & environment* 115, 201-218.

Magid, J., Tiessen, H., and Condron, L. (1996). Dynamics of organic phosphorus in soils under natural and agricultural ecosystems. *In* "Humic substances in terrestrial ecosystems" (A. Piccolo, ed.), pp. 429-466. Elsevier, Amsterdam.

Mahmoud, E., and Abd El-Kader, N. (2015). Heavy metal immobilization in contaminated soils using phosphogypsum and rice straw compost. *Land Degradation & Development* 26, 819-824.

Mamedov, A., Shainberg, I., Wagner, L., Warrington, D., and Levy, G. (2010). Infiltration and erosion in soils treated with dry PAM, of two molecular weights, and phosphogypsum. *Soil Research* 47, 788-795.

Manoharan, V., Loganathan, P., Parfitt, R., and Tillman, R. (1996). Changes in soil solution composition and aluminium speciation under legume based pastures in response to long term phosphate fertiliser applications. *Soil Research* 34, 985-998.

Manoharan, V., Loganathan, P., Tillman, R., and Parfitt, R. (2007). Interactive effects of soil acidity and fluoride on soil solution aluminium chemistry and barley (Hordeum vulgare L.) root growth. *Environmental Pollution* 145, 778-786.

Margenot, A. J., Sommer, R., and Parikh, S. J. (2018). Soil phosphatase activities across a liming gradient under long-term managements in Kenya. *Soil Science Society of America Journal* 82, 850-861.

Marion, G., and Babcock, K. (1976). Predicting specific conductance and salt concentration in dilute aqueous solutions. *Soil Science* 122, 181-187.

Marques, J., Teixeira, W., Schulze, D., and Curi, N. (2002). Mineralogy of soils with unusually high exchangeable Al from the western Amazon Region. *Clay Minerals* 37, 651-661.

Marschner, H. (1995). "Mineral nutrition of higher plants," Academic Pres, London, UK.

Martin-Hendrie, D. L. (2019). Liming effects on legume production and phosphorus availability in acid South Island hill and high country soils: A thesis submitted in partial fulfilment of the requirements for the Degree of Doctor of Philosophy at Lincoln University, Lincoln University.

Martini, J., and Mutters, R. (1984). Effect of liming and fertilization on sulfur availability, mobility, and uptake in cultivated soils of south carolina1. *Soil science* 138, 403-410.

Martins, A. P., de Oliveira Denardin, L. G., Tiecher, T., Borin, J. B. M., Schaidhauer, W., Anghinoni, I., de Faccio Carvalho, P. C., and Kumar, S. (2020). Nine-year impact of grazing management on soil acidity and aluminum speciation and fractionation in a long-term no-till integrated crop-livestock system in the subtropics. *Geoderma* 359, 113986.

Mason, S. D., McLaughlin, M. J., Johnston, C., and McNeill, A. (2013). Soil test measures of available P (Colwell, resin and DGT) compared with plant P uptake using isotope dilution. *Plant and soil* 373, 711-722.

Masud, M., Li, J.-Y., and Xu, R.-k. (2015). Application of alkaline slag and phosphogypsum for alleviating soil acidity in an Ultisol profile: a short-term leaching experiment. *Journal of soils and sediments* 15, 365-373.

Matschonat, G., and Vogt, R. (1998). Significance of the total cation concentration in acid forest soils for the solution composition and the saturation of exchange sites. *Geoderma* 84, 289-307.

Maxwell, T., Moir, J., and Edwards, G. (2012). Sulphur and lime response of four adventive annual clovers grown in a New Zealand high country soil under glasshouse conditions. *New Zealand Journal of Agricultural Research* 55, 47-62.

Maxwell, T. M., Moir, J., and Edwards, G. (2013). Phosphorus response and efficiency of four adventive annual clovers grown in a New Zealand high country soil under glasshouse conditions. *New Zealand Journal of Agricultural Research* 56, 203-214.

Maxwell, T. M., Moir, J. L., and Edwards, G. R. (2010). Influence of environmental factors on the abundance of naturalised annual clovers in the South Island hill and high country. *In* "Proceedings of the New Zealand Grassland Association", Vol. 72, pp. 165-170.

Maxwell, T. M. R., Moir, J. L., and Edwards, G. R. (2016). Grazing and soil fertility effect on naturalized annual clover species in new zealand high country. *Rangeland Ecology & Management* 69, 444-448.

Mazzilli, B., Palmiro, V., Saueia, C., and Nisti, M. (2000). Radiochemical characterization of Brazilian phosphogypsum. *Journal of Environmental Radioactivity* 49, 113-122.

McCallum, H. M., Park, K. J., O'brien, M. G., Gimona, A., Poggio, L., and Wilson, J. D. (2015). Soil pH and organic matter content add explanatory power to Northern Lapwing Vanellus vanellus distribution models and suggest soil amendment as a conservation measure on upland farmland. *Ibis* 157, 677-687.

McCormack, M. L., Dickie, I. A., Eissenstat, D. M., Fahey, T. J., Fernandez, C. W., Guo, D., Helmisaari, H. S., Hobbie, E. A., Iversen, C. M., and Jackson, R. B. (2015). Redefining fine roots improves

understanding of below-ground contributions to terrestrial biosphere processes. *New Phytologist* 207, 505-518.

McDougall, K. L., Morgan, J. W., Walsh, N. G., and Williams, R. J. (2005). Plant invasions in treeless vegetation of the Australian Alps. *Perspectives in Plant Ecology, Evolution and Systematics* 7, 159-171.

McDowell, R., and Condron, L. (2000). Chemical nature and potential mobility of phosphorus in fertilized grassland soils. *Nutrient Cycling in Agroecosystems* 57, 225-233.

McDowell, R., and Condron, L. (2001). Influence of soil constituents on soil phosphorus sorption and desorption. *Communications in Soil Science and Plant Analysis* 32, 2531-2547.

McDowell, R., Mahieu, N., Brookes, P., and Poulton, P. (2003). Mechanisms of phosphorus solubilisation in a limed soil as a function of pH. *Chemosphere* 51, 685-692.

McIntosh, P., and Backholm, G. (1981). "A reconnaissance soil survey of the Otago uplands [New Zealand]," Rep. No. 0110-649X. Ministry of Primary Industries. Invermay Agricultural Research Centre (NZ).

McKay Fletcher, D. M., Ruiz, S., Dias, T., Petroselli, C., and Roose, T. (2020). Linking root structure to functionality: the impact of root system architecture on citrate-enhanced phosphate uptake. *New Phytologist*.

McLaren, T. I., Smernik, R. J., McLaughlin, M. J., McBeath, T. M., McCaskill, M. R., Robertson, F. A., and Simpson, R. J. (2020). Soil phosphorus pools with addition of fertiliser phosphorus in a long-term grazing experiment. *Nutrient Cycling in Agroecosystems* 116, 151-164.

McLean, E. (1976). Chemistry of soil aluminum. *Communications in soil science and plant analysis* 7, 619-636.

Mehlich, A. (1964). Influence of Sorbed Hydroxyl and Sulfate on Liming Efficiency, pH, and Conductivity 1. *Soil Science Society of America Journal* 28, 496-499.

Menzies, N., Bell, L., and Edwards, D. (1994). Exchange and solution phase chemistry of acid, highly weathered soils. I. Characteristics of soils and the effects of lime and gypsum amendments. *Soil Research* 32, 251-267.

Mesić, M., Brezinščak, L., Zgorelec, Ž., Perčin, A., Šestak, I., Bilandžija, D., Trdenić, M., and Lisac, H. (2016). The application of phosphogypsum in agriculture. *Agriculturae Conspectus Scientificus* 81, 7-13.

Mimmo, T., Ghizzi, M., Marzadori, C., and Gessa, C. (2008). Organic acid extraction from rhizosphere soil: effect of field-moist, dried and frozen samples. *Plant and soil* 312, 175-184.

Miotto, A., Tiecher, T., Kaminski, J., Brunetto, G., De Conti, L., Tiecher, T. L., Martins, A. P., and Rheinheimer dos Santos, D. (2020). Soil acidity and aluminum speciation affected by liming in the conversion of a natural pasture from the Brazilian Campos Biome into no-tillage system for grain production. *Archives of Agronomy and Soil Science* 66, 138-151.

Mitran, T., Meena, R. S., Lal, R., Layek, J., Kumar, S., and Datta, R. (2018). Role of soil phosphorus on legume production. *In* "Legumes for Soil Health and Sustainable Management", pp. 487-510. Springer.

Mkhonza, N. P., Buthelezi-Dube, N. N., and Muchaonyerwa, P. (2020). Effects of lime application on nitrogen and phosphorus availability in humic soils. *Sci Rep* 10, 8634.

Mogollón, J., Beusen, A., Van Grinsven, H., Westhoek, H., and Bouwman, A. (2018). Future agricultural phosphorus demand according to the shared socioeconomic pathways. *Global Environmental Change* 50, 149-163.

Moir, J., Hedley, M., Mackay, A., and Tillman, R. (1997). The effect of fertiliser history on nutrient accumulation and plant-available nutrient supply in legume-based pasture soils. *In* "XVII International Grassland Congress. Eds. Buchanan-Smith, JG", pp. 68-69.

Moir, J., Jordan, P., Moot, D., and Lucas, R. (2016). Phosphorus response and optimum pH ranges of twelve pasture legumes grown in an acid upland New Zealand soil under glasshouse conditions. *Journal of Soil Science and Plant Nutrition* 16, 438-460.

Moir, J., and Moot, D. (2014). Medium-term soil pH and exchangeable aluminium response to liming at three high country locations. *In* "New Zealand Grassland Association Conference", Vol. 76, pp. 41-45. Journal of New Zealand Grasslands, Alexandra, NZ.

Moir, J., Scotter, D., Hedley, M., and Mackay, A. (2000). A climate-driven, soil fertility dependent, pasture production model. *New Zealand Journal of Agricultural Research* 43, 491-500.

Moir, J. L., and Moot, D. J. (2010). Soil pH, exchangeable aluminium and lucerne yield responses to lime in a South Island high country soil. *In* "Proceedings of the New Zealand Grassland Association", Vol. 72, pp. 191-196.

Mokolobate, M., and Haynes, R. (2002). Comparative liming effect of four organic residues applied to an acid soil. *Biology and fertility of soils* 35, 79-85.

Moot, D. (2013). An overview of dryland legume research in New Zealand. *Crop and Pasture Science* 63, 726-733.

Moot, D., Mills, A., Lucas, D., and Scott, W. (2009). Country pasture/forage resource profiles: New Zealand. *FAO Report. Rome: Food and Agriculture Organization* 61.

Moot, D. J., and Pollock, K. M. (2014). Perennial lupin establishment and yield when sown at five different rates at Glenmore Station, Lake Tekapo. *In* "Proceedings of the New Zealand Grassland Association", pp. 53-60.

Morris, S., and Kenyon, P. (2014). Intensive sheep and beef production from pasture—A New Zealand perspective of concerns, opportunities and challenges. *Meat science* 98, 330-335.

Morton, J., Gray, M., and Gillingham, A. (2005). Soil and pasture responses to lime on dry hill country in central Hawke's Bay, New Zealand. *New Zealand Journal of Agricultural Research* 48, 143-150.

Morton, J., and Roberts, A. (1999). Fertiliser use on New Zealand sheep and beef farms. *New Zealand Fertiliser Manufacturers' Research Association: Auckland, New Zealand*.

Morton, J. D. (2020). A review of research on the effect of lime on New Zealand soils and pastures. *New Zealand Journal of Agricultural Research* 63, 189-201.

Morton, J. D., Grace, N. D., O'Connor, M. B., New Zealand Pastoral Agriculture Research, I., and New Zealand Fertiliser Manufacturers' Research, A. (1999). "Use of trace elements in New Zealand pastoral farming : the principles and practice of meeting trace element requirements of pastures and animals," Auckland N.Z. : New Zealand Fertiliser Manufacturers' Research Association, Auckland] N.Z.

Morton, J. D., and Moir, J. L. (2018). Soil aluminium toxicity in New Zealand pastoral farming–a review. *Journal of New Zealand Grasslands* 80, 129-136.

Müller, J., Gödde, V., Niehaus, K., and Zörb, C. (2015). Metabolic adaptations of white lupin roots and shoots under phosphorus deficiency. *Frontiers in plant science* 6, 1014.

Murphy, J., and Riley, J. P. (1962). A modified single solution method for the determination of phosphate in natural waters. *Analytica chimica acta* 27, 31-36.

Murphy, P. N., and Sims, J. (2012). Effects of lime and phosphorus application on phosphorus runoff risk. *Water, Air, & Soil Pollution* 223, 5459-5471.

Nannipieri, P., Giagnoni, L., Landi, L., and Renella, G. (2011). Role of phosphatase enzymes in soil. *In* "Phosphorus in action" (E. Bünemann, A. Oberson and E. Frossard, eds.), Vol. 26, pp. 215-243. Springer, Berlin, Heidelberg.

Nayak, A., Mishra, V., Sharma, D., Jha, S., Singh, C., Shahabuddin, M., and Shahid, M. (2013). Efficiency of phosphogypsum and mined gypsum in reclamation and productivity of rice–wheat cropping system in sodic soil. *Communications in soil science and plant analysis* 44, 909-921.

Nayak, S., Mishra, C., Guru, B., and Rath, M. (2011). Effect of phosphogypsum amendment on soil physico-chemical properties, microbial load and enzyme activities. *Journal of environmental biology* 32, 613.

Neto, J. F., Franzluebbers, A. J., Crusciol, C. A. C., Rigon, J. P. G., Calonego, J. C., Rosolem, C. A., do Nascimento, C. A. C., and Ribeiro, L. C. (2021). Soil carbon and nitrogen fractions and physical attributes affected by soil acidity amendments under no-till on Oxisol in Brazil. *Geoderma Regional* 24, e00347.

Neumann, G., and Römheld, V. (2007). The release of root exudates as affected by the plant physiological status. *In* "The Rhizosphere: Biochemistry and organic substances at the soil-plant interface" (R. Pinton, Z. Varanini and P. Nannipieri, eds.), Vol. 2, pp. 23-72. CRC Press, New York.

Newbould, P., and Rangeley, A. (1984). Effect of lime, phosphorus and mycorrhizal fungi on growth, nodulation and nitrogen fixation by white clover (*Trifolium repens*) grown in UK hill soils. *Plant and Soil* 76, 105-114.

Niemi, R., and Vepsäläinen, M. (2005). Stability of the fluorogenic enzyme substrates and pH optima of enzyme activities in different Finnish soils. *Journal of microbiological methods* 60, 195-205.

NIST (1995). Standard Reference Material 1573a ,Tomato leaves. *In* "Certificate of analysis", pp. 5. National Institute of standards and technology, Gaithersburg, USA.

Nobile, C., Bravin, M., Becquer, T., and Paillat, J.-M. (2020). Phosphorus sorption and availability in an andosol after a decade of organic or mineral fertilizer applications: Importance of pH and organic carbon modifications in soil as compared to phosphorus accumulation. *Chemosphere* 239, 124709.

Nordstrom, D. K. (1982). The effect of sulfate on aluminum concentrations in natural waters: some stability relations in the system Al2O3-SO3-H2O at 298 K. *Geochimica et Cosmochimica Acta* 46, 681-692.

Nuruzzaman, M., Lambers, H., Bolland, M. D., and Veneklaas, E. J. (2006). Distribution of carboxylates and acid phosphatase and depletion of different phosphorus fractions in the rhizosphere of a cereal and three grain legumes. *Plant and Soil* 281, 109-120.

Oberson, A., Friesen, D. K., Rao, I. M., Bühler, S., and Frossard, E. (2001). Phosphorus transformations in an Oxisol under contrasting land-use systems: the role of the soil microbial biomass. *Plant and Soil* 237, 197-210.

Oburger, E., and Jones, D. L. (2018). Sampling root exudates–mission impossible? *Rhizosphere* 6, 116-133.

Oehl, F., Frossard, E., Fliessbach, A., Dubois, D., and Oberson, A. (2004). Basal organic phosphorus mineralization in soils under different farming systems. *Soil Biology and Biochemistry* 36, 667-675.

Olsen, S. R. (1954). "Estimation of available phosphorus in soils by extraction with sodium bicarbonate." US Department of Agriculture, Washington, USA.

Olsen, S. R., Cole, C. V., and Watanabe, F. S. (1954). "Estimation of available phosphorus in soils by extraction with sodium bicarbonate," USDA, Washington.

Olykan, S. T., Lucas, R. J., Teixeira, C. S., Subtil, R. A., and Moot, D. J. (2018). Establishment, production and regeneration of subterranean clovers in the Mackenzie Basin, New Zealand. New Zealand Grassland Association.

Otieno, H. M., Chemining'Wa, G. N., and Zingore, S. (2018). Effect of farmyard manure, lime and inorganic fertilizer applications on soil pH, nutrients uptake, growth and nodulation of soybean in acid soils of western Kenya. *J Agr Sci* 10, 199-208.

Palmer, D. A., and Wesolowski, D. J. (1992). Aluminum speciation and equilibria in aqueous solution: II. The solubility of gibbsite in acidic sodium chloride solutions from 30 to 70 C. *Geochimica et Cosmochimica Acta* 56, 1093-1111.

Pang, J., Ryan, M. H., Tibbett, M., Cawthray, G. R., Siddique, K. H., Bolland, M. D., Denton, M. D., and Lambers, H. (2010a). Variation in morphological and physiological parameters in herbaceous perennial legumes in response to phosphorus supply. *Plant and Soil* 331, 241-255.

Pang, J., Tibbett, M., Denton, M. D., Lambers, H., Siddique, K. H., Bolland, M. D., Revell, C. K., and Ryan, M. H. (2010b). Variation in seedling growth of 11 perennial legumes in response to phosphorus supply. *Plant and Soil* 328, 133-143.

Paradelo, R., Virto, I., and Chenu, C. (2015). Net effect of liming on soil organic carbon stocks: A review. *Agriculture, Ecosystems & Environment* 202, 98-107.

Parfitt, R., Schipper, L., Baisden, W., and Elliott, A. (2006). Nitrogen inputs and outputs for New Zealand in 2001 at national and regional scales. *Biogeochemistry* 80, 71-88.

Paul, E., and Clark, F. (1996). Soil microbiology and biochemistry. –Academic Press. *New York, USA*.

Pausch, J., and Kuzyakov, Y. (2018). Carbon input by roots into the soil: quantification of rhizodeposition from root to ecosystem scale. *Global Change Biology* 24, 1-12.

Pavan, M. A., Bingham, F., and Pratt, P. (1984). Redistribution of exchangeable calcium, magnesium, and aluminum following lime or gypsum applications to a Brazilian Oxisol. *Soil Science Society of America Journal* 48, 33-38.

Pearse, S. J., Veneklaas, E. J., Cawthray, G., Bolland, M. D., and Lambers, H. (2007). Carboxylate composition of root exudates does not relate consistently to a crop species' ability to use phosphorus from aluminium, iron or calcium phosphate sources. *New Phytologist* 173, 181-190.

Pearse, S. J., Veneklaas, E. J., Cawthray, G. R., Bolland, M. D., and Lambers, H. (2006). Carboxylate release of wheat, canola and 11 grain legume species as affected by phosphorus status. *Plant and Soil* 288, 127-139.

Peng, Q., Wu, M., Zhang, Z., Su, R., He, H., and Zhang, X. (2020). The interaction of arbuscular mycorrhizal fungi and phosphorus inputs on selenium uptake by alfalfa (*Medicago sativa L.*) and selenium fraction transformation in soil. *Frontiers in plant science* 11, 966.

Penn, C. J., and Camberato, J. J. (2019). A critical review on soil chemical processes that control how soil pH affects phosphorus availability to plants. *Agriculture* 9, 120.

Percival, H., Giddens, K., Lee, R., and Whitton, J. (1996). Relationships between soil solution aluminium and extractable aluminium in some moderately acid New Zealand soils. *Soil Research* 34, 769-779.

Perrott, K. (1992). Effect of exchangeable calcium on fractionation of inorganic and organic soil phosphorus. *Communications in soil science and plant analysis* 23, 827-840.

Peryea, F., and Kittrick, J. (1988). Relative solubility of corundum, gibbsite, boehmite, and diaspore at standard state conditions. *Clays and Clay Minerals* 36, 391-396.

Peters, S., Visser, A. E., Weisskopf, M., Kioumourtzoglou, M.-A., and Vermeulen, R. (2020). Metals and neurodegeneration. *In* "Oxford Textbook of Neurologic and Neuropsychiatric Epidemiology" (C. Brayne, V. L. Feigin, L. J. Launer and G. Lorgoscino, eds.), pp. 417-424. Oxford University Press, Oxford, UK.

Petersen, W., and Böttger, M. (1991). Contribution of organic acids to the acidification of the rhizosphere of maize seedlings. *Plant and Soil* 132, 159-163.

Pietri, J. A., and Brookes, P. (2008). Relationships between soil pH and microbial properties in a UK arable soil. *Soil Biology and Biochemistry* 40, 1856-1861.

Polglase, P., and Attiwill, P. (1992). Nitrogen and phosphorus cycling in relation to stand age of Ecucalyptus regnans F. Muell. *Plant and soil* 142, 157-166.

Poorter, H., Bühler, J., van Dusschoten, D., Climent, J., and Postma, J. A. (2012). Pot size matters: a meta-analysis of the effects of rooting volume on plant growth. *Functional Plant Biology* 39, 839-850.

Price, G. (2006). "Australian soil fertility manual," Fertilizer Industry Federation of Australia, Inc. & CSIRO, Collingwood, Australia.

Quiquampoix, H. (2005). Enzymatic hydrolysis of organic phosphorus. *In* "Organic phosphorus in the environment" (B. L. Turner, E. Frossard and D. S. Baldwin, eds.), pp. 89-112. CAB International, Wallingford, UK.

Radcliffe, J. (1982). Effects of aspect and topography on pasture production in hill country. *New Zealand Journal of Agricultural Research* 25, 485-496.

Rahman, R., and Upadhyaya, H. (2020). Aluminium Toxicity and Its Tolerance in Plant: A Review. *Journal of Plant Biology*, 1-21.

Raiesi, F., and Beheshti, A. (2014). Soil specific enzyme activity shows more clearly soil responses to paddy rice cultivation than absolute enzyme activity in primary forests of northwest Iran. *Applied Soil Ecology* 75, 63-70.

Ramaekers, L., Remans, R., Rao, I. M., Blair, M. W., and Vanderleyden, J. (2010). Strategies for improving phosphorus acquisition efficiency of crop plants. *Field Crops Research* 117, 169-176.

Rayment, G. E., and Higginson, F. R. (1992). "Australian laboratory handbook of soil and water chemical methods," Inkata Press Pty Ltd, Melbourne, Australia.

Rayment, G. E., and Lyons, D. J. (2011). Exchange acidity (H^+ + Al^{3+}) by 1 M potassium chloride. *In* "Soil chemical methods: Australasia", Vol. 3, pp. 335-338. CSIRO publishing, Collingwood, Victoria, Australia.

Razavi, B. S., Zhang, X., Bilyera, N., Guber, A., and Zarebanadkouki, M. (2019). Soil zymography: Simple and reliable? Review of current knowledge and optimization of the method. *Rhizosphere* 11, 100161.

Rechcigl, J., Edmisten, K., Wolf, D., and Reneau, R. (1988). Response of alfalfa grown on acid soil to different chemical amendments. *Agronomy Journal* 80, 515-518.

Reinhard, H., Rupp, H., Sager, F., Streule, M., and Zoller, O. (2006). Quinolizidine alkaloids and phomopsins in lupin seeds and lupin containing food. *Journal of Chromatography A* 1112, 353-360.

Rengel, Z. (1992). Role of calcium in aluminium toxicity. *New Phytologist* 121, 499-513.

Rengel, Z. (2003). "Handbook of soil acidity," Marcel Dekker, Inc., New York, USA.

Rentería-Villalobos, M., Vioque, I., Mantero, J., and Manjón, G. (2010). Radiological, chemical and morphological characterizations of phosphate rock and phosphogypsum from phosphoric acid factories in SW Spain. *Journal of hazardous materials* 181, 193-203.

Reussi Calvo, N. I., Wyngaard, N., Orcellet, J., Sainz Rozas, H. R., and Echeverría, H. E. (2018). Predicting field-apparent nitrogen mineralization from anaerobically incubated nitrogen. *Soil Science Society of America Journal* 82, 502-508.

Richardson, A. E., Hocking, P. J., Simpson, R. J., and George, T. S. (2009). Plant mechanisms to optimise access to soil phosphorus. *Crop and Pasture Science* 60, 124-143.

Richardson, A. E., Lynch, J. P., Ryan, P. R., Delhaize, E., Smith, F. A., Smith, S. E., Harvey, P. R., Ryan, M. H., Veneklaas, E. J., and Lambers, H. (2011). Plant and microbial strategies to improve the phosphorus efficiency of agriculture. *Plant and soil* 349, 121-156.

Richardson, A. E., and Simpson, R. J. (2011). Soil microorganisms mediating phosphorus availability update on microbial phosphorus. *Plant physiology* 156, 989-996.

Roach, C., Nemaia, E., Ledgard, S., Brier, G., and McLay, C. (1996). Effects of long-term differences in fertiliser history on hill country: seasonal pasture production, legume growth and soil phosphorus status. *In* "Proceedings of the New Zealand Grassland Association", pp. 105-109.

Roberts, A., Webb, T., Morton, J., O'connor, M., and Edmeades, D. (1994). Building a solid foundation-sulphur, phosphorus and potassium requirements for the sedimentary soils of North Canterbury. *In* "Proceedings of the New Zealand Grassland Association", Vol. 56, pp. 7-12.

Robles-Aguilar, A. A., Pang, J., Postma, J. A., Schrey, S. D., Lambers, H., and Jablonowski, N. D. (2019). The effect of pH on morphological and physiological root traits of *Lupinus angustifolius* treated with struvite as a recycled phosphorus source. *Plant and soil* 434, 65-78.

Robson, A., and Abbott, L. (1989). The effect of soil acidity on microbial activity in soils. *In* "Soil acidity and plant growth" (A. Robson, ed.), pp. 139-166. Academic Press Inc., San Diego, CA.

Robson, A. D., and Abbot, L. K. (2012). The effect of soil acidity on microbial activity in soils. *In* "Soil acidity and plant growth" (A. Robson, ed.), pp. 139–166. Academic Press Inc, San Diego, CA.

Rose, T. J., Hardiputra, B., and Rengel, Z. (2010). Wheat, canola and grain legume access to soil phosphorus fractions differs in soils with contrasting phosphorus dynamics. *Plant and Soil* 326, 159-170.

Rout, G., Samantaray, S., and Das, P. (2001). Aluminium toxicity in plants: a review. *Agronomie* 21, 3-21.

Rutherford, P., Dudas, M., and Arocena, J. (1995). Radium in phosphogypsum leachates. *Journal of Environmental Quality* 24, 307-314.

Ryan-Salter, T. P., Black, A., Andrews, M., and Moot, D. J. (2014). Identification and effectiveness of rhizobial strains that nodulate *Lupinus polyphyllus*. New Zealand Grassland Association.

Ryan, J., and Smillie, G. (1975). Liming in relation to soil acidity and P fertilizer efficiency. *Communications in Soil Science and Plant Analysis* 6, 409-419.

Ryan, P., Delhaize, E., and Jones, D. (2001). Function and mechanism of organic anion exudation from plant roots. *Annual review of plant biology* 52, 527-560.

Saadaoui, E., Ghazel, N., Ben Romdhane, C., and Massoudi, N. (2017). Phosphogypsum: potential uses and problems–a review. *International Journal of Environmental Studies* 74, 558-567.

Sade, H., Meriga, B., Surapu, V., Gadi, J., Sunita, M. S. L., Suravajhala, P., and Kavi Kishor, P. B. (2016). Toxicity and tolerance of aluminum in plants: tailoring plants to suit to acid soils. *BioMetals* 29, 187-210.

Saggar, S., Hedley, M., and White, R. (1990). A simplified resin membrane technique for extracting phosphorus from soils. *Fertilizer Research* 24, 173-180.

Sainz Rozas, H., Calviño, P. A., Echeverría, H. E., Barbieri, P. A., and Redolatti, M. (2008). Contribution of anaerobically mineralized nitrogen to the reliability of planting or presidedress soil nitrogen test in maize. *Agronomy journal* 100, 1020-1025.

Sanaullah, M., Razavi, B. S., Blagodatskaya, E., and Kuzyakov, Y. (2016). Spatial distribution and catalytic mechanisms of β-glucosidase activity at the root-soil interface. *Biology and fertility of soils* 52, 505-514.

Sandral, G. A., Price, A., Hildebrand, S. M., Fuller, C. G., Haling, R. E., Stefanski, A., Yang, Z., Culvenor, R. A., Ryan, M. H., and Kidd, D. R. (2019). Field benchmarking of the critical external phosphorus requirements of pasture legumes for southern Australia. *Crop and Pasture Science* 70, 1080-1096.

Santner, J., Prohaska, T., Luo, J., and Zhang, H. (2010). Ferrihydrite containing gel for chemical imaging of labile phosphate species in sediments and soils using diffusive gradients in thin films. *Analytical chemistry* 82, 7668-7674.

Santner, J., Zhang, H., Leitner, D., Schnepf, A., Prohaska, T., Puschenreiter, M., and Wenzel, W. W. (2012). High-resolution chemical imaging of labile phosphorus in the rhizosphere of Brassica napus L. cultivars. *Environmental and Experimental Botany* 77, 219-226.

Sato, S., and Comerford, N. B. (2005). Influence of soil pH on inorganic phosphorus sorption and desorption in a humid Brazilian Ultisol. *Revista Brasileira de Ciência do Solo* 29, 685-694.

Ščančar, J., and Milačič, R. (2006). Aluminium speciation in environmental samples: a review. *Analytical and bioanalytical chemistry* 386, 999-1012.

Scanlan, C. (2015). Phosphorus Deficiency in Narrow Leafed Lupins. Vol. 2021. Western Australian Department of Agriculture.

Scanlan, C. A., Brennan, R. F., D'Antuono, M. F., and Sarre, G. A. (2017). The interaction between soil pH and phosphorus for wheat yield and the impact of lime-induced changes to soil aluminium and potassium. *Soil research* 55, 341-353.

Scherer, H., and Sharma, S. (2002). Phosphorus fractions and phosphorus delivery potential of a luvisol derived from loess amended with organic materials. *Biology and Fertility of Soils* 35, 414-419.

Schmitt, M., Watanabe, T., and Jansen, S. (2016). The effects of aluminium on plant growth in a temperate and deciduous aluminium accumulating species. *AoB PLANTS* 8, 1-13.

Schoeneberger, P., Wysocki, D., and Benham, E. (2012). "Field book for describing and sampling soils," third/Ed. Natural Resources Conservation Service, USDA, National Soil Survey Center, Lincoln, Nebraska, USA.

Schroth, G., Seixas, R., Da Silva, L., Teixeira, W., and Zech, W. (2000). Nutrient concentrations and acidity in ferralitic soil under perennial cropping, fallow and primary forest in central Amazonia. *European Journal of Soil Science* 51, 219-231.

Scott, D. (2014). The rise to dominance over two decades of *Lupinus polyphyllus* among pasture mixtures in tussock grassland trials. *In* "Proceedings of the New Zealand Grassland Association", pp. 47-52.

Scott, D., Keoghan, J., Cossens, G., Maunsell, L., Floate, M., Wills, B., and Douglas, G. (1985). Limitations to pasture production and choice of species. *New Zealand Grassland Association: Research and Practice Series* 3, 9-15.

Shah, A. S., Wakelin, S. A., Moot, D. J., Blond, C., Laugraud, A., and Ridgway, H. J. (2021). *Trifolium repens* and *T. subterraneum* modify their nodule microbiome in response to soil pH. *Journal of Applied Microbiology*.

Shann, J., and Bertsch, P. (1993). Differential cultivar response to polynuclear hydroxo-aluminum complexes. *Soil Science Society of America Journal* 57, 116-120.

Shi, S., Condron, L., Larsen, S., Richardson, A. E., Jones, E., Jiao, J., O'Callaghan, M., and Stewart, A. (2011). In situ sampling of low molecular weight organic anions from rhizosphere of radiata pine (Pinus radiata) grown in a rhizotron system. *Environmental and Experimental Botany* 70, 131-142.

Shuman, L. (1990). Comparison of exchangeable Al, extractable Al, and Al in soil fractions. *Canadian Journal of Soil Science* 70, 263-275.

Siao, W., Coskun, D., Baluška, F., Kronzucker, H. J., and Xu, W. (2020). Root-apex proton fluxes at the centre of soil-stress acclimation. *Trends in Plant Science* 25, 794-804.

Sikora, F. (2002). Evaluating and quantifying the liming potential of phosphate rocks. *Nutrient Cycling in Agroecosystems* 63, 59-67.

Silva, S. (2012). Aluminium Toxicity Targets in Plants. *Journal of Botany*.

Simonsson, M., Östlund, A., Renfjäll, L., Sigtryggsson, C., Börjesson, G., and Kätterer, T. (2018). Pools and solubility of soil phosphorus as affected by liming in long-term agricultural field experiments. *Geoderma* 315, 208-219.

Sims, T. J., and Pierzynski, G. M. (2005). Chemistry of phosphorus in soils. *In* "Chemical processes in soils" (M. Tabatabai and D. Sparks, eds.), Vol. 8, pp. 151-192. SSSA book series, Madison Wisconsin, USA.

Sinclair, A., Crooks, B., and Coull, M. (2014). Soils information, texture and liming recommendations. *Technical Note TN656, SRUC Aberdeen*.

Singh, S., Tripathi, D. K., Singh, S., Sharma, S., Dubey, N. K., Chauhan, D. K., and Vaculík, M. (2017). Toxicity of aluminium on various levels of plant cells and organism: a review. *Environmental and Experimental Botany* 137, 177-193.

Six, L., Smolders, E., and Merckx, R. (2013). The performance of DGT versus conventional soil phosphorus tests in tropical soils—maize and rice responses to P application. *Plant and Soil* 366, 49-66.

Sjöstedt, C. S., Gustafsson, J. P., and Köhler, S. J. (2010). Chemical equilibrium modeling of organic acids, pH, aluminum, and iron in Swedish surface waters. *Environmental science & technology* 44, 8587-8593.

Skujiņš, J., and Burns, R. (1976). Extracellular enzymes in soil. *CRC critical reviews in microbiology* 4, 383-421.

Smith, C., Peoples, M., Keerthisinghe, G., James, T., Garden, D., and Tuomi, S. (1994). Effect of surface applications of lime, gypsum and phosphogypsum on the alleviating of surface and subsurface acidity in a soil under pasture. *Soil Research* 32, 995-1008.

Smith, R., Martell, A., and Motekaitis, R. (2004). NIST standard reference database 46 version 8.0: NIST critically selected stability constants of metal complexes database. US National Institute of Standards and Technology, Gaithersburg, USA.

Soon, Y., Haq, A., and Arshad, M. (2007). Sensitivity of nitrogen mineralization indicators to crop and soil management. *Communications in soil science and plant analysis* 38, 2029-2043.

Soon, Y. K., and Arshad, M. A. (2005). Tillage and liming effects on crop and labile soil nitrogen in an acid soil. *Soil and Tillage Research* 80, 23-33.

Soon, Y. K., Clayton, G. W., and Rice, W. A. (2001). Tillage and previous crop effects on dynamics of nitrogen in a wheat–soil system. *Agronomy Journal* 93, 842-849.

Sorn-Srivichai, P., Tillman, R. W., Syers, J. K., and Cornforth, I. S. (1984). The effect of soil pH on Olsen bicarbonate phosphate values. *Journal of the Science of Food and Agriculture* 35, 257-264.

Spiers, G., and McGill, W. (1979). Effects of phosphorus addition and energy supply on acid phosphatase production and activity in soils. *Soil Biology and Biochemistry* 11, 3-8.

Sposito, G. (2008). "The chemistry of soils," 2/Ed. Oxford University Press.

Stewart, A., Kerr, G., Lissaman, W., and Rowarth, J. (2000). "Pasture and forage plants for New Zealand," 4th/Ed. New Zealand Grassland Association, Dunedin, New Zealand.

Stockdale, E., and Rees, R. (1994). Relationships between biomass nitrogen and nitrogen extracted by other nitrogen availability methods. *Soil Biology and Biochemistry* 26, 1213-1220.

Su, C., and Evans, L. (1996). Soil solution chemistry and alfalfa response to CaCO3 and MgCO3 on an acidic gleysol. *Canadian Journal of Soil Science* 76, 41-47.

Sugihara, S., Tomita, Y., Nishigaki, T., Kilasara, M., Wasaki, J., and Funakawa, S. (2016). Effects of different phosphorus-efficient legumes and soil texture on fractionated rhizosphere soil phosphorus of strongly weathered soils. *Biology and Fertility of Soils* 52, 367-376.

Sumner, M. E., and Noble, A. D. (2003). Soil acidification: the world story. *In* "Handbook of soil acidity" (Z. Rengel, ed.), pp. 1-28. Marcel Dekker, Inc., New York, USA.

Sun, B., Gao, Y., Wu, X., Ma, H., Zheng, C., Wang, X., Zhang, H., Li, Z., and Yang, H. (2020). The relative contributions of pH, organic anions, and phosphatase to rhizosphere soil phosphorus mobilization and crop phosphorus uptake in maize/alfalfa polyculture. *Plant and Soil*, 1-17.

Suzaki, T., Yoro, E., and Kawaguchi, M. (2015). Leguminous plants: inventors of root nodules to accommodate symbiotic bacteria. *International review of cell and molecular biology* 316, 111-158.

Tabatabai, M. A. (1994). Soil enzymes. *In* "Methods of Soil Analysis: Part 2—Microbiological and Biochemical Properties" (R. Weaver, S. Angle, P. Bottomley, D. Bezdicek, S. Smith, A. Tabatabai and A. Wollum, eds.), Vol. 5, pp. 775-833. Soil Science Society of America, Madison, WI.

Taher, M. (2007). Influence of thermally treated phosphogypsum on the properties of Portland slag cement. *Resources, Conservation and Recycling* 52, 28-38.

Takamoto, A., Takahashi, T., and Togami, K. (2021). Effect of changes in the soil calcium-to-magnesium ratio by calcium application on soybeans, Glycine max (L.) Merr., growth. *Soil Science and Plant Nutrition*, 1-11.

Tamás, L., Huttová, J., Mistrík, I., Šimonovic, M., and Široká, B. (2006). Aluminium-induced drought and oxidative stress in barley roots. *Journal of plant physiology* 163, 781-784.

Tanaka, A., Tadano, T., Yamamoto, K., and Kanamura, N. (1987). Comparison of Toxicity to Plants among Al8+, AlSO4+, and Al-F Complex Ions. *Soil Science and Plant Nutrition* 33, 43-55.

Tang, C., Longnecker, N., Thomson, C., Greenway, H., and Robson, A. (1992). Lupin (*Lupinus angustifolius L.*) and pea (Pisum sativum L.) roots differ in their sensitivity to pH above 6.0. *Journal of Plant Physiology* 140, 715-719.

Tang, C., and Rengel, Z. (2003). Role of plant cation/anion uptake ratio in soil acidification. *Handbook of soil acidity. Marcel Dekker, New York*, 57-81.

Tang, C., and Robson, A. D. (1993). pH above 6.0 reduces nodulation in *Lupinus* species. *Plant and Soil* 152, 269-276.

Tang, C., Unkovich, M., and Bowden, J. (1999). Factors affecting soil acidification under legumes. III. Acid production by N2-fixing legumes as influenced by nitrate supply. *New Phytologist* 143, 513-521.

Tayibi, H., Choura, M., López, F. A., Alguacil, F. J., and López-Delgado, A. (2009). Environmental impact and management of phosphogypsum. *Journal of environmental management* 90, 2377-2386.

Taylor, M. D., Kim, N. D., Briggs, R. M., Taylor, A., and Guinto, D. F. (2012). An investigation into the process of dealumination in soils of the Auckland, Bay of Plenty and Waikato regions of New Zealand. *Applied clay science* 64, 18-24.

Thomas Sims, J., and Pierzynski, G. M. (2005). Chemistry of phosphorus in soils. *Chemical processes in soils* 8, 151-192.

Tiessen, H., and Moir, J. (2008). Characterization of available P by sequential extraction. *In* "Soil sampling and methods of analysis" (M. R. Carter and E. G. Gregorich, eds.), pp. 293–306. Taylor and Francis Group, Boca Raton Fl, USA.

Tipping, E. (2005). Modelling Al competition for heavy metal binding by dissolved organic matter in soil and surface waters of acid and neutral pH. *Geoderma* 127, 293-304.

Tipping, E., and Hurley, M. A. (1988). A model of solid-solution interactions in acid organic soils, based on the complexation properties of humic substances. *Journal of Soil Science* 39, 505-519.

Tiziani, R., Puschenreiter, M., Smolders, E., Mimmo, T., Herrera, J. C., Cesco, S., and Santner, J. (2020). Millimeter-resolution mapping of citrate exuded from soil grown roots using a novel, low-invasive sampling technique. *bioRxiv*.

Toal, M., Yeomans, C., Killham, K., and Meharg, A. (2000). A review of rhizosphere carbon flow modelling. *Plant and Soil* 222, 263-281.

Toma, M., Sumner, M., Weeks, G., and Saigusa, M. (1999). Long-term effects of gypsum on crop yield and subsoil chemical properties. *Soil Science Society of America Journal* 63, 891-895.

Tozer, K., Douglas, G., Dodd, M., and Müller, K. (2021). Vegetation Options for Increasing Resilience in Pastoral Hill Country. *Frontiers in Sustainable Food Systems* 5, 8.

Tozer, K. N., Marshall, A. J., Cameron, C. A., and Edwards, G. R. (2013). Salt (NaCl) application as a tool to reduce the annual grass weed, ripgut brome, in New Zealand South Island hill/high country pastures. *New Zealand Journal of Agricultural Research* 56, 117-129.

Trasar-Cepeda, M. C., Carballas, T., Gil-Sotres, F., and De Blas, E. (1991). Liming and the phosphatase activity and mineralization of phosphorus in an andic soil. *Soil Biology and Biochemistry* 23, 209-215.

Tunney, H., Sikora, F., Kissel, D., Wolf, A., Sonon, L., and Goulding, K. (2010). A comparison of lime requirements by five methods on grassland mineral soils in Ireland. *Soil use and management* 26, 126-132.

Turner, B., and Blackwell, M. (2013). Isolating the influence of pH on the amounts and forms of soil organic phosphorus. *European Journal of Soil Science* 64, 249-259.

Turner, B. L. (2010). Variation in pH optima of hydrolytic enzyme activities in tropical rain forest soils. *Applied and Environmental Microbiology* 76, 6485-6493.

Turner, B. L., Cade-Menun, B. J., Condron, L. M., and Newman, S. (2005). Extraction of soil organic phosphorus. *Talanta* 66, 294-306.

Turner, B. L., and Haygarth, P. M. (2005). Phosphatase activity in temperate pasture soils: Potential regulation of labile organic phosphorus turnover by phosphodiesterase activity. *Science of the Total Environment* 344, 27-36.

Turner, B. L., Mahieu, N., and Condron, L. M. (2003). The phosphorus composition of temperate pasture soils determined by NaOH–EDTA extraction and solution 31P NMR spectroscopy. *Organic Geochemistry* 34, 1199-1210.

Turner, B. L., Papházy, M. J., Haygarth, P. M., and McKelvie, I. D. (2002). Inositol phosphates in the environment. *Philosophical Transactions of the Royal Society of London B* 357, 449-469.

Turner, L., and Kramer, J. (1991). Sulfate ion binding on goethite and hematite. *Soil Science* 152, 226-230.

USDA (2014). "Keys to Soil Taxonomy," 12th /Ed. United States Department of Agriculture, Natural Resources Conservation Service, Washington, D.C.

Valentinuzzi, F., Mimmo, T., Cesco, S., Al Mamun, S., Santner, J., Hoefer, C., Oburger, E., Robinson, B., and Lehto, N. (2015). The effect of lime on the rhizosphere processes and elemental uptake of white lupin. *Environmental and Experimental Botany* 118, 85-94.

Valeur, I., and Nilsson, I. (1993). Effects of lime and two incubation techniques on sulfur mineralization in a forest soil. *Soil Biology and Biochemistry* 25, 1343-1350.

Valeur, I., Nilsson, S. I., Andersson, S., and Sjöberg, G. (2002). Net sulphur mineralization in forest soils as influenced by different lime application rates. *Soil Biology and Biochemistry* 34, 1291-1298.

Valtonen, A., Jantunen, J., and Saarinen, K. (2006). Flora and lepidoptera fauna adversely affected by invasive *Lupinus polyphyllus* along road verges. *Biological conservation* 133, 389-396.

van Hees, P., Lundström, U., Danielsson, R., and Nyberg, L. (2001). Controlling mechanisms of aluminium in soil solution–an evaluation of 180 podzolic forest soils. *Chemosphere* 45, 1091-1101.

Van Hees, P., Lundström, U., Starr, M., and Giesler, R. (2000). Factors influencing aluminium concentrations in soil solution from podzols. *Geoderma* 94, 289-310.

Vazquez, E., Benito, M., Espejo, R., and Teutscherova, N. (2019). Effects of no-tillage and liming amendment combination on soil carbon and nitrogen mineralization. *European Journal of Soil Biology* 93, 103090.

Venter, A., Beukes, D., Claassens, A., and Van Meirvenne, M. (2004). Temporal and spatial relations of plant element uptake and yield of a lucerne stand. *South African Journal of Plant and Soil* 21, 157-165.

Venter, H. J. (2017). "Soil acidity in the Kakanui ranges." Fertiliser and Lime Research Centre, Massey University, Palmerston North, New Zealand.

Vitorello, V. A., Capaldi, F. R., and Stefanuto, V. A. (2005). Recent advances in aluminum toxicity and resistance in higher plants. *Brazilian Journal of Plant Physiology* 17, 129-143.

Vogel, C., Sekine, R., Steckenmesser, D., Lombi, E., Herzel, H., Zuin, L., Wang, D., Félix, R., and Adam, C. (2019). Combining diffusive gradients in thin films (DGT) and spectroscopic techniques for the determination of phosphorus species in soils. *Analytica Chimica Acta* 1057, 80-87.

Volf, M. R., and Rosolem, C. A. (2020). Soil P Diffusion and Availability Modified by Controlled-Release P Fertilizers. *Journal of Soil Science and Plant Nutrition*, 1-11.

Von Uexküll, H., and Mutert, E. (1995). Global extent, development and economic impact of acid soils. *Plant and soil* 171, 1-15.

Vyshpolsky, F., Qadir, M., Karimov, A., Mukhamedjanov, K., Bekbaev, U., Paroda, R., Aw-Hassan, A., and Karajeh, F. (2008). Enhancing the productivity of high-magnesium soil and water resources

in Central Asia through the application of phosphogypsum. *Land Degradation & Development* 19, 45-56.
Wachendorf, C. (2015). Effects of liming and mineral N on initial decomposition of soil organic matter and post harvest root residues of poplar. *Geoderma* 259, 243-250.
Waldrop, M. P., Balser, T. C., and Firestone, M. K. (2000). Linking microbial community composition to function in a tropical soil. *Soil Biology and Biochemistry* 32, 1837-1846.
Wan, W., Hao, X., Xing, Y., Liu, S., Zhang, X., Li, X., Chen, W., and Huang, Q. (2020). Spatial differences in soil microbial diversity caused by pH-driven organic phosphorus mineralization. *Land Degradation & Development*.
Wang, A. S., Angle, J. S., Chaney, R. L., Delorme, T. A., and McIntosh, M. (2006a). Changes in soil biological activities under reduced soil pH during Thlaspi caerulescens phytoextraction. *Soil Biology and Biochemistry* 38, 1451-1461.
Wang, B., Shen, J., Tang, C., and Rengel, Z. (2008). Root morphology, proton release, and carboxylate exudation in lupin in response to phosphorus deficiency. *Journal of Plant Nutrition* 31, 557-570.
Wang, G., Zhang, Q., Du, W., Lin, R., Li, J., Ai, F., Yin, Y., Ji, R., Wang, X., and Guo, H. (2021). In-situ immobilization of cadmium-polluted upland soil: A ten-year field study. *Ecotoxicology and Environmental Safety* 207, 111275.
Wang, H., Bolan, N., Hedley, M., and Horne, D. (1999). The influence of surface incorporated lime and gypsiferous by-products on surface and subsurface soil acidity. I. Soil solution chemistry. *Soil Research* 37, 165-180.
Wang, J. (2020). Utilization effects and environmental risks of phosphogypsum in agriculture: A review. *Journal of Cleaner Production*, 123337.
Wang, P., Bi, S., Ma, L., and Han, W. (2006b). Aluminum tolerance of two wheat cultivars (Brevor and Atlas66) in relation to their rhizosphere pH and organic acids exuded from roots. *Journal of agricultural and food chemistry* 54, 10033-10039.
Wang, Y., Krogstad, T., Clarke, J. L., Hallama, M., Øgaard, A. F., Eich-Greatorex, S., Kandeler, E., and Clarke, N. (2016). Rhizosphere organic anions play a minor role in improving crop species' ability to take up residual phosphorus (P) in agricultural soils low in P availability. *Frontiers in Plant Science* 7, 1664.
Wang, Y., Krogstad, T., Clarke, N., Øgaard, A. F., and Clarke, J. L. (2017). Impact of phosphorus on rhizosphere organic anions of wheat at different growth stages under field conditions. *AoB Plants* 9.
Wang, Y., and Lambers, H. (2020). Root-released organic anions in response to low phosphorus availability: recent progress, challenges and future perspectives. *Plant and Soil* 447, 135-156.
Wang, Y., Yu, W., Cao, Y., Cai, Y., Lyi, S. M., Wu, W., Kang, Y., Liang, C., and Liu, J. (2020). An exclusion mechanism is epistatic to an internal detoxification mechanism in aluminum resistance in Arabidopsis. *BMC plant biology* 20, 1-12.
Wang, Z., Yu, K., Lv, S., Niklas, K. J., Mipam, T. D., Crowther, T. W., Umaña, M. N., Zhao, Q., Huang, H., and Reich, P. B. (2019). The scaling of fine root nitrogen versus phosphorus in terrestrial plants: a global synthesis. *Functional Ecology* 33, 2081-2094.
Wasaki, J., Yamamura, T., Shinano, T., and Osaki, M. (2003). Secreted acid phosphatase is expressed in cluster roots of lupin in response to phosphorus deficiency. *Plant and Soil* 248, 129-136.
Watkinson, J., and Kear, M. (1994). High performance ion chromatography measurement of sulfate in 20 m M phosphate extracts of soil. *Communications in soil science and plant analysis* 25, 1015-1033.
Wen, Z., Li, H., Shen, J., and Rengel, Z. (2017). Maize responds to low shoot P concentration by altering root morphology rather than increasing root exudation. *Plant and Soil* 416, 377-389.
Wheeler, D., and O'Connor, M. (1998). Why do pastures respond to lime? *In* "Proceedings of the New Zealand Grassland Association", pp. 57-61.
White, R., and Taylor, A. (1977). Effect of pH on phosphate adsorption and isotopic exchange in acid soils at low and high additions of soluble phosphate. *Journal of Soil Science* 28, 48-61.
Whitley, A., Moir, J., and Almond, P. (2019). A meta-analysis of exchangeable aluminium in New Zealand soils using the National Soils Database. *Soil Research* 57, 113-123.

Whitley, A., Moir, J., Almond, P., and Moot, D. (2016). Soil pH and exchangeable aluminium in contrasting New Zealand high and hill country soils. *Hill Country–Grassland Research and Practice Series* 16, 169-172.

Whitley, A. E. (2018). Investigations of soil extractable aluminium and toxicity in New Zealand soils: A thesis submitted in partial fulfilment of the requirements for the Degree of Doctor of Philosophy at Lincoln University. Lincoln University.

Whitley, A. E., Moir, J. L., Lehto, N. J., and Paramashivam, D. (2020). Extractable aluminium in New Zealand Andisols and Inceptisols. *Geoderma Regional* 22, 1-8.

Wigley, K., Ridgway, H. J., Humphries, A. W., Ballard, R. A., and Moot, D. J. (2018). Increased lucerne nodulation in acid soils with Sinorhizobium meliloti and lucerne tolerant to low pH and high aluminium. *Crop and Pasture Science* 69, 1031-1040.

Wu, J., Huang, M., Xiao, H.-A., Su, Y.-R., Tong, C.-L., Huang, D.-Y., and Syers, J. K. (2007). Dynamics in microbial immobilization and transformations of phosphorus in highly weathered subtropical soil following organic amendments. *Plant and soil* 290, 333-342.

Yan, F., Zhu, Y., Müller, C., Zörb, C., and Schubert, S. (2002). Adaptation of H+-pumping and plasma membrane H+ ATPase activity in proteoid roots of white lupin under phosphate deficiency. *Plant physiology* 129, 50-63.

Yerima, B. P. K., Enang, R. K., Kome, G. K., and Van Ranst, E. (2020). Exchangeable aluminium and acidity in Acrisols and Ferralsols of the north-west highlands of Cameroon. *Geoderma Regional* 23, 1-9.

Yu, Y. W., Fraser, M., and Evans, J. (2011). Long-term effects on sward composition and animal performance of reducing fertilizer inputs to upland permanent pasture. *Grass and Forage Science* 66, 138-151.

Zhang, X., Dippold, M. A., Kuzyakov, Y., and Razavi, B. S. (2019a). Spatial pattern of enzyme activities depends on root exudate composition. *Soil Biology and Biochemistry* 133, 83-93.

Zhang, X., Long, Y., Huang, J., and Xia, J. (2019b). Molecular mechanisms for coping with Al toxicity in plants. *International journal of molecular sciences* 20, 1551.

Zhang, Y., Peng, Y., Yun-Feng, P., Xue-Xian, L., Fan-Jun, C., and Chun-Jian, L. (2012). Fine root patterning and balanced inorganic phosphorus distribution in the soil indicate distinctive adaptation of maize plants to phosphorus deficiency. *Pedosphere* 22, 870-877.

Zhao, Z., Wang, S., White, P. J., Wang, Y., Shi, L., and Xu, F. (2020). Boron and Phosphorus Act Synergistically to Modulate Absorption and Distribution of Phosphorus and Growth of Brassica napus. *Journal of Agricultural and Food Chemistry* 68, 7830-7838.

Zheng, S. J., Ma, J. F., and Matsumoto, H. (1998). Continuous secretion of organic acids is related to aluminium resistance during relatively long-term exposure to aluminium stress. *Physiologia Plantarum* 103, 209-214.

Zhu, Y., Yan, F., Zörb, C., and Schubert, S. (2005). A Link Between Citrate and Proton Release by Proteoid Roots of White Lupin (*Lupinus albus L.*) Grown Under Phosphorus-deficient Conditions? *Plant and Cell Physiology* 46, 892-901.

Zobel, R. W., Kinraide, T. B., and Baligar, V. C. (2007). Fine root diameters can change in response to changes in nutrient concentrations. *Plant and Soil* 297, 243-254.