Response of 12 Pasture Legumes to Phosphorus and Lime Additions when Grown in a High Country Soil Under Glasshouse Conditions

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A dissertation
submitted in partial fulfilment
of the requirements for the Degree of
Bachelor of Agricultural Science with Honours

at
Lincoln University

by
Peter Robert Jordan

Lincoln University
2011

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Abstract

Legumes are the main source of plant available nitrogen in high country pastures through nitrogen fixation. High country soils are typically acidic and have low fertility compared with intensive high fertility low land systems. Therefore the legumes commonly used in New Zealand perform poorly in these high country environments. The optimum P and pH status for growth of many potential alternative legume species for this environment are unknown.

Twelve novel legume species were grown for a period of 42 weeks under glasshouse conditions at Lincoln University in an acidic high country soil (Ashwick stoney / bolder silt loam) from the Lees Valley (North Canterbury). Phosphorus was applied at eight rates (0, 10, 30, 60, 100, 250, 500, 1500 mg P kg^{-1} soil) while lime (100% $CaCO_3$) was applied at five rates (0, 2, 5, 8, 15 t ha^{-1} equivalent). Plants were harvested monthly post establishment and the yield was determined. Annual species grew on average for 25 weeks, while perennial species grew for 42 weeks. Herbage was analysed for macro and micro element content and uptake. Soils were analysed for available P content and pH at the end of the experiment.

Phosphorus increased the yield of both annual and perennial legume species through the increase in plant available P. Optimum Olsen P for maximum yield differed between species as did P use efficiency. Persian clover was the highest yielding annual species (13.6 g DM pot^{-1}) followed by subterranean clover > arrowleaf clover > balansa clover, while gland clover was the lowest yielding annual species (1.2 g DM pot^{-1}). Lotus was the highest yielding perennial species (15.0 g DM pot^{-1}) followed by tagasaste > lucerne > Caucasian clover > falcata lucerne > strawberry clover, while white clover was the lowest yielding perennial species (8.2 g DM pot^{-1}). As a measure of P use efficiency, the Olsen P at which biological optimum (97%) yields were achieved varied between species, ranging from 21 µg ml^{-1}

(tagasaste) to 174 µg ml^{-1} (gland clover). Gland, balansa and arrowleaf clovers had the lowest P use efficiency, while tagasaste, white and Persian clovers had the highest. Arrowleaf, subterranean and balansa clovers gave the greatest increase in yield at low P inputs (100 mg P kg^{-1}).

Lime increased the yield of both annual and perennial species up to a point, beyond which yield decreased. This increase in yield was primarily driven by an increase in plant available P and Mo, while decreases in yield were driven by the decrease in P and B availability at high pH values. Persian clover was the highest yielding annual species (8.5 g DM pot^{-1}) while gland clover was the lowest yielding annual species (0.7 g DM pot^{-1}). Tagasaste was the highest yielding perennial species (11.0 g DM pot^{-1}) while white clover was the lowest yielding perennial species (5.0 g DM pot^{-1}). Lotus and tagasaste were the highest yielding species under acidic conditions, while lotus, lucerne and Persian clover yielded the most under alkaline conditions. Strawberry clover, lucerne and falcata lucerne showed the greatest response from relatively small inputs of lime (2 t lime ha^{-1}). A pH induced boron deficiency was identified in some species (subterranean, balansa, Persian, Caucasian and arrowleaf clovers), which also contributed to reduced yields at high pH.

The optimum Olsen P and pH level for maximum yield has been identified for these 12 legume species. For many species examined in this experiment, this represents new and valuable information. The practical implications of the results are discussed. Further research using field trials is required to confirm these results under natural climatic and physical conditions.

Keywords:
Chamaecytisus proleferus, DM yield, *Lotus pedunculatus*, *Medicago falcate*, *Medicago sativa*, nutrient efficiency, nutrient uptake, soil pH, *Trifolium aumbiguum*, *Trifolium fragiferum*, *Trifolium glanduliferum*, *Trifolium michelianum*, *Trifolium repens*, *Trifolium resupinatum*, *Trifolium subterraneum*, *Trifolium vesiculosum*.

Acknowledgements

This dissertation is the result of one year's work, in which I have been assisted by many people from within and outside the university. I wish to express my sincere appreciation to the following people for their support and assistance with this research project.

To my supervisor and lecturers, I have been privileged to work alongside such a world renowned team of scientists with such knowledge and expertise. Not only have I enjoyed my honours year, but have achieved academically what I would have never thought possible, due to the guidance of my supervisor and lecturers at Lincoln University.

Dr Jim Moir for contributing so much time and effort to this study. Your constant advice, support and guidance throughout this last year in both the practical and theoretical aspects of this study have been greatly appreciated and were invaluable to the success of my final year. You have been a great supervisor, and I have learnt so much from you.

Professor Derrick Moot for your help and advice not only in this last year, but throughout my studies. Your passion not only for the sciences, but for teaching them is amazing, and greatly appreciated.

Mr Richard Lucas for your advice throughout this experiment. Your knowledge of plants is astounding and I appreciate you sharing this with me.

Agam Nangul, Malcolm Smith and the Glasshouse staff for technical assistance throughout this experiment. The care you took with this experiment was much appreciated and critical to the success of this study.

Angela Reid and Julia Bellamy for assistance with the herbage digest as well as Lynne Clucas for ICP analyses.

Claire Marshall for all your help, encouragement and support. I appreciate that not just anyone would pretend to be enthusiastic about spending the weekend helping me weed pots.

To my parents Lindsay and Robyn Jordan, and siblings Mark and Cathy for your support and interest throughout my studies. The many phone calls were much appreciated.

As well as everyone else not previously mentioned that made this project possible and this year so enjoyable!

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Chapter 1
Introduction

The hill and high country of New Zealand is extensively farmed and in terms of productivity often has a short, moisture limited production season. The soils are typically acidic, with low available phosphorus (P) and sulphur (S) which influences the growth and persistence of legumes (Haynes & Williams 1993; Moir *et al.* 2000).

Legumes play a key role in New Zealand agriculture, contributing nitrogen (N) to the system as well as increasing the quality of feed, for improved animal performance (Brown & Green 2003). N is often the most limiting nutrient for pastures, while the amount of N fixed from the atmosphere and added to the soil by legumes is significant and may be the only nitrogen input to the system. There is extensive literature on the optimum growth conditions of white clover (*Trifolium repens*) (Black *et al.* 2000; During & Brier 1973; Lowther & Adams 1970), the most commonly sown clover in New Zealand but it is poorly suited to dryland low fertility conditions. Subterranean clover (*Trifolium subterranean*) has also been researched for this environment (Brown *et al.* 2006; Hayes *et al.* 2008a). However, farmers require a more diverse range of both perennial and annual legume species for their dryland farming systems (Brown & Green 2003). There is little available information on many other potential species for the hill country environment in terms of optimum soil or nutrient conditions for growth.

Phosphorus is required at higher concentrations for legumes to grow and compete with grasses (Caradus 1980). Adequate plant-available soil P is critical for their production and persistence. P has been aerially applied to the high country in the form of super phosphate for over 50 years (Gillingham *et al.* 1999). However, due to the economics of this practice most high and hill country soils are deficient in P. For this reason species which perform well in low P soils are valuable for this type of production system and environment.

Plants can also be affected by acidic (low pH) soil conditions (Edmeades & Ridley 2003), particularly in terms of nutritional requirements, which affects yield. Many legumes are particularly sensitive to low pH. The application of lime is used to increase soil pH. However this practice is often uneconomic on these extensive properties due to the high application costs. Plants which are able to thrive in low pH soils or with low inputs of lime and P, would be well suited to this environment and could be of major benefit to these farming systems.

This experiment was carried out under glasshouse conditions as a pot trial to allow for the accurate manipulation of soil conditions (P fertility status and pH) and accurate yield measurements under controlled climatic conditions so the effect of P and pH could be accurately assessed.

The objective of this study was to determine the effects of P or lime addition on the growth and nutrient uptake of 12 legume species grown in an acid high country soil from the Lees Valley in North Canterbury. The hypothesis being tested in this experiment is that the growth and nutrient uptake will differ between species with different soil P levels and different soil pH conditions.

Chapter 2

Literature Review

2.1 Introduction

New Zealand high country is a challenging environment for plant growth, where soils are typically nutrient poor and acidic (Moir & Moot 2010). Pasture legumes are a critical part of these high country farming systems, in terms of nitrogen input from biological N_2 fixation, which along with climate, is the key driver of overall pasture yield (Caradus *et al.* 1996). Further, pasture legumes provide high quality feed to improve livestock production (Caradus *et al.* 1996; Hofmann *et al.* 2007).

Phosphorus is second only to N as the key macro nutrient driving the productivity of legume based grazed pasture systems in New Zealand hill and high country (Moir *et al.*, 1997). Historically, P has been applied to high country as single super phosphate (SSP), made possible through aerial topdressing. This has substantially increased the productivity of high country. Different plant species are more or less efficient at extracting and utilizing soil P and some require higher P levels than other species. Legume species which are productive yet more P efficient could be of substantial value in low input high country systems.

Soil pH also plays a critical role in the growth of plants, having a major effect on their performance and persistence. The major effects of pH come from the interactions with other elements, potentially causing deficiencies and toxicities in plants at ‘high’ or ‘low’ pHs (Edmeades & Ridley 2003). From previous research it is evident that some plants perform better than others at different pH levels. This indicates a range of suitability of such species for acid or alkaline field soil environments.

This review of literature focuses on the current knowledge on the effects of soil phosphorus and pH levels on pasture legumes, with particular reference to dryland legumes in New Zealand high country.

2.2 Phosphorus

Phosphorus is a critical nutrient for productive developed pastures, particularly for legume growth and persistence in the sward. In many cases legumes are the only source of nitrogen input via fixation to the system to alleviate the chronic N deficiency in hill and high country

(Lambert *et al.* 1988). More phosphorus has been applied to New Zealand soils than any other single nutrient (McLaren & Cameron 1990) while around 20 x 10^9 kg of P is applied every year worldwide (Cramer 2010).

2.2.1 Phosphorus cycling in grazed pastures

Fertilizer P, in the form of single superphosphate (SSP) is flown onto many large areas of New Zealand's high country as the sole input of P to the system (Gillingham *et al.* 1999). Shown below in Figure 2.1 is the P cycle. A significant amount of the total P that is removed from the soil by plants and then grazed is returned to the system in the form of dung and decomposing plant material. Approximately 20 – 35% of the P taken from the soil is lost from hill and high country systems as meat, or dung deposited to the races or yards. Also in hill country environments, around 60% of the nutrients can be deposited on the stock camps which only make up around 13 – 30% of the land (Kemp *et al.* 1999), there are also significant amounts deposited onto stock tracks (Gillingham *et al.* 1980). Virtually no P is found in the urine of grazing animals. A significant amount of fertilizer P is lost when fixed into non-labile forms. Leaching is thought to be less than 1 kg ha^{-1} $year^{-1}$, so is considered negligible except for through very sandy soils (McLaren & Cameron 1990). The interactions of the soil solution, labile and non- labile forms are discussed in Section 2.2.3.1.

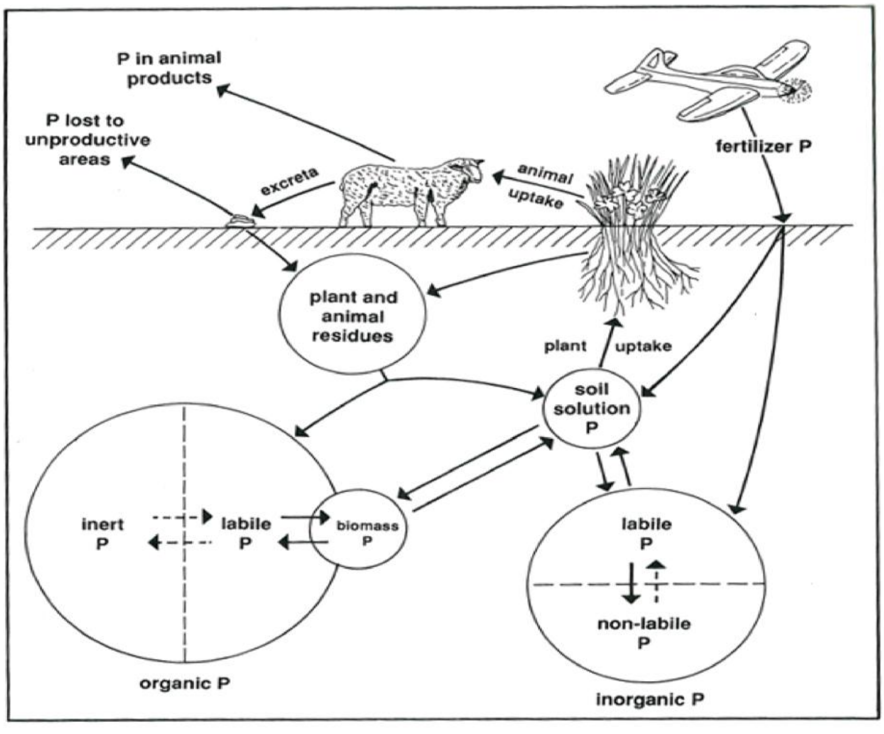

Figure 2.1: The phosphorus cycle in a grazed system (McLaren & Cameron 1990).

2.2.2 Plant P

2.2.2.1 Function

Phosphorus is considered deficient in the plant at levels less than 0.30% of DM (Morton & Roberts 1999). It is a constituent of ATP (adenosine triphosphate) and ADP (adenosine diphosphate), the main energy sources for the majority of cellular functions. Phosphorus is also a component of nucleic acids and therefore DNA (the building blocks of all living organisms), as well as phospholipids which make up the cell membranes and several essential co-enzymes which are required for the protein's biological activity (Raven *et al.* 1992).

2.2.2.2 Acquisition

Within the rhizosphere, P is likely to be the least available nutrient as it can be fixed by inorganic colloids at both alkaline and acidic pH values. Plants use phosphorus in the form of monovalent phosphate (P*i*) anions ($H_2PO_4^-$) and less rapidly as the divalent anion (HPO_4^{2-}) (Sailsbury & Ross 1992; Syers *et al.* 2008). Plants are able to acquire P*i* better under deficiency as they may produce and secrete organic acids into the rhizosphere, which induce the solubilisation of P*i* (Pinton *et al.* 2007; Syers *et al.* 2008). Figure 2.2 illustrates the changes in speciation in relation to pH change. As hill country soils are typically acidic, the majority of phosphate is likely to be in the form of $H_2PO_4^-$.

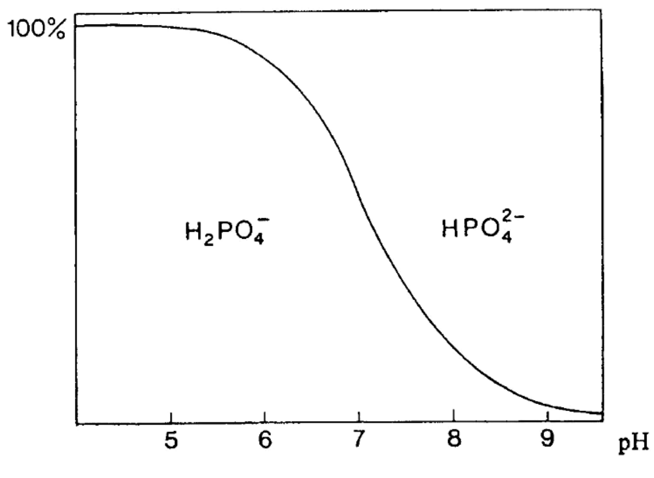

Figure 2.2: Ratio between $H_2PO_4^-$ and HPO_4^{2-} in relation to pH (Mengel & Kirkby 2001).

Plant roots primarily take up P from the soil by the two processes of mass flow and diffusion (Barber 1984). The amount of P taken up as mass flow is dependent on the water uptake of the plant and also the concentration of P in the soil solution. Diffusion is the main way that P makes its way to the root surface because of the concentration gradient (Syers *et al.* 2008).

2.2.3 Soil P

Phosphate is present in different forms in soil, such as labile and non-labile P fractions. This affects the bio-availability of the P to plants. P is also lost from the system, while large amounts of P have been added to the high country of New Zealand through the use of particularly single super phosphate. This has lead to an increase in the Olsen P values of our hill country (Lambert *et al.* 1988).

2.2.3.1 P fractions

Phosphorus is present in soil in both organic and inorganic forms. The P can either be in the soil solution, or in a solid phase as both labile and non-labile fractions (Figure 2.3) (Hedley *et al.* 1982). P in the soil solution is normally fully available for plant uptake, while more than 90% of the P in the soil is insoluble or in fixed forms. Non-labile forms of soil P include, primary phosphate minerals such as Calcium, Iron and Aluminium (Al) phosphates and also humus (organic / carbon based) P. The labile soil P fraction is available to plants, and is bound by the soil particle surface or in phosphate precipitations which are in equilibrium with the soil solution (Mengel & Kirkby 2001).

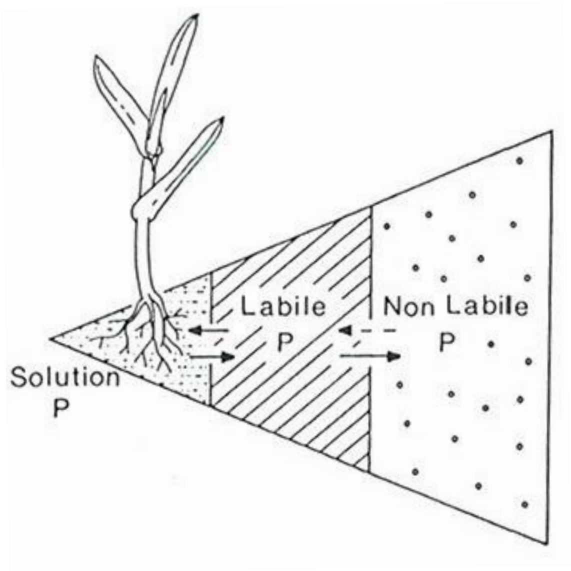

Figure 2.3: The interactions between the three important phosphorus fractions for plant nutrition (Mengel & Kirkby 2001).

Therefore, soil P moves between labile and non-labile phases (Figure 2.3). When the plant takes P from the soil solution, P is released from the solid state labile pool into the soil solution (Syers *et al.* 2008).

2.2.3.2 Bioavailability

The P content of soil ranges from 0.02 to 0.15% P depending on the degree of weathering of the different parent materials and leaching that has occurred (McLaren & Cameron 1990). Even at high soil P levels, there may only be 1 kg P ha^{-1} available in the soil solution, yet a crop may take up 20 - 40 kg P ha^{-1} growing $season^{-1}$. P is made available through the desorption from the solid (non-labile) state in the soil, provided there is sufficient P on the adsorption sites (Syers *et al.* 2008).

2.2.4 Phosphorus losses

Phosphorus is lost from the pasture based system both above and below ground (Figure 2.1). P removed in animal products can account for 15% of the P taken up by the animals, with the remainder redeposited to the system in dung (Saggar *et al.* 1990). On high country properties, P is shifted to camp sites and stock tracks, as stock graze the steep slopes, but spend more time on the less steep areas of the paddock (Gillingham 1980). Therefore just 10% of the total P is redeposited onto the steep slopes despite these making up 28% of the area in this experiment (Saggar *et al.* 1990). Soil P is also 'lost' from the labile pool through mineralization into non-labile forms (McLaren & Cameron 1990).

2.2.5 Nutrient accumulation from long-term SSP application

Single super phosphate (SSP) has been the main phosphate fertilizer used in New Zealand since pastoral farming was developed over 100 years ago. It contains between 8.5 and 10% P and 10 to 12% sulphur (S). SSP is made by treating ground phosphate rock with sulphuric acid (Gowariker *et al.* 2009). Moir *et al.* (1997) examined the long term effects of single super phosphate application on nutrient accumulation in the hill country of the east coast of the North Island of New Zealand. Results indicated that overall, the long term application of SSP had resulted in large increases in total soil P, with 1000 µg P g^{-1} accumulating in the 0 to 75 mm soil horizon. Soil N and S was also shown to have accumulated as a result of the application of SSP, but to a lesser degree (proportionally) than that of P. This was possibly due to greater leaching losses of S and N. This agrees with the findings of Lambert *et al.* (1988) who found that under high SSP inputs (490 kg ha^{-1} yr^{-1}) 47% of the fertilizer P was still present in the 0 to 75 mm soil samples taken each year. In terms of soil P fraction, 72% of this P was in the inorganic form.

2.2.6 Pasture response to added P in New Zealand

Higher historic SSP inputs are generally associated with higher soil Olsen P status, and increased dry matter production in the New Zealand hill country (Moir *et al.*, 2000). Figure 2.4 illustrates a general relationship between soil Olsen P and grass yield for a particular soil type (Mengel and Kirkby, 2001). This example shows that above an Olsen P value of 20 µg ml^{-1} there is no extra DM produced, indicating that the pastures are strongly responsive to P additions (are P deficient) below an Olsen P of 20 µg ml^{-1}. Above an Olsen P of 20 µg ml^{-1}, the pastures exhibited 'luxury' P uptake, indicating that factors other than soil labile P were limiting yield. The 'optimum' soil Olsen P level for pasture varies with site-specific soil type (Morton & Roberts 2001), in combination with site-specific climatic variables (Moir *et al.*, 2000). Edmeades *et al.* (2006) found that the 97% relative yields of pasture ranged between an Olsen P of 12 and 50 µg ml^{-1} depending on the soil type, while the production curves were similar for all soil types. This difference in optimum Olsen P was due to the differences in P buffering capacity and soil volume weight.

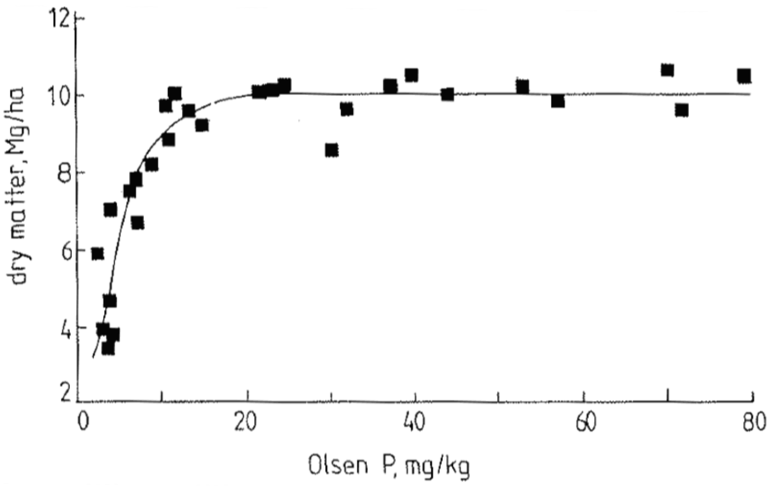

Figure 2.4: The effect of Olsen P on grass yield (Mengel & Kirkby 2001).

Gillingham *et al.* (1998) studied the effect of SSP applications to hill country on yields of grass and legume sward components. Table 2.1 shows that improved P status of the soil resulted in greater production of clover and therefore grass, increasing the total yield by 55%. The maintenance P requirement for the high fertility block was 13 kg P ha^{-1} $year^{-1}$ more than that for the low P input block which gave an increased yield of 100 kg DM kg^{-1} P. Moir *et al.* (1997) found that the accumulation of P was lower at low rainfall sites, most likely due to the lower fertilizer inputs. Mackay *et al.* (1988) found that regardless of the fertilizer history (high and low), the application of P and S stimulated legume growth in both summer and winter. Also, they found that 95% of relative maximum yield was produced at an Olsen P of 35 – 40 µg ml^{-1} in hill country soils in the Wairarapa region (North Island).

Table 2.1: Effect of P fertilizer on grass, clover and total DM production (kg ha^{-1}) (Olsen P = µg ml^{-1}) (*=significant **=highly significant *=very highly significant) (Gillingham *et al.* 1998).**

	Grass	Clover	Total
Low P (Olsen P 9)	2006	316	2322
High P (Olsen P 28)	2474	1111	3585
Significance (P)	**	***	***

2.2.7 Phosphorus status of the high country in New Zealand

The phosphorus status of hill country pastures differs with soil type, slope and aspect related factors which govern the potential production of that area (Gillingham *et al.* 1999; Lambert *et al.* 1983). New Zealand hill country generally has a low P status, affecting development and maintenance of these properties (Percival *et al.* 1984).

During the mid 1980's when farming subsidies in New Zealand were removed by government, the amount of fertilizer inputs to the hill country was heavily reduced. The amount of fertilizer applied in New Zealand was 50% to 60% lower in the late 1980's than it was in the 1970's (Perrott 1992). There has been substantial work carried out looking at the effect of this reduction in inputs to the system, particularly the effect on Olsen P levels over time (Dodd & Ledgard 1999; Lambert *et al.* 1990; Lambert *et al.* 1998). The impacts of withholding single super phosphate inputs for 15 years on hill country properties has seen decreases in the pasture production of the land by between 10 and 42% with a significant decrease in the proportion of the sown species being present (Dodd & Ledgard 1999).

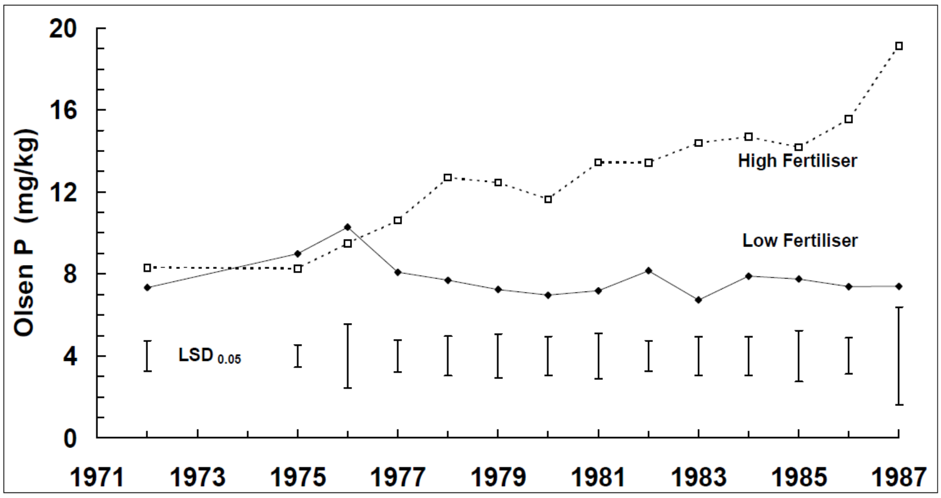

Figure 2.5: Soil Olsen P levels for low and high fertilizer treatments during 1972-1987 on a hill country property (Lambert *et al.* 1998)

Figure 2.5 demonstrates the effect of a reduction in fertiliser inputs on soil Olsen P status (Lambert et al., 1998). For the low input system (125 kg SSP ha^{-1} yr^{-1}) the Olsen P remained at approximately 8 mg kg^{-1} over the 16 year measurement period. Considering that in the late 1980's many farmers were not applying any superphosphate, much of the high country in New Zealand still has low phosphorus levels (Lambert *et al.* 1990).

2.3 Soil pH

pH is a measure of the concentration of hydrogen ions present in the soil solution and is calculated using:

Equation 1: pH

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

The optimum soil pH is the pH at which the plant yield is maximised, and this differs between plant species. This is not necessarily at neutral (pH = 7) soil conditions as once believed (Edmeades & Ridley 2003). Different soils are able to buffer the pH change differently depending on the base saturation of the soil (Edmeades & Ridley 2003). Soil pH decreases from the weathering of parent materials, leaching of bases, the formation of soluble acids, the release of hydrogen ions by plant roots, aluminium hydrolysis and fertilizer application which accelerate the cycling of nitrogen, sulphur, carbon and phosphorus, all releasing H^+ ions into the soil (Bolan & Hedley 2003).

The pH of the soil has a significant impact on the availability of nutrients in the soil as shown in Figure 2.6. Given that most of the high country in New Zealand has a pH below 5.5 (acidic) this can make the plants deficient in nutrients such as P, Mo, Ca, Mg or suffer from toxicity from nutrients such as Al, Mn, Cu or Zn. Phosphorus availability is of particular interest as it increases in availability up to pH 6.5 then decreased beyond this point. (Figure 2.6). Boron availability generally decreases with increasing pH (Sherrell 1983).

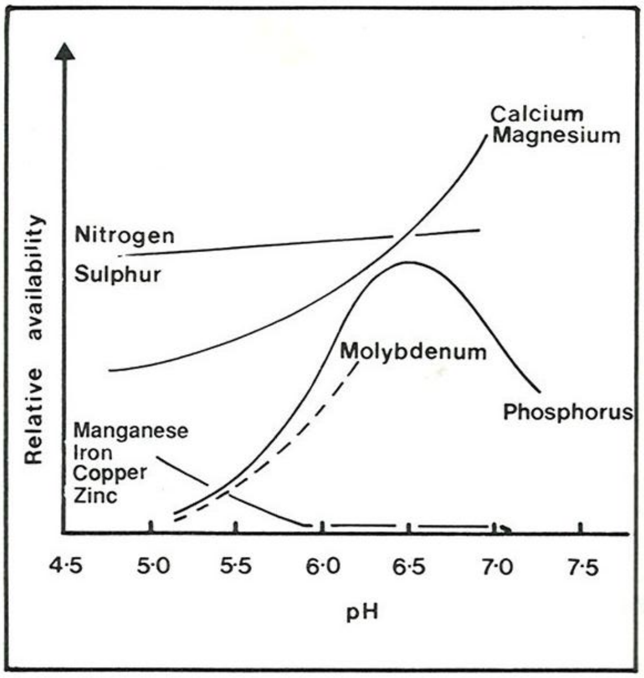

Figure 2.6: The effect of pH on the relative availability of nutrients (McLaren & Cameron 1990).

2.3.1 Effect of pH on soil and plants

pH plays a vital role in the availability of many essential nutrients in the soil solution, and therefore affects the production of plants. The main limiting nutrients under acidic conditions are molybdenum and P, while the most likely nutrient to become toxic is aluminium. Lime, and therefore an increase in pH of the soil, can result in an increase in the concentration of the bioavialable P in the soil. This increase in pH allows the plant to take up more P and reduces the toxicity from Al and Mn, and also increases the amount of P available in the soil through P sparing (Wheeler & O'Connor 1998).

2.3.1.1 P availability

Phosphorus availability is heavily affected by pH due to chemical changes in the soil, in both acidic and alkaline conditions. At a low pH, P adsorption occurs with Al and Fe minerals, and P becomes unavailable. At a high pH, Ca and Mg phosphates form, fixing the P into forms that are unavailable to plants (Sanyal & Datta 1991).

2.3.1.2 Al toxicity

Al_2O_3 makes up around 15% of the earth's crust and along with silicone (Si) is the major element making up the primary and secondary lattices of the clay minerals (Mengel & Kirkby

2001). At a pH below 5.5, Al competes with other cations for exchange sites. While in neutral soils the Al concentration is around 400 µg l^{-1}, at pH 4.4 the Al concentration rises to 5700 µg L^{-1} (Kabata-Pendias 2001). This increase in Al is often the cause of crop losses rather than the excess of H^+ ions, as many plant species are highly sensitive to even low concentrations of Al (Kabata-Pendias 2001). Al^{3+} dominates at pH 4 to 5 in the soil while $Al(OH)^{2+}$ and $Al(OH)_2^+$ dominate at pH 5.5 to 7. The total Al content is dependent on the parent material but only the Al that is easily mobile and exchangeable plays a role in the soil fertility (Kabata-Pendias 2001).

Aluminium toxicity causes overall stunting of plants, dark green leaves, purpling of stems, the death of leaf tips and damage to the root systems of plants by inhibiting the root cell elongation and division which then results in decreased water and nutrient uptake (Kabata-Pendias 2001; Langer *et al.* 2009). Some plants are more susceptible to yield losses at the same exchangeable Al level, for example lucerne is more affected than subterranean clover. It has been suggested that leaf analysis could be useful in subterranean clover but not for lucerne to detect toxicity issues (Bouma *et al.* 1981). The pH, exchangeable Al and Al saturation are widely used for the prediction of Al toxicity in soils, although most of the threshold values for yield losses are soil and species dependant.

Al toxicity rarely occurs in soils with a pH greater than 5.5 and is usually a problem in the sub soil. This can cause issues with the roots not penetrating into the sub soil when root elongation is reduced due to reduced mitotic activity. This results in more drought prone plants and affects the plants ability to access nutrients from the soil (Roy *et al.* 1988; Wheeler & O'Connor 1998). The critical level where Al becomes toxic is dependent on the plant species, soil organic matter content, P status and electrolyte concentration in the soil (Kabata-Pendias 2001). It has been shown that despite the acidification of the high country in the South Island of New Zealand from pH 5.79 in 1978 to 5.25 in 1996, that on average the Al concentrations were still below the threshold thought to limit growth in legumes (Adams *et al.* 1999).

Al tolerance is genetically controlled. Therefore there are possibilities to breed more tolerant plants. This is thought to be heavily associated with NH_4^+ tolerance because nitrification is strongly inhibited in acid soils (Kabata-Pendias 2001).

The interaction between Al and P is related to the formation of soluble Al phosphates in the soil. It has been observed that the presence of Al^{3+} at low concentrations was responsible for a reduced uptake of P and NO_3^- in white clover. Therefore Al toxicity can appear like P

deficiency and P or Si can be used to detoxify excess Al. Al^{3+} can also cause Ca and Mg deficiency in the plant, these too can be applied to lessen the effect of the excess Al.
Aluminium is a significant factor for plants in acidic conditions, and many details relating to aluminium toxicity are, in fact, not well known.

2.3.1.3 Mo availability

Molybdenum (Mo) is normally found in agricultural soils in the range of 0.8 – 3.3 mg kg^{-1} of soil (McLaren & Cameron 1990). However levels can vary greatly depending on the parent material and pH of the soils. Adsorption of molybdate (the form taken up by plants) is strongly correlated with concentrations of Fe and Al oxides. This means that heavily weathered soils of low pH are particularly prone to Mo deficiency. There is evidence that molybdate is bound and transported across the plasma membrane by phosphate binding and transport sites while the uptake is strongly increased in P stressed plants. Mo is located primarily in the phloem of the plant and is moderately mobile, but the translocated form of Mo is unknown. (Kabata-Pendias 2001; Kubota *et al.* 1963). Molybdenum-phosphorus interactions are highly variable depending on these soil factors and are also related to the plant metabolic processes (Kabata-Pendias 2001).

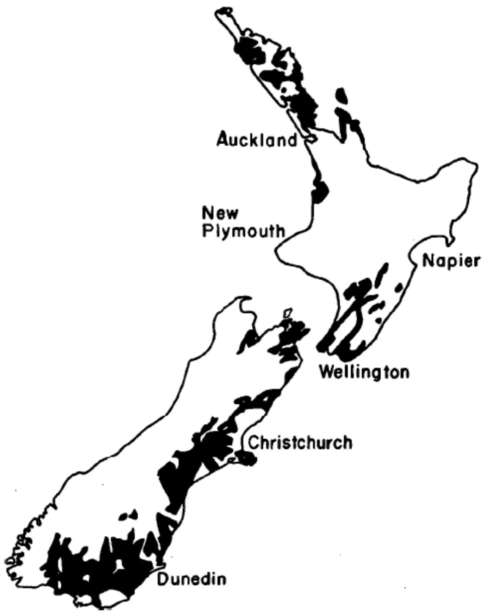

Figure 2.7: Distribution of molybdenum deficient soils in New Zealand (Sherrell & Metherell 1986).

Figure 2.7 illustrates molybdenum deficient areas in New Zealand. These are primarily along the south and east coasts of the South Island and the most southern and northern areas of the

North Island. This includes some high country areas, so Mo fertilizer may be required in addition to lime. Molybdenum is deficient in clover plants at concentrations less than 0.10 ppm (Morton *et al.* 1999).

Table 2.2: Effect of pH on the concentration of Mo (mg kg^{-1}) in grass and clover in a mixed sward (Mills 1987).

	pH					
	5.0	5.5	6.0	6.5	7.0	7.5
Grass	1.1	1.6	2.7	4.0	4.3	5.2
Clover	0.9	1.3	2.7	3.9	5.7	5.9

Molybdenum is a constituent of nitrate reductase, nitrogenase, oxidases and molybdoferredoxin and is used for N_2 fixation from the atmosphere, NO_3^- reduction and valence changes so is critical in leguminous plants (Kabata-Pendias 2001; Sherrell & Metherell 1986).

Molybdenum levels are strongly related to soil pH (Section 2.3). In most areas, molybdenum deficiency can be remedied by raising the pH of the soil. However some soils are still deficient at higher pH's, in which case Mo is applied in the form of fertilizer (Sherrell & Metherell 1986). Table 2.2 shows pH significantly increases the amount of Mo found in both clover and grass tissue, driven by the increased availability in the soil (Mills 1987).

Molybdenum is essential for legumes for N_2 fixation, as it is part of the enzyme nitrogenase which reduces atmospheric N_2 into plant available NH_3. When molybdenum is severely deficient in the soil, the nodules will be relatively small and green, rather than pink, and they do not fix nitrogen. Therefore leguminous plants that are deficient in Mo, show the same signs as nitrogen deficiency with yellow leaves and lower yields (Anderson & Spencer 1950). In a white clover stand, deficiency can be uneven and appear as yellow chequered spots against a healthy green background (Kubota *et al.* 1963). Legumes with a large seed will take longer to show signs of deficiency when sown into a deficient soil as these plants have more Mo stored in the seed (Kabata-Pendias 2001).

While liming can be used to increase the pH, and therefore increase Mo availability, large quantities of lime can make the Mo unavailable because the Mo is adsorbed onto the $CaCO_3$ (Gupta 1997).

2.3.1.4 Trace element effects

Boron is essential in all plants as it is closely associated with cell division and development in the growth regions in the plant. B deficiency occurs in soils that are low in organic matter, or in alkaline soils. Liming therefore reduces the availability of B in the soil and thus plants can become deficient. The root growth is the first process to be affected and they become stunted. Deficient clover plants (< 14 – 20 ppm B) can show signs such as red and purple colouration and thickened leaves, while lucerne (< 13 -18 ppm B) plants can have yellow to orange tints (Dear & Weir 2004; Morton *et al.* 1999).

Zinc is an essential part of over 300 enzymes in plants. Deficiency mainly occurs on soils with a high pH (Figure 2.6), due to changes in the chemical nature of the soil. Lucerne is deficient at levels lower than 15 µg g^{-1} DM and deficiency leads to chlorosis in older leaves, as well as stunted plants due to reduced stem elongation (Mengel & Kirkby 2001; Morton *et al.* 1999).

2.3.2 The pH status of the high country in NZ

The hill country of New Zealand is mostly made up of yellow brown earths, which are generally acidic with a pH of 5.5 or less, while heavily leached areas are often below pH 5.0 (McLaren & Cameron 1990). Around 500,000 ha of farmed high country in New Zealand has low soil pH values (Moir & Moot 2010). In many high country areas it is thought to be uneconomic to apply large amounts of lime aerially to decrease the acidity of the soils, and therefore they fail to produce high yields and the legume content is reduced (Craighead 2005).

Soil acidification occurs not only through the weathering of parent material, but also from other chemical and physical factors in the soil. Of significant importance in New Zealand is the acidification of the soils due to nitrate leaching, which enters the system from the nitrogen fixation by legumes. This is caused by N entering the system as N_2 and leaving the system as NO_3^- which causes a net increase of H^+ ions in the soil (Bolan *et al.* 1991). Carbon assimilation also produces H^+ ions which are disposed of through the roots, and hence into the soil solution (Raven 1985). During the process of N fixation, H^+ ions are exported into the rhizosphere when actively fixing N_2 (Haynes 1983).

Single superphosphate causes direct and indirect acidification of the soil through the fertilizer reaction with the soil, but also the increased legume growth and thus increased nitrogen fixation, and accelerated nitrogen cycling and leaching in the soil (Bolan & Hedley 2003).

The soil can be acidified through carbon cycling in the soil. In an experiment by Monaghan *et al.* (1998) they looked at the effect that white clover, lotus, lucerne and Caucasian clover had on the acidification of the soils. They found that the acidification that occurred was due to the excess of cations over anions taken from the soil into the plant resulting in soil acidification.

2.3.2.1 Liming options

Lime is used to neutralize the hydrogen ions, put simply this is possible due to the reaction of limestone with water.

Equation 2: How lime increses the pH of soils

$$CaCO_3 + H_2O \Leftrightarrow Ca^{2+} + HCO_3^- + OH^-$$

There are four alternative types of lime available, with the most commonly used, ground limestone ($CaCO_3$), commonly referred to as ‘AgLime’. The quality of this lime differs from site to site but is usually in the range of 60 – 95% $CaCO_3$. The reactivity of the lime also varies between mining sites. This is caused by the hardness of the original rock used and the particle size applied. Quick lime (CaO) is seldom used in agriculture as is slaked Lime ($Ca(OH_2)$), although the latter is often used in horticulture (McLaren & Cameron 1990). Moir and Moot (2010) carried out a field experiment and found no significant difference between the effect of quicklime and limestone on either soil pH or the exchangeable Al in the soil. Dolomite ($CaCO_3.MgCO_3$) is a mixture of calcium and magnesium carbonates, and weight for weight has a higher neutralizing power than ground lime. This is useful in soils deficient in magnesium as it contains between 11 and 13% Mg (McLaren & Cameron 1990). Dolomite is however not the main liming material used because of cost. In 2011, dolomite cost 285% more than ground limestone from the same site (Ravensdown 2011).

The rate of lime required to change the pH of the soil differs depending on the buffering capacity of the soil and so can differ substantially between soil types. As a general rule, on many soils, 10 t ha^{-1} of lime will raise the pH by 1 unit (McLaren & Cameron 1990). However, this value is a generalization and the high variability of high country soils requires that site-specific soil properties are considered to determine appropriate liming rates (Moir and Moot, 2010).

2.3.3 Pasture growth response to liming

Edmeades (1983) reported that across 140 lime application trials, at a soil pH of 5.0 and 5.5 lime increased pasture production by 5 and 10% respectively. Wheeler and O'Connor (1998) state that pasture responds to lime because of the reduction in aluminium toxicity. The increase in plant availability of phosphorus and molybdenum is also critical to the pasture response. Soil moisture may also contribute to this response to a lesser degree. Jiayou *et al.* (1993) found that lime application at a rate of 2 t ha^{-1} increased the herbage mass from 4 to 15% compared with the control. Lime also increased the white clover content in the sward from 25 to 58%. Liming increased the soil moisture by 1.5% which was said to be because of the improved soil structure and reduced water repellence of hill soils (Jackson & Gillingham 1985; Jiayou *et al.* 1993).

2.3.3.1 Lime transport in soil

Lime is a relatively insoluble material and the pH increasing ions such as OH^-, HCO_3^- and CO_3^- take many years to travel through the soil, therefore if possible it is best to incorporate the lime into the soil if cultivating (Edmeades & Ridley 2003). For surface applied lime to work effectively it needs to travel right through the root zone. It is proposed that this could occur by chemical, biological or physical processes. This includes earthworms, the breakdown of plant material over time and particles making their way through the pores in the soil (Scott *et al.* 2000). Edmeades and Ridley (2003) concluded that surface liming or the incorporation of lime does reduce the acidity below the depth of incorporation. Moir and Moot (2010) found in a field trial that surface-applied lime had the greatest effect on the pH in the top soil horizon where pH increased on average 0.15 units t^{-1} lime applied, and less impact was measured further down the soil profile. They also found that significant reductions (to plant safe levels) in the exchangeable Al can be made in the top 0.1 m of the soil with as little as 2 t lime ha^{-1} on that high country soil.

2.4 Dryland Pasture Legumes

There is a wide range of dryland legumes used in New Zealand, all with different optimum growing conditions, but all with similar roles in the high country.

2.4.1 The role of legumes in the high country

Legumes in high country provide the only source of nitrogen for sward growth, via N fixation. In this process, atmospheric N_2 is 'fixed' to NH_3 through the symbiotic relationship between the rhizobia and the plant that occurs in the nodules of the plant root, while providing a high quality feed to improve the performance of grazing animals (Caradus *et al.* 1996; Hofmann *et al.* 2007). The nitrogen that is fixed by the legumes can be used by the companion (grass) species in the pasture, to increase total pasture DM yield. Dryland legumes are also used to produce high quality feed at different times of the year compared with the grass species sown in the high country (Stevens & McCorkindale 2002). On average legumes produce between 26 and 34 kg N t^{-1} of legume DM produced (Goh & Bruce 2005). Trying to find legumes that can perform and persist in the harsh high country environment is difficult, given the acidic conditions, low plant available phosphorus, and frequent moisture deficits.

2.4.2 Legume species and their specific growth conditions

Arrowleaf clover (*Trifolium vesiculosum* L*)* is a late maturing annual legume which is intolerant to waterlogging but grows well in most soil types with soil pHs between 5.0 and 7.0. It is deep rooted and produces a small hard seed so second year performance can be poor (Evans & Mills 2008). In an experiment carried out at Lincoln University, arrowleaf clover produced 9800kg DM ha^{-1} over 211 days, compared with 3370 kg DM ha^{-1} and 1790 kg DM ha^{-1} for subterranean and white clovers respectively (Evans & Mills 2008).

Balansa clover (*T. michelianum*) is able to withstand winter waterlogging (Dear *et al.* 2003). Monks *et al.* (2008) state that under the optimum conditions balansa clover will be productive for three to four years in hill country conditions with the correct management. Within the pH range of 4.2 and 6.0 balansa clover produced the highest yield at pH 5.1, on an Australian soil under glasshouse conditions (Table 2.3). However no information on the optimum P and pH zones were available for New Zealand conditions.

Table 2.3: The effect of soil pH on relative yields (%) of legume shoot DM grown under glasshouse conditions in an Australian acidic sandy loam soil (Evans *et al.* 1990). Different letters indicate significance (P = 0.05).

Legume	Soil pH			
	4.2	4.7	5.1	6.0
Subterranean clover	94a	100a	96a	95a
Balansa clover	69b	90a	100a	91a
Persian clover	44b	90a	99a	100a

Caucasian clover (*T. ambiguum*) is a slow establishing, long lived perennial pasture legume (Stevens & McCorkindale 2002). It is well adapted to high altitude areas at 800m ASL and may out-perform white clover on low phosphorus soils (Black *et al.* 2000). Black *et al.* (2000) found that Caucasian clover in a pasture with ryegrass held a higher legume content than white clover over three years when the ryegrass was direct drilled once the legume was established. The result was higher livewight gains in sheep, regardless of the fertility status of the site. An increase in P inputs had very little effect on the establishment of Caucasuan clover, as illustrated in Figure 2.8.

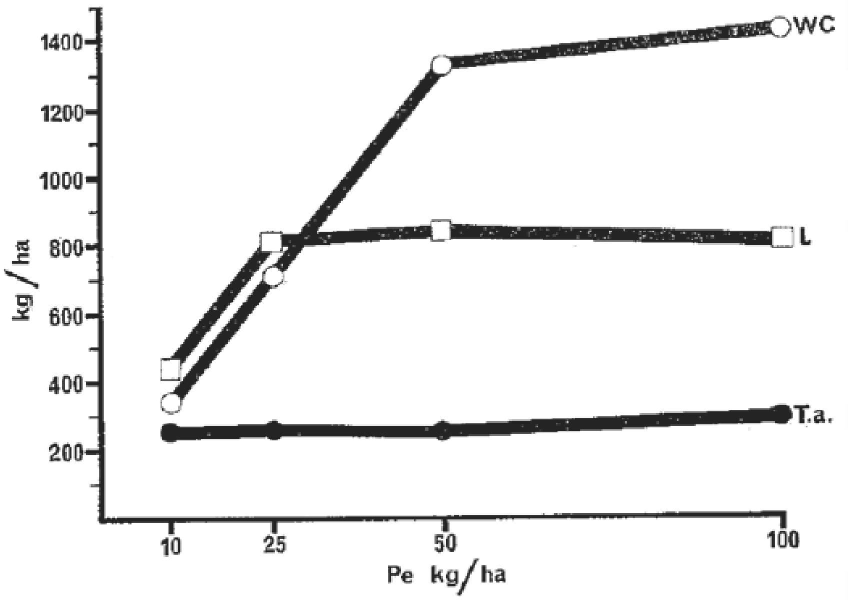

Figure 2.8: The effects of establishment P rate on DM production for white clover, 'Maku' lotus and *Trifolium ambiguim* (Lucas *et al.* 1980).

Caradus (1980) investigated the effect of P (300 and 2000 ppm) on plant yield under glasshouse conditions, on a Stratford sandy loam soil. During six weeks of growth of the established plants, with 1 plant per 1 l pot, significant effects from P application were found

(Table 2.4). Lotus and subterranean clover were the highest yielding species at a low P rate (3.6 and 3.5 g DM pot^{-1}), while Caucasian and white clovers were the lowest (0.3 and 0.8 g DM pot^{-1}). At 2000 mg P kg^{-1} lotus and subterranean clover were still the highest yielding.

Table 2.4: Yield accumulated (g DM pot^{-1}) after 6 weeks growth for the given species (Caradus 1980).

Species	P treatment	
	300 mg P kg^{-1}	2000 mg P kg^{-1}
Strawberry clover	1.2	1.7
Caucasian clover	0.3	0.8
Lotus	3.6	6.0
Subterranean clover	3.5	6.7
White clover	0.8	5.5

Gland clover (*T. glanduliferum*) is tolerant of waterlogging (Dear *et al.* 2003). No literature is available for the optimum P and pH conditions for this species under New Zealand conditions. Gland clover is reportedly less tolerant than subterranean clover of acid conditions (Hayes *et al.* 2008b).

Lotus (*Lotus pedunculatus*) has been shown to out-perform white clover by up to three times in yield in the same conditions in soils with a pH of less than 5.2 with the same phosphorus inputs (Scott & Lowther 1982). Lotus, while being a P efficient legume, still requires superphosphate inputs for establishment and survival. In a trial with a pH 4.9 and Olsen P of 9 µg ml^{-1} establishment was poor with no P inputs. With 10 to 20 kg super phosphate ha^{-1} at establishment and each following year, yields were doubled. It was also found that in an environment with pH 4.5 and Olsen P 9 µg ml^{-1}, 0.5 t lime ha^{-1} saw a significant increase in the numbers of plants established, but 1 t lime ha^{-1} saw no further increase. Yield differences were gone by the third year, with the lime only affecting establishment (Scott & Mills 1981). It can be seen from Figure 2.8 that the P rate at establishment saw a sharp increase at low rates, it then reached a plateau. In Figure 2.9 it is evident that lotus was the highest yielding plant over the four years, and increased its yield linearly with increasing P. Table 2.4 also shows this significant increase in yield with P inputs.

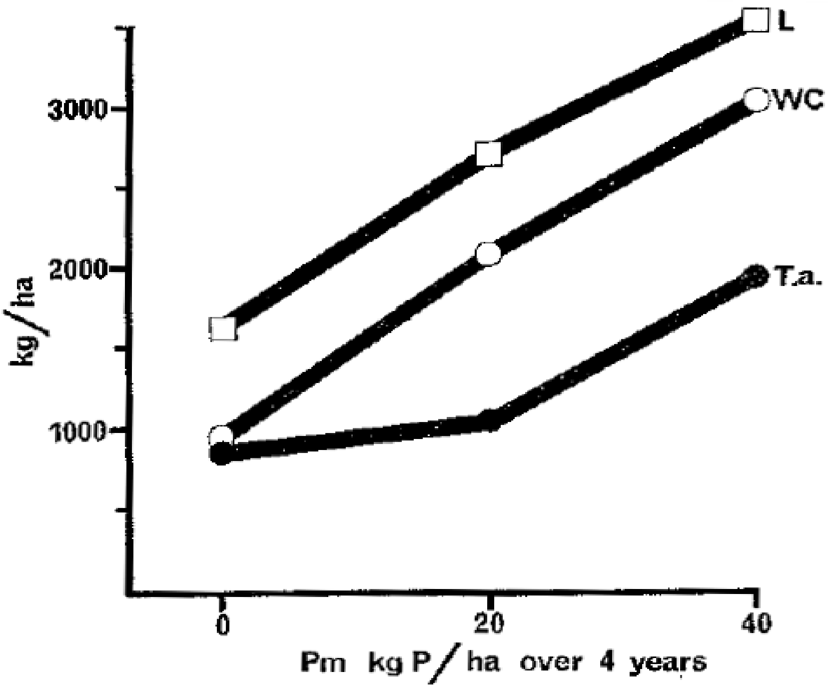

Figure 2.9 The effects of maintenance P rate on DM production for white clover, 'Maku' lotus and *Trifolium ambiguim* (Lucas *et al.* 1980).

Lucerne *(Medicago sativa)* is a deep rooted perennial pasture legume which has drought tolerance and can therefore keep producing well into the late spring and summer. Brown *et al.* (2006) found that in an experiment carried out at Lincoln University, lucerne supported the highest stock production during late spring and summer compared to other pasture mixes. Lucerne produced the highest drymass at 4.3 g $plant^{-1}$ at a soil P supply of 24 µg g^{-1} (Figure 2.10). Lucerne is known to be very sensitive to Al, and in a pot trial run by Scott *et al.* (2008) lucerne yield increased from 0.1 g DM pot^{-1} up to 0.26 g DM pot^{-1} from a pH of 4.15 to 5.45.

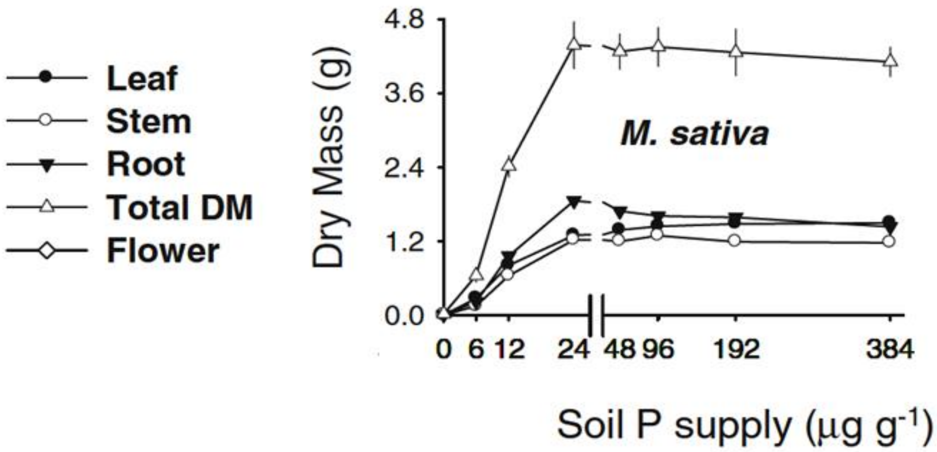

Figure 2.10: Drymass of *M.Sativa* grown for 8 weeks and supplied with 8 levels of P (Pang *et al.* 2009).

Persian clover (*T. Resupinatum*) is also adversely affected by pH (Table 2.3). However there is no literature available for New Zealand conditions on the optimum pH and P conditions for growth.

Falcata lucerne *(M. falcata)* has no information available in regard to its optimum pH and phosphorus conditions.

Strawberry clover (*T. fragiferum*) will produce significantly more yield under high P inputs (Table 2.4). It grows well in waterlogged soils within a pH range of 4.8- 8.0. It is also tolerant of temperatues from 0 to over 35^{o}C. Summer production relies on the soil moisture, and a reduction in DM yield of 66% has been reported under drought conditions which was comparable with white clover (64% reduction) (Hofmann *et al.* 2007).

Subterranean clover (*T. Subterranium)* is well suited to moderately acidic soils that are well drained (Dear *et al.* 2003). Subterranean clover was the most tolerant of high Al in the soil in an experiment by Hayes *et al.* (2008b). There was no significant change in DM production between pH 4.2 and 6.0 (Table 2.3), but a significant increase in yield which occurs with an increased P status of the soil (Table 2.4).

Tagasaste (*Chamaeyctisus proliferus*) is a small drought tolerant perennial shrub with a deep taproot that grows to 3 – 5 m high and a similar diameter. It can be useful as a cattle or sheep feed in the summer and early autumn (Douglas *et al.* 1998). It should be established on well drained soils and sown into warmer soils in spring to avoid frost damange. You can encourage low branching by grazing or cutting main shoots on small plants (Townsend & Radcliffe 1987). No scientific literature is available on the optimum pH and P conditions for growth under New Zealand conditions.

White clover (*T. Repens)* is commonly found in New Zealand farming systems which receive reliable rainfall and is adapted to a wide range of soil types. Ryegrass white clover pasture mixes are the most commonly used pasture mix in New Zealand (Brown *et al.* 2006; Goh & Bruce 2005). White clover also withstands hard grazing and is frost tolerant. It does however have poor drought and heat resistance, so does well under high rainfall or irrigated environments (Wurst 2004). It can be seen in Figure 2.11 that with increasing P inputs, there was little effect during winter and autumn, although in spring there was over a three fold increase in the DM produced by the white clover. P concentration also had a large effect on the establishment rate (Figure 2.11), while an increase in the maintenance saw a linear response in DM production (Figure 2.9). Due to this poor drought tolerance, and low P use efficiency, it is not suitable for use in many high country areas of New Zealand.

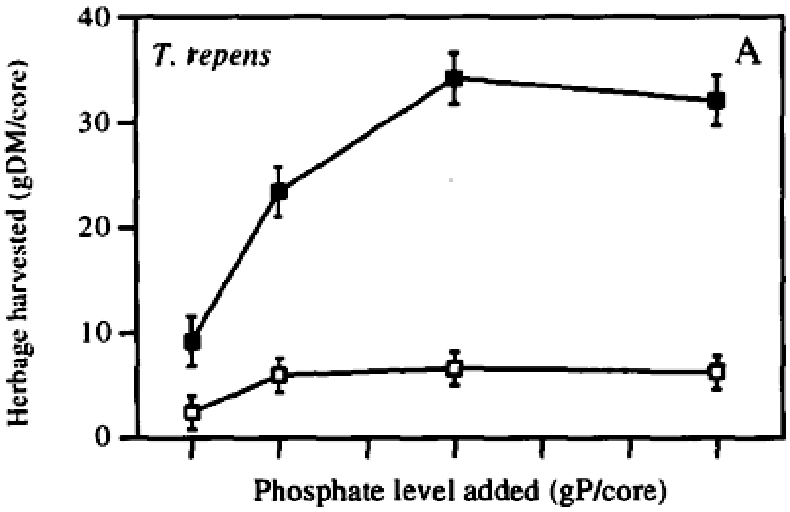

Figure 2.11: White clover drymatter response to increased P inputs, Open squares = autumn winter, Black squares = spring adapted from (Dodd & Orr 1995b).

2.5 Conclusions

- Phosphorus is essential for plant growth and function, but very little is known about the optimum P levels required for many of the legumes which may be suitable for use in New Zealand high country.
- pH has a major effect on plant growth and development, and many high country soils are acidic (<pH 5.5). An increase in soil acidity results in toxic levels of plant available Al, which restricts root growth and yield. pH also affects the availability of several macro and micro elements essential for plant growth and N fixation in pasture legumes.
- Legumes make up an integral part of high country pastures, fixing nitrogen from the atmosphere and providing high quality feed in the pasture. Information on the key environmental requirements of alternative pasture legumes is key for the continued productivity and profitability of these these farming systems.

From this review of current literature it is apparent that there is little information available on the optimum soil phosphorus pH and levels for maximum growth for many dryland legumes for use in New Zealand high and hill country. Therefore, the objective of this experiment was to determine the yield and nutrient uptake response of 12 pasture legume species to P and lime additions on an acidic South Island high country soil.

Chapter 3
Materials and Methods

3.1 Soil collection and preparation

Soil (0–0.2 m depth) was collected from a hill/ high country site (48° 08' 25" South. 172° 11' 20" East) on 'Mt Pember Station' in the Lees Valley, North Canterbury, New Zealand in May 2010. The soil is a high country yellow brown shallow stony soil (NZ classification: Orthic brown soil, (Hewitt 1998); USDA: Dystrochrept, (Soil Survey Staff 1998)). The soil type is an Ashwick stoney/ boldery silt loam (mean top soil depth of 0.2 m silt loam over gravels). The altitude is 430 m a.s.l. with a mean annual rainfall of 600 mm, which mainly falls in winter and early spring. This high country site has had no regular farm management strategy (fertiliser inputs) for improving soil fertility. Therefore it is likely that the site had received no fertilizer or lime since the start of livestock grazing on the property over 100 years ago. The site has undeveloped pasture, dominant in low fertility species such as browntop (*Agrostis tenuis*) and very low (< 1%) legume content. The soil was prepared by passing it through a 4 mm sieve while field moist, removing all plant material, and then mixing thoroughly. Soil subsamples were taken from the bulk sample, prepared by air-drying at 30°C for 7 days and 2 mm sieved. Analyses were then conducted before commencement of the experiment.

3.2 Soil chemical analysis

Standard and advanced soil fertility analyses were conducted on the soil from the site and the results are presented in Table 3.1. Soil pH was measured at a water: soil ratio of 2.5:1 (Blackmore *et al.* 1987). The method of Olsen *et al.* (1954) was used to measure available soil P, while phosphate retention was determined using the methods of both Blakemore *et al.*(1972) and Saunders *et al.* (1965). Extractable soil sulphate was determined using the method of Searle (1979). Soil extractable cations were measured using the method of Schollenberger & Simon (1945), while the cation exchange capacity was determined using the method of Hesse (1971). Total carbon (C) and N contents were determined by the Dumas method of combustion (Horneck & Miller 1998) using an Elementar 'Vario' MAX CN Analyzer (Elementar Analysensystane, GmbH). Total phosphorus concentration was determined using an acid digest (Kjeldahl digest procedure (Blackmore *et al.* 1987)) and analysed for Total P concentration by molybdenum blue using an FIA (Flow Injection Analyser; Tecator Inc., Sweden). Exchangeable aluminium was measured using the 0.02

$CaCL_2$ extraction method (Edmeades *et al.* 1983) then measured by ICP-OES (Varian 720-ES ICP-OES; Varian Inc., Victoria, Australia). Reserve magnesium was measured using the method of Metson (1975) and reserve potassium was measured using the methods of Blackmore *et al.* (1972) and Metson *et al.* (1968; 1956). Anaerobic mineralisable N was measured using a modified method of Waring and Bremner (1964) and Keeney and Bremner (1966).

Table 3.1: Initial average soil test results for the Lees Valley high country yellow brown shallow stony soil used in this experiment.

Soil Analysis	Initial Value
pH	5.1
Olsen P	9 µg ml^{-1}
Sulphate Sulphur	9 µg g^{-1}
Ext. Org. Sulphur	8 µg g^{-1}
Reserve Potassium	5.95 me $100g^{-1}$
P Retention	55%
Anaerobic MinN	125 kg ha^{-1}
Organic Matter	9.2% w/w
Exchangeable Aluminium	13.9 mg kg^{-1}
Reserve Magnesium	23.6 me $100g^{-1}$
Total Nitrogen	0.41% w/w
Total Carbon	5.35% w/w
Carbon/Nitrogen	13:1
Total Phosphorus	904 mg kg^{-1}
CEC	15 me $100g^{-1}$
Calcium	1.40 me $100g^{-1}$
Magnesium	0.49 me $100g^{-1}$
Potassium	0.41 me $100g^{-1}$
Sodium	0.14 me $100g^{-1}$
Base Saturation (Total)	16.1%

3.3 Experimental design and trial management

3.3.1 Trial design and setup

A pot trial was conducted under glasshouse conditions at the Lincoln University glasshouse facility. A complete randomized block design was used. This pot trial examined 12 species of pastoral legumes. Eight rates of phosphorus and five rates of lime were used. These were as follows in Tables 3.2 and 3.3:

Table 3.2: Phosphorus rates and treatment codes used in experiment.

Phosphorus rate (mg P kg^{-1} soil)	Phosphorus rate (kg ha^{-1})	Treatment code
0	0	P0
10	8	P1
30	24	P2
60	48	P3
100	80	P4
250	199	P5
500	397	P6
1500	1191	P7

Table 3.3: Lime (100% $CaCO_3$, laboratory grade) rates and treatment codes used in the experiment.

Lime rate (t lime ha^{-1})	Lime rate (mg $CaCO_3$ kg^{-1} soil)	Treatment code
0	0	L0
2	2364	L1
5	6585	L2
8	10536	L3
15	19755	L4

Each treatment was replicated four times to give a total of 624 pots.

The phosphorus treatment pots received basal lime at a rate of 5.0 t ha^{-1} equivalent, in the form of pure (100%) laboratory grade $CaCO_3$.

Pots, 746 cm^3 in volume (9.5 cm deep 10 cm diameter) were used. This is a standard pot size used in studies of this type. Field moist soil (600 g) was weighed for each pot and placed into a plastic bag. The appropriate lime or phosphorus treatment was weighed and then added to the bag and mixed thoroughly. This bag was then emptied into the labelled pot. The pots were placed in saucers, which stored any leachate, if drainage did occur, so that nutrients were not lost from the soil/ plant system. These pots were then placed in the glasshouse and watered lightly on the 2nd June 2010. The final soil surface level was approximately 20 mm below the top of the pot.

3.3.2 Glasshouse conditions

This pot trial was carried out in the Aluminex glasshouse, located at the Lincoln University Nursery on Farm Road. The glass house was heated and also had a fan system to cool in the summer. The temperature was monitored automatically every two hours for the trial period. The mean daily temperature for the trial period was 19.4°C with daily average temperatures ranging between 16.2 and 23.9°C.

3.3.3 Pre-Sowing germination trial

In June 2010 the germination rate of all selected legume species was determined before sowing of the main experiment. This was conducted to test the viability of the seed, to ensure that the final plant density was achieved for all species. Twenty-five seeds for each species were placed in a Petri dish, which contained moist tissue paper. The annual species were incubated at 15°C while the perennial species were incubated at 20 °C due to different optimum temperatures for germination (Moot *et al.* 2000). Each day, any germinated seeds were removed once the radicle was twice the length of the seed and recorded. The germination percent for each species was calculated and is presented in Table 3.4. The number of seeds sown in each pot was then adjusted accordingly using the germination rate information, as follows; 10 seeds pot^{-1} for most species, excluding lotus and tagasaste (20 seeds pot^{-1}), and falcata lucerne (60 seeds pot^{-1}).

Table 3.4: Final germination rate of species, used in this experiment incubated at 15 or 20ºC.

Species	Final germination %
Arrowleaf clover (*Trifolium vesiculosum*)	65%
Balansa clover (*Trifolium michelianum*)	83%
Caucasian clover (*Trifolium aumbiguum*)	77%
Gland clover (*Trifolium glanduliferum*)	72%
Lotus (*Lotus pedunculatus*)	37%
Lucerne (*Medicago sativa*)	81%
Persian clover (*Trifolium resupinatum*)	96%
Falcata lucerne (*Medicago falcate*)	10%
Subterranean clover (*Trifolium subterraneum*)	94%
Strawberry clover (*Trifolium fragiferum*)	81%
Tagasaste (*Chamaecytisus proleferus*)	33%
White clover (*Trifolium repens*)	73%

3.3.4 Plant establishment

The 12 legume species sown are commonly used in NZ high country, or could potentially be grown there. Seeds were sown at 10 seeds pot^{-1} for most species (see Section 3.3.3) on June 3rd 2010 and 35 ml (2-3 mm) of the Lees Valley soil was placed on top and firmed down gently. The pots then received a light watering and were randomised in four blocks (on four different tables in the glasshouse). The legume species examined were:

- Arrowleaf clover (*Trifolium vesiculosum*; West Coast Seed (Aus) cv. 'Cefalu')
- Balansa clover (*Trifolium michelianum*; Seed Mark cv. 'Bolta')
- Caucasian clover (*Trifolium aumbiguum*; Pgg Wrightsons cv. 'Endura 3')
- Gland clover (*Trifolium glanduliferum*; Kiwi Seed Co. cv. 'Prima')
- Lotus (*Lotus pedunculatus*; Tai Tapu, Selwyn district cv. 'Maku')
- Lucerne (*Medicago sativa*; Seed Force cv. 'Force 4')
- Persian clover (*Trifolium resupinatum*; Specialty Seeds cv. 'Enrich')
- Falcata lucerne (*Medicago falcate*; Kiwi Seed Co.)
- Subterranean clover (*Trifolium subterraneum*; Kiwi Seed Co. cv. 'Mt Barker')
- Strawberry clover (*Trifolium fragiferum;* Gentos cv. 'Lucila')
- Tagasaste (*Chamaecytisus proleferus;* collected near Lincoln, Selwyn district)
- White clover *(Trifolium repens*; Grasslands cv. 'Nomad')

The tagasaste seeds were scarified before sowing. This scarification was done by immersing the seed in boiling water for ten minutes, then bleach for ten seconds, and rinsed five times with water. Some tagasaste plants which failed to emerge were re-sown with pre-germinated seedlings soon after the initial sowing. All pots were thinned to the final plant density of 5 plants pot^{-1} on the 17th July 2010. All seeds used in the experiment were uncoated (no lime and/or trace element coating).

Plate 3.1: One replicate of the glasshouse experiment (26.7.10), showing newly established seedlings.

3.3.5 Inoculation

The pots were inoculated with commercial (diluted peat culture) rhizobia strains (‘Nodulaid’; Becker Underwood Ltd, Australia). Rhizobia strains appropriate to each legume species were applied on both the 29th of June (16 days post germination) and the 4th of October 2010 to ensure that an active soil rhizobia population was present. The ‘Group’ (strains) of inoculums applied to the different legume species are below in Table 3.5;

Table 3.5: Rhizobia strains 'group' applied to the legume species in this experiment.

Species	Group
Arrowleaf clover (*Trifolium vesiculosum*)	'C'
Balansa clover (*Trifolium michelianum*)	'C'
Caucasian clover (*Trifolium aumbiguum*)	'CC238b' (species specific)
Gland clover (*Trifolium glanduliferum*)	'C'
Lotus (*Lotus pedunculatus*)	'D'
Lucerne (*Medicago sativa*)	'AL'
Persian clover (*Trifolium resupinatum*)	'C'
Falcata lucerne (*Medicago falcate*)	'AL'
Subterranean clover (*Trifolium subterraneum*)	'C'
Strawberry clover (*Trifolium fragiferum*)	'B'
Tagasaste (*Chamaecytisus proleferus*)	Mixture ('B', 'C' , 'D', 'AL')
White clover (*Trifolium repens*)	'B'

3.3.6 Pot management

The pots were weeded throughout the experiment to remove any other plants from the soil. Plant counts were carried out throughout the experiment and any pots with plants missing were sown with new seeds and noted. Fungicide (Elliott 'Super six') was applied on two occasions to prevent and control powdery mildew. In the case if any plants died, which was very rare, they were recorded and more seed was sown into the pot.

3.3.7 Basal nutrient application

Basal nutrient solution was applied to the pots throughout the experiment. The nutrient solution (Booking 1976; Caradus & Snaydon 1986) which was applied to the lime treatment pots was a 'complete solution', containing all macro and trace elements required for plant growth. The P treatment pots received a modified version of this solution, which contained no P. These solutions were applied on a regular basis (twice monthly over winter, then weekly thereafter during rapid growth in spring and early summer) to ensure adequate plant nutrition. In terms of key macro nutrients, the pots received approximately 200 kg S ha^{-1} and 500 kg K ha^{-1} equivalent from the nutrient solutions. The lime pots received approximately 200 kg P ha^{-1}, which increased the soil Olsen P to an optimum level of approximately 30 µg P mL^{-1}.

On the 16th July 2010 (four weeks post germination), a small quantity of N (30 kg N ha^{-1} equivalent) nutrient solution was also applied, in the form of ammonium nitrate (NH_4NO_3).

This was applied to all pots to overcome any plant N deficiencies during the seedling establishment phase, and again on the 23rd September 2010 as the plants were pale and there was an obvious nitrogen deficiency. Beyond this point, plants were dependant on N sourced from N fixation or soil N for growth.

3.3.8 Soil moisture and watering management

The pots were watered daily to 35-40% volumetric water capacity which represented a soil which was neither waterlogged nor dry. Several pots were randomly selected from each block every week and weighed, to check that the target soil moisture content was being achieved and maintained. In general, this watering regime allowed for adequate soil moisture to cope with high plant growth rates, while at the same time avoiding waterlogging and nutrient leaching from the pots.

Plate 3.2: The established glasshouse experiment 11 days before the first harvest (4.9.10).

3.4 Measurements

3.4.1 Shoot yield

Plants were harvested every four to five weeks, so as to maximise shoot yield, but prevent plants from flowering (becoming reproductive). A total of seven harvests were performed, on the 15/16 of September, 19/20 October, 24/25 November, 14/15 December 2010, and 17/18

January, 14/15 February and 23/24 March 2011. Generally the annual species had three harvests before going to seed and dying as expected from the physiology of annual legume species. In addition three species (arrowleaf, balansa and gland clovers) had a shorter total growth period and for that reason were harvested only once or twice during the experiment, hence these three species were lower yielding for that reason. The different species were harvested using different cutting techniques, dependant on the growth habit of the particular species. These were as follows:

- White clover - harvested at pot height and around the rim of the pot (20 mm).
- Arrowleaf clover - first harvest, was cut as pot height (20 mm), leaving the growing point. After the first harvest it was harvested where the internodal distance was greater than 40 mm, on primary and secondary nodes if flowering. Otherwise harvested leaving two leaves per shoot.
- Persian clover - harvested where the internodal distance was greater than 40 mm, on primary and secondary nodes if flowering. If not, older leaves were trimmed (leaving at least two large new leaves on the plant).
- Caucasian clover - harvested leaving the growing point in the centre and the youngest large leaf.
- Balansa clover - harvested where the internodal distance was greater than 40 mm, on primary and secondary nodes if flowering. If not, trimmed the older leaves (leaving at least two large new leaves on the plant).
- Subterranean clover - harvested at pot height (20 mm) and around the rim of the pot.
- Gland clover - harvested at a height of 100 mm flat across the top.
- Strawberry clover - harvested leaving the growing point in the centre and two of the large young leaves.
- Lucerne - cut at 60- 70 mm height, flat across.
- Falcata lucerne - cut at 60- 70 mm height, flat across.
- Tagasaste – harvested at a height of 100 mm.
- Lotus - harvested at pot height (20 mm) and around the rim of the pot.

Once harvested all herbage samples were placed in a small paper bag and oven dried at 70°C for 48 hours. These herbage samples were then weighed on a four decimal place balance to obtain the dry weight of each sample. This weight represented shoot yield (g DM pot^{-1}) for the given growth period.

Plate 3.3: Harvesting a pot of tagasaste plants, cutting the plants at 10cm above the top of the pot.

3.4.2 Plant tissue analysis

The herbage samples were bulked on an individual pot basis for the first three harvests (harvests 1-3) and also for the second three harvests (harvests 3-6). All bulked samples then underwent nutrient analysis as follows.

The bulked samples were finely ground and acid digested. Dried, finely ground herbage (0.1000 g) was digested in 2 ml of nitric acid (HNO_3) on a heating block at 110°C for two hours, then topped up to 5 ml with deionised water for analysis. Digest samples were analysed for a complete range of elements (excluding N) using ICP-OES analysis (Varian 720-ES ICP-OES; Varian Inc., Victoria, Australia).

3.4.3 Final soil sampling

Selected soil from pots was dried (30°C, 7 days) on the 20th of March 2011 for the purpose of final soil analyses. Once dry, the soil was sieved (2 mm) and a sub-sample taken from each. Soil samples were then sent to the commercial lab ARL (Napier) for analysis. The range of soil analyses and procedures carried out were similar to those outlined in Section 3.2.

3.5 Statistical analysis

All data were tested for treatment effects by conducting an analysis of variance (ANOVA) using Genstat version 13.0 (VSN International). For the P treatment pots, the model included plant species, P rate and species × P rate interaction as fixed effects, with shoot yield, shoot P concentration and P uptake as random effects. For the lime treatment pots, the model included plant species, lime rate and species × lime rate interaction as fixed effects, with shoot yield, shoot P concentration and uptake and shoot Mo and B concentration as random effects. In some instances a statistically significant species × (P rate / lime rate) interaction was detected. Therefore regression analysis (curve fitting) was used to understand these relationships, where appropriate using Sigmaplot 11.0 (Systat Software Inc. GmbH. 2008).

Chapter 4
Results

4.1 Phosphorus experiment

4.1.1 Yield

A strong difference ($P < 0.001$) in dry matter (DM) yield was observed with increasing rates of phosphorus. The mean total shoot yield ranged from 0.2 g DM pot^{-1} (gland clover) to 11.3 g DM pot^{-1} (lotus) (Table 4.1). The mean DM yield across all P treatments for the annual species ranged from 0.2 g DM pot^{-1} (gland clover) to 10.1 g DM pot^{-1} (Persian clover). Gland clover was the lowest yielding species overall, and the other annual species produced more DM ($P < 0.001$). In terms of the perennial species, mean DM yields across all P treatments ranged from 6.1 g DM pot $^{-1}$ (white clover) to 11.3 g DM pot^{-1} (lotus). Lotus and tagasaste were much more productive than the other perennial species. In most cases the perennials produced a greater total DM yield than the annual clovers. The maximum yield across all species was at the 500 mg P kg^{-1} P rate (Olsen P = 58) with 8.2 g DM pot^{-1}. On average the greatest increase in DM yield was between 30 and 60 mg P kg^{-1} soil (1.0 g DM pot^{-1}). There was a strong ($P < 0.001$) species by P rate interaction. This interaction effect indicates that the way in which the plants responded to the increased P rate differed among species. For this reason the relationship between increased P rate and DM yield was examined in more detail using regression analysis of the data for individual species.

Most annual species followed a typical 'rise to maximum' yield response curve with increasing P rate. Yield was strongly associated (R^2 = 0.93 – 0.98) with P rate (Figure 4.1, Figure 4.2). The exception was gland clover, where the relationship between yield and P rate was best explained by an exponential curve function (R^2 = 0.97; Figure 4.1). The perennial species all followed a 'rise to maximum' curve with increasing P inputs, and again, these relationships were very strong (R^2 = 0.90 – 0.98; Figure 4.1, Figure 4.2). At 0 mg P kg^{-1} lotus, Persian clover and tagasaste were the highest yielding species at 8.0, 7.0 and 6.4 g DM pot^{-1}. Gland, arrowleaf, balansa and subterranean clovers were the lowest yielding species at 0, 0.5, 0.5 and 0.9 g DM pot^{-1}. At 1500 µg P pot^{-1} lotus, tagasaste and lucerne were the highest yielding species at 15.0, 13.0 and 12.1 g DM pot^{-1}. Gland, balansa and arrowleaf clovers were the lowest yielding at 1.2, 1.4 and 1.8 g DM pot^{-1}. At just 100 µg P kg^{-1} arrowleaf clover, subterranean clover and balansa clover gave the greatest response in yield from the control treatment.

4.1.2 Phosphorus uptake

The mean phosphorus content of shoots increased (P < 0.001) with increasing P inputs. Across all plant species, % P increased from 0.157% P at 0 mg P kg^{-1} to 0.473% P for 1500 mg P kg^{-1} (Table 4.1). Between species the mean shoot P concentration ranged from 0.079% (gland clover) to 0.288% (white clover). At 0 mg P kg^{-1} white clover and Caucasian clover had the highest P concentration at 0.220% and 0.227% while Persian clover had the lowest of the species that yielded, at 0.129%. At 1500 mg P kg^{-1} arrowleaf, balansa and strawberry clovers all had significantly higher P concentrations at 0.793%, 0.551% and 0.514% respectively than gland clover, Caucasian clover and tagasaste which had the lowest P concentrations at 0.347%, 0.377% and 0.377%.

The overall P uptake of the herbage across all species increased (P < 0.001) with increasing P inputs from 6.6 mg P pot^{-1} at 0 mg P kg^{-1} to 3.6 mg P pot^{-1} at 1500 mg p kg^{-1}. At 0 mg P kg^{-1} lotus, Caucasian clover, lucerne and tagasaste had the highest P uptake, at 13.5, 10.3, 10.0 and 9.9 mg P pot^{-1} respectively. Of the species that yielded, arrowleaf and balansa clovers took up the least P at 0.7 and 0.9 mg P pot^{-1}. At 1500 mg P kg^{-1} lotus, lucerne and tagasaste took up the most P at 66.7, 53.3 and 48.8 mg P pot^{-1} while gland and balansa clovers took up the least at 4.2 and 13.2 mg P pot^{-1}. There was a significant interaction between yield and herbage P concentration so regression analysis was used to further examine the relationships for each species.

The yield of the annual clovers increased with increasing P concentration in the shoot following a strong ‘rise to maximum’ relationship with the exception of Persian clover and gland clover (Figure 4.3, Figure 4.4). Yield was strongly associated (R^2 = 0.88 – 0.97) with P concentration. Persian clover increased to a point, then decreased in yield beyond a herbage concentration of 0.29% P. Gland clover followed an exponential increase (R^2 = 0.98). Arrowleaf clover had the sharpest increase in yield with increasing herbage P concentration (Figure 4.3). The perennial clovers all increased in yield with increasing P concentration in the herbage following a strong ‘rise to maximum’ (R^2 = 0.67 – 0.98) curve (Figure 4.3, Figure 4.4). Lotus and white clover had the sharpest increase in yield with increasing herbage P content.

4.1.3 Maximum yields (97%)

The 97% maximum DM yield of each species was calculated as a measure of biological maximum relative yield, and values are presented in Table 4.2. The maximum yield (97%)

along with the P rate at this yield indicates P use efficiency. Of the annual species the greatest 97% maximum yield ranged from 1.1 g DM pot^{-1} (gland clover) achieved at 1465 mg P kg^{-1} to 12.9 g DM pot^{-1} (Persian clover) achieved at 369 mg P kg^{-1} (Table 4.1). Subterranean clover achieved a 97% maximum yield of 4.2 g DM pot^{-1} at just 336 mg P kg^{-1}. Of the perennial species the 97% yield ranged from 7.8 g DM pot^{-1} at 203 mg P kg^{-1} (white clover) to 14.3 g DM pot^{-1} at 440 mg P kg^{-1} soil (lotus). Interestingly, tagasaste produced 12.6 g DM pot^{-1} at just 132 mg P kg^{-1} soil while white clover produced 7.8 g DM pot^{-1} at 202 mg P kg $soil^{-1}$. The shoot P concentration at 97% yield was highest for falcata lucerne at 0.392% while tagasaste was the lowest at 0.233% P.

4.1.4 Phosphorus input effect on Olsen P

Figure 4.5 illustrates the effect that the various P input levels had on the soil Olsen P values. There was a linear increase in Olsen P from 7 µg ml^{-1} at 0 mg P kg^{-1}, to 181 µg ml^{-1} at 1500 mg P kg^{-1}. This was an increase in Olsen P of 0.12 µg P ml^{-1} per mg P applied kg^{-1} soil. Between 0 mg P kg^{-1} and 10 mg P kg^{-1} the Olsen P increased 0.20 µg P ml^{-1} per mg P applied kg^{-1} soil. While between 500 mg P kg^{-1} and 1000 mg P kg^{-1} the Olsen P increased by 0.12 µg P ml^{-1} per mg P applied kg^{-1} soil.

Table 4.1: Values of shoot yield, P concentration, P uptake, Mo concentration and B concentration for 12 pasture legume species, grown under glasshouse conditions in a NZ high country soil supplied with increasing rates of P (8 levels of P; ranging from 0 to 1500 mg P kg^{-1} soil).

Species		Mean Shoot Yield (g DM Pot^{-1})	Mean Shoot P Concentration (% P)	Mean Shoot P Uptake (mg P Pot^{-1})	Mean Shoot Mo Concentration (ppm)	Mean Shoot B Concentration (ppm)
Arrowleaf clover		1.84	0.260	5.9	0.61	5.37
Balansa clover		1.41	0.261	4.2	0.84	8.45
Caucasian clover		7.13	0.271	20.3	0.10	10.01
Gland clover		0.20	0.079	0.61	0.54	3.33
Lotus		11.25	0.240	28.8	0.40	14.42
Lucerne		9.36	0.228	22.7	0.58	12.30
Persian clover		10.06	0.210	22.2	0.63	15.81
Falcata lucerne		7.04	0.257	19.0	0.51	13.58
Subterranean clover		2.81	0.249	7.9	0.60	8.66
Strawberry clover		6.31	0.241	16.3	0.81	11.97
Tagasaste		10.68	0.217	24.3	0.68	6.33
White clover		6.14	0.288	18.8	0.82	12.98
Grand Mean		6.18	0.233	15.9	0.67	10.27
Species	SEM	0.159	0.0053	0.56	0.059	0.553
	LSD (5%)	0.442	0.0148	1.56	0.163	1.540
P Rate	0	3.89	0.157	6.6	0.62	8.91
	10	4.42	0.165	7.7	0.65	9.48
	30	4.99	0.177	9.4	0.57	8.57
	60	5.98	0.188	12.0	0.56	9.12
	100	6.6	0.203	14.4	0.65	8.65
	250	7.37	0.226	17.6	0.68	10.93
	500	8.17	0.278	23.5	0.79	11.18
	1500	8.05	0.473	36.2	0.83	13.30
	SEM	0.130	0.0043	0.46	0.048	0.452
	LSD (5%)	0.361	0.0121	1.27	0.133	0.639
P	Species	***	***	***	***	***
	P Rate	***	***	***	***	***
	Sp*P Rate	***	***	***	***	***

*** Significant at $P < 0.001$ level

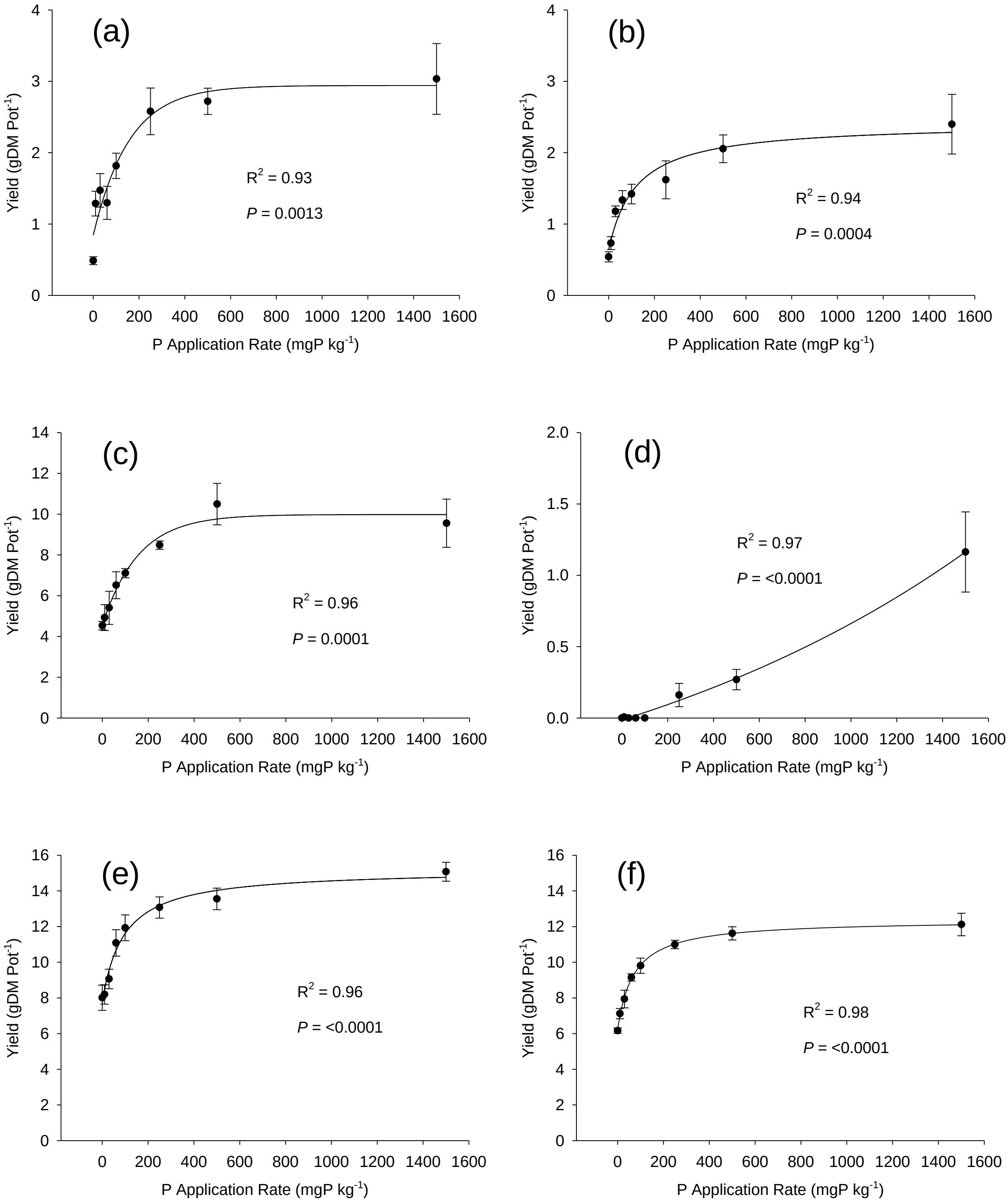

Figure 4.1: Total accumulated shoot dry matter (DM) yield response of pasture legume species (a) arrowleaf clover (*T. Vesiculosum L.*), (b) balansa clover (*T. michelianum*), (c) Caucasian clover (*T. ambiguum*), (d) gland clover (*T. glanduliferum*), (e) lotus (*L. pedunculatus*), (f) lucerne (*M. sativa*) to increasing levels of soil phosphorus (8 levels of P; ranging from 0 to 1500 mg P kg^{-1}soil), grown in 4 mm-sieved NZ high country soil. Data are mean values ± SEM (*n*=4), with *p* and r^2 values for fitted curve showing data trend (Line equation for all graphs are in Appendix 1).

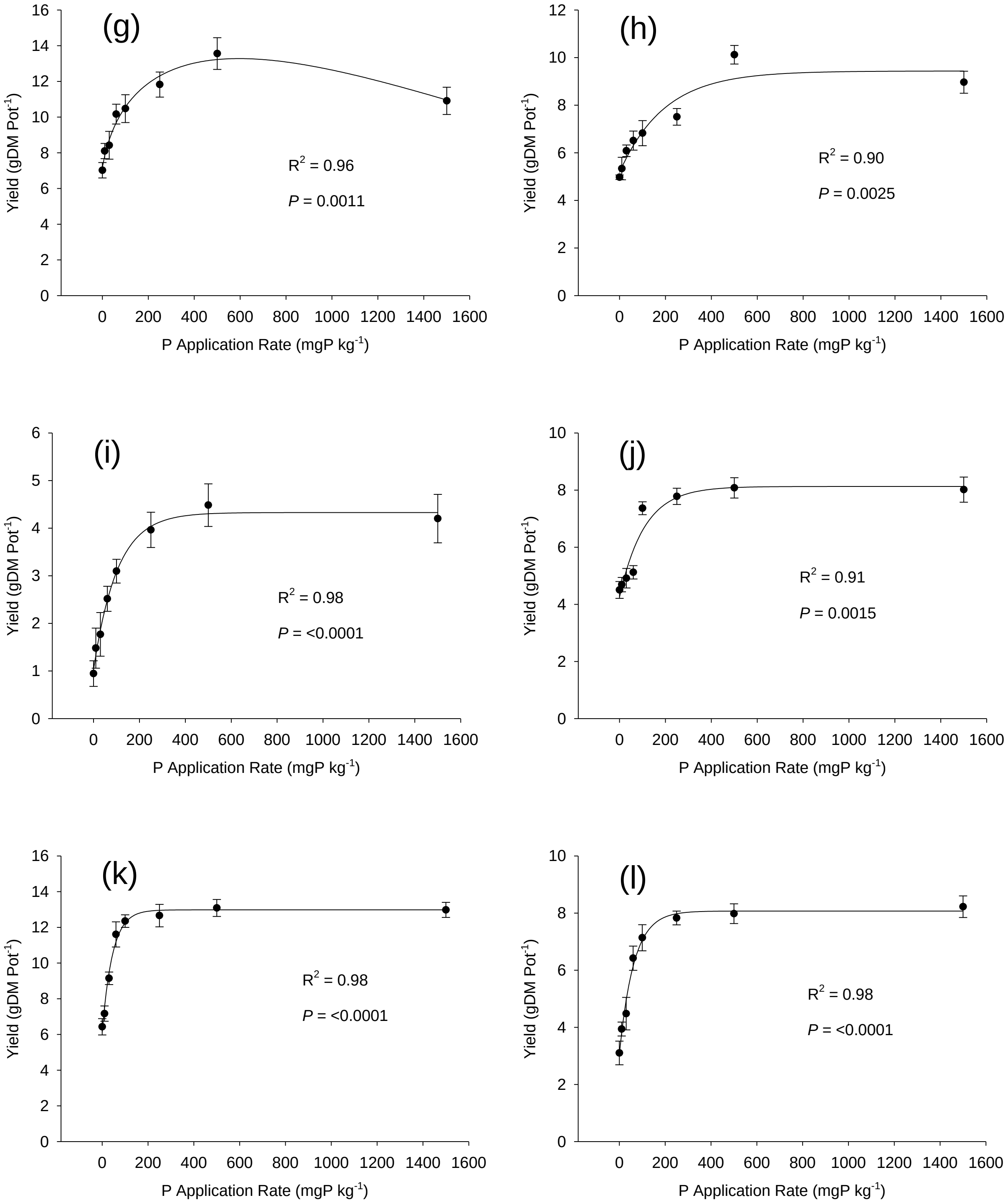

Figure 4.2: Total accumulated shoot dry matter (DM) yield response of pasture legume species (g) Persian clover (*T resupinatum*), (h) falcata lucerne (*M. falcata*), (i) subterranean clover (*T. subterranium*), (j) strawberry clover (*T. fragiferum*), (k) tagasaste (*C. proliferus*), (l) white clover (*T. Repens*) to increasing levels of soil phosphorus (8 levels of P; ranging from 0 to 1500 mg P kg^{-1}soil), grown in 4 mm-sieved NZ high country soil. Data are mean values ± SEM (*n*=4), with *p* and r^2 values for fitted curve showing data trend (Line equation for all graphs are in Appendix 1).

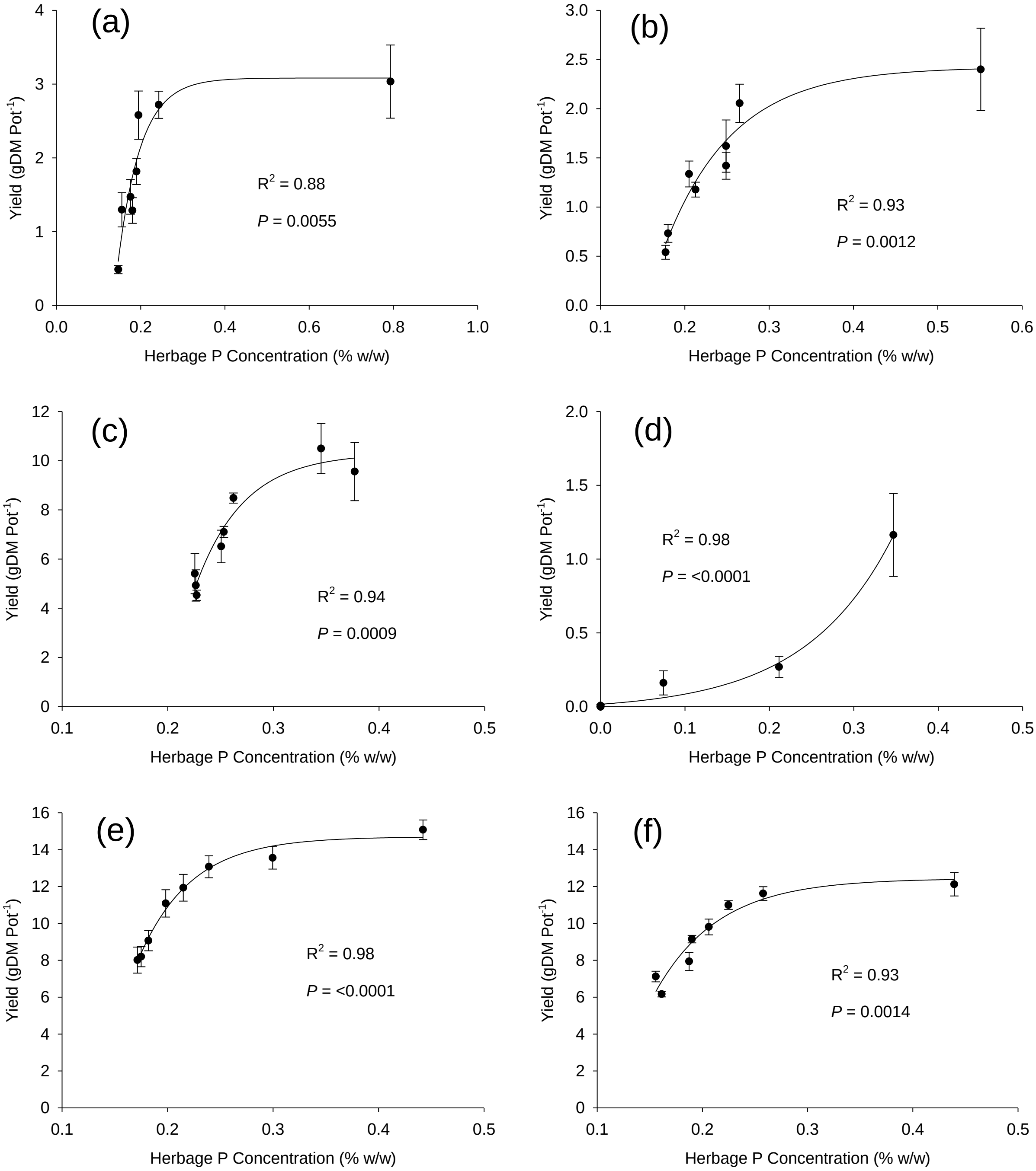

Figure 4.3: Total accumulated shoot dry matter (DM) yield response of pasture legume species (a) arrowleaf clover (*T. Vesiculosum L.*), (b) balansa clover (*T. michelianum*), (c) Caucasian clover (*T. ambiguum*), (d) gland clover (*T. glanduliferum*), (e) lotus (*L. pedunculatus*), (f) lucerne (*M. sativa*) to increasing herbage P concentrations across all P treatments (8 levels of P; ranging from 0 to 1500 mg P kg^{-1}soil), grown in 4 mm-sieved NZ high country soil. Data are mean values ± SEM (*n*=4), with *p* and r^2 values for fitted curve showing data trend (Line equation for all graphs are in Appendix 2).

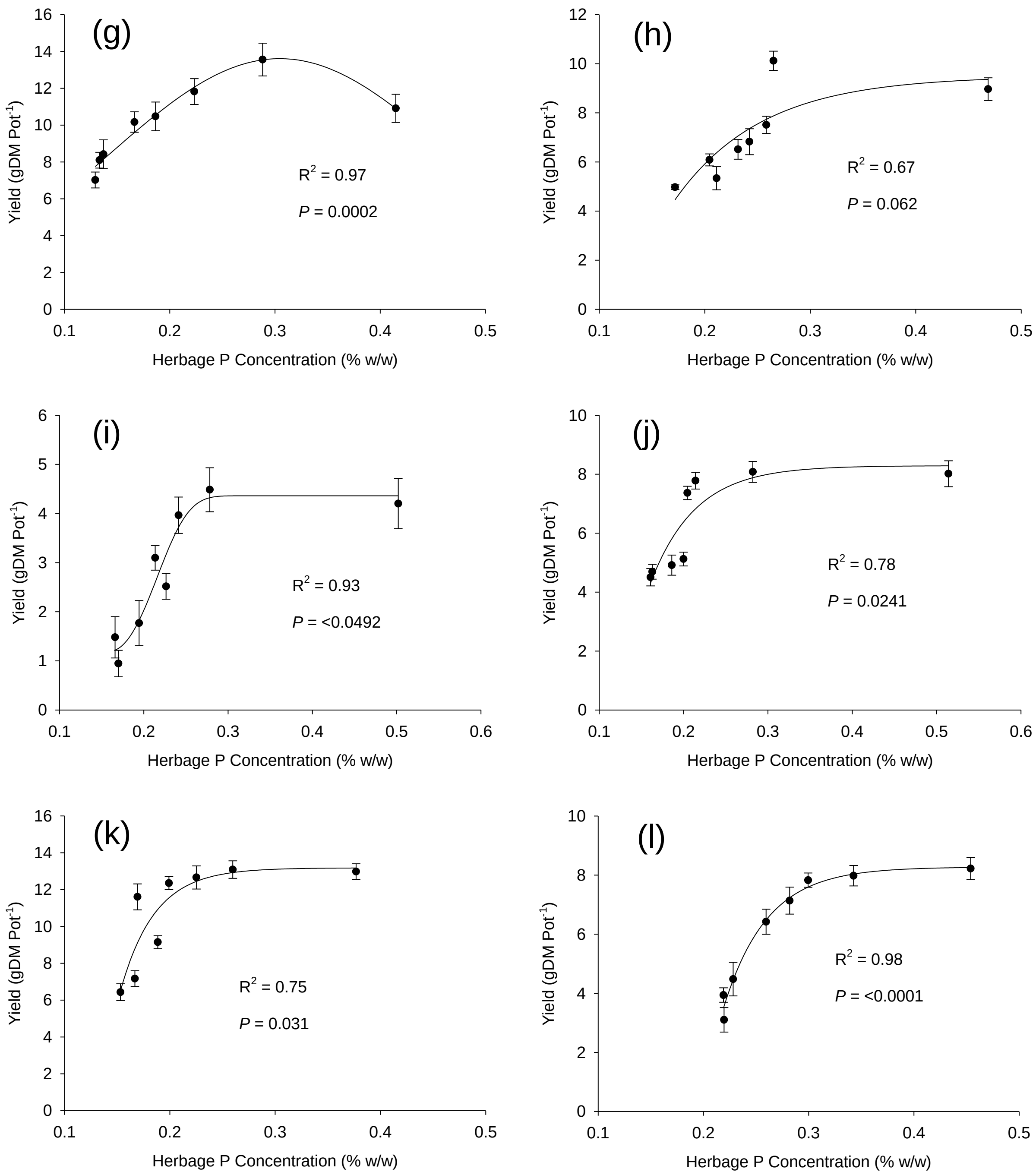

Figure 4.4: Total accumulated shoot dry matter (DM) yield response of pasture legume species (g) Persian clover (*T. resupinatum*), (h) falcata lucerne (*M. falcata*), (i) subterranean clover (*T. subterranium*), (j) strawberry clover (*T. fragiferum*), (k) tagasaste (*C. proliferus*), (l) white clover (*T. Repens*) to increasing herbage P concentrations across all P treatments (8 levels of P; ranging from 0 to 1500 mg P kg^{-1}soil), grown in 4 mm-sieved NZ high country soil. Data are mean values ± SEM (*n*=4), with *p* and r^2 values for fitted curve showing data trend (Line equation for all graphs are in Appendix 2).

Table 4.2: Rate of P application, equivalent Olsen P and shoot P concentration at which 97% of maximum yield was observed for each pasture legume species.

Species	P rate (mg P kg^{-1} soil)	Olsen P (µg mL^{-1})	97% Maximum Yield (g DM pot^{-1})	Shoot P Concentration (%)
Arrowleaf clover	517	66	2.84	0.277
Balansa clover	888	108	2.20	0.355
Caucasian clover	439	57	9.66	0.324
Gland clover	1467	175	1.13	0.345
Lotus	440	57	14.27	0.310
Lucerne	669	83	11.73	0.288
Persian clover	369	48	12.89	0.251
Falcata lucerne	536	68	9.11	0.392
Subterranean clover	336	45	4.19	0.263
Strawberry clover	302	41	7.88	0.281
Tagasaste	132	21	12.56	0.233
White clover	202	29	7.82	0.321

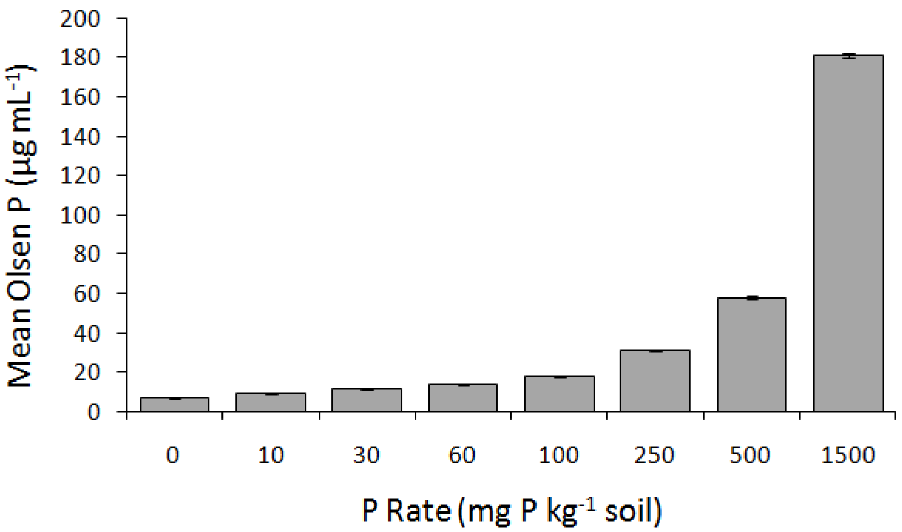

Figure 4.5: Olsen P values (*µ*g P mL^{-1}) of a NZ high country soil supplied with increasing levels of P after final legume herbage harvest. Values are means ± SEM (*n*=6) of Olsen P values from pots across all pasture legume species within each P treatment level (P rate) (pH of all = 6.0).

4.2 Lime experiment

4.2.1 Yield

A strong difference ($P < 0.001$) in DM yield was found as lime rate increased. The mean total shoot yield across all lime treatments ranged from 0.2 g DM pot $^{-1}$ (gland clover) to 11.0 g DM pot^{-1} (tagasaste) (Figure 4.6, Figure 4.7). Of the annual species, Persian clover was the highest yielding species at 8.5 g DM pot^{-1} while gland clover was the lowest (0.2 g DM pot^{-1}) followed by balansa clover (1.4 g DM pot^{-1}). For perennial species, mean yields across all lime treatments ranged from 3.9 g DM pot $^{-1}$ (falcata lucerne) to 9.6 g DM pot^{-1} (lotus). In general the perennials gave a total yield greater than the annual clovers. The mean maximum yield across all species was for a lime rate of 5 t ha^{-1} (pH = 6.0) at 5.7 g DM pot^{-1}. Beyond this point the yields began to decrease ($P < 0.001$). The largest increase in yield across all species (3.0 g DM pot^{-1}) was between 0 and 2 t lime ha^{-1}. There was also a strong ($P < 0.001$) species by lime rate interaction. This interaction effect indicates that the way in which the plants responded to the increased P rate differed among species. For this reason the relationship between increased lime rate and DM yield was examined in more detail using regression analysis of the data for individual species.

All annual species increased in yield to a maximum point, at either 2 or 5 t lime ha^{-1}, then declined with further lime application (R^2 = 0.82 – 0.97) as the soils became more alkaline (Figure 4.6, Figure 4.7). Arrowleaf clover stayed at near maximum (90%) yield across a greater range of lime rates (5.7 – 6.7) than the other annual species (Figure 4.6). The perennial species all increased sharply in yield with increasing lime inputs, then declined beyond around 4 t lime ha^{-1} (R^2 = 0.95 – 0.98). The exception was white clover which increased in yield sharply and then remained at that yield up to the maximum lime application rate (15 t lime ha^{-1}). Lotus, lucerne, strawberry clover and white clover remained near maximum yield across a wider range of lime rates then the other perennial species.

At 0 t lime ha^{-1} (pH = 5.0) tagasaste and lotus were the highest yielding species at 8.2 and 7.5 g DM pot^{-1}. Strawberry clover, balansa clover and lucerne were the lowest yielding at just 0.1, 0.2 and 0.2 g DM pot^{-1}. At 15 t lime ha^{-1} lotus, lucerne and Persian clover were the highest yielding species at 9.6, 6.5 and5.5 g DM pot^{-1}. Gland clover, arrowleaf clover, balansa clover and subterranean clover were the lowest yielding species at 0.0, 0.3, 0.4 and 0.8 g DM pot^{-1}.

4.2.2 Phosphorus uptake

Phosphorus uptake by the plants was affected by lime inputs to the soil. The main effect of lime was that the average concentration of phosphorus in the plant tissue increased (P < 0.001) from 0.184% at 0 t lime ha^{-1} up to 0.226% at 5 t lime ha^{-1}, then decreased back down to 0.182% at 15 t lime ha^{-1} across all species (Table 4.3).

At 0 t lime ha^{-1} balansa clover, falcata lucerne, and Caucasian clover had the highest % P at 0.216%, 0.212 % and 0.212%. Lucerne, strawberry clover and Persian clover had the lowest % P in the shoot herbage, at just 0.138%, 0.146% and 0.147%. At 15 t lime ha^{-1} falcata lucerne, tagasaste and lotus has the highest %P at 0.271%, 0.264% and 0.252%. Gland clover and arrowleaf clover had the lowest % P at 0.000% and 0.057% (Figure 4.6, Figure 4.7). This increased (P < 0.001) with yield, whereby the total P uptake by the herbage across all species increased from 4.0 mg P pot^{-1} at 0 t lime ha^{-1} up to 13.1 mg P pot^{-1}. It then declined to 7.5 mg P pot^{-1} at 15 t lime ha^{-1} across all species. At 0 t lime ha^{-1} lotus and tagasaste had the greatest uptake at 15.6, and 13.4 mg P pot^{-1}. Strawberry clover, lucerne and balansa clover had the lowest uptake at 0.2, 0.3 0.4 mg P pot^{-1}. At 15 t lime ha^{-1} lotus and lucerne had the highest P uptake at 24.0 and 15.1 mg P pot^{-1}. Gland, arrowleaf and balansa clovers took up the least P at 0.0, 0.2 and 0.4 mg P pot^{-1}.

4.2.3 Trace elements

The soil pH had a large effect on shoot molybdenum (Mo) concentrations. Mean Mo concentrations increased (P < 0.001) across all species with increasing lime rate, from 0.1 ppm (0 t lime ha^{-1}) up to 1.8 ppm (15 t lime ha^{-1}). Within species, across all lime treatments Caucasian clover had the greatest mean shoot Mo concentration (2.27 ppm), while gland clover had the lowest (0.23 ppm) (Table 4.3). At 0 t lime ha^{-1} gland and white clovers had the highest Mo concentration in the herbage at 0.25 and 0.21 ppm. Lotus and tagasaste had the lowest Mo concentrations in the herbage at 0.02 and 0.03 ppm. At 15 t lime ha^{-1} Caucasian and white clovers had the greatest Mo content in the herbage at 6.12 and 3.52 ppm. Gland and arrowleaf clovers had the lowest Mo concentration at 0.00 and 0.40 ppm.

Herbage boron (B) concentrations were also affected by the pH of the soil. Mean B concentrations decreased (P < 0.001) from 20.84 ppm (0 t lime ha^{-1}) down to 6.70 ppm (15 t lime ha^{-1}) across all species. Within species, across all lime treatments, lotus had the highest mean B concentration (17.1 ppm) while arrowleaf clover contained the lowest (6.40 ppm) (Table 4.3). At 0 t lime ha^{-1} lucerne and falcata lucerne had the highest B content at 31.60 and

30.74 ppm. Arrowleaf clover, tagasaste, gland clover and balansa clover had the lowest B content at 14.76, 15.13, 16.19 and 16.97 ppm. At 15 t lime ha^{-1} white clover, Persian clover and lotus had the highest B content at 11.68, 11.15 and 10.73 ppm. Gland clover, arrowleaf clover and tagasaste had the lowest B concentrations at 0.00, 0.75 and 4.06 ppm.

4.2.4 Maximum yields (97%)

Of the annual species the greatest 97% maximum yield ranged from 1.1 g DM pot^{-1} (gland clover) at 2 t lime ha^{-1} to 8.1 g DM pot^{-1} (Persian clover) achieved at 2 t lime ha^{-1} (Table 4.4). Of the perennial species the 97% yield ranged from 4.8 g DM pot^{-1} at 3 t lime ha^{-1} (white clover) to 11.2 g DM pot^{-1} at 2 t lime ha^{-1} (tagasaste).

The species had different ranges which were identified for the 90% yield. This is the pH range 10% either side of the maximum yield for each species. This indicates the optimum pH range for each species for growth. The species with the least tolerance for pH range was balansa clover (pH 5.7 - 5.9), while the species with the greatest tolerance was white clover (5.4 - 7.5) (Table 4.4). Gland clover, Persian clover, lotus, tagasaste and white clover were all able to produce 90% yields at pH = < 5.4, while lotus, arrowleaf clover, lucerne, white clover and strawberry clover produced > 90% yields at a pH < 6.4.

4.2.5 Lime input effect on pH

As expected, the variable lime rates applied in this experiment significantly increased the pH of the soil (Figure 4.8). The pH increased from 5.0 at 0 t ha^{-1} to 7.5 at 15 t ha. From pH 5.0 to pH 7.5 the pH increased by 0.17 pH unit per t lime ha^{-1}. This response changed with liming rate. Between 5 and 8 t lime ha^{-1} 1 t lime ha^{-1} moved the pH by 0.3 units and between 8 and 15 t lime ha^{-1} the pH only shifted 0.09 units per t lime ha^{-1}.

Table 4.3: Values of shoot yield, P concentration, P uptake, Mo concentration and B concentration by twelve pasture legume species, grown under glasshouse conditions in a NZ high country soil supplied with increasing rates of lime (5 levels of lime; ranging from 0 to 15 t lime kg^{-1}soil).

Species		Mean Shoot Yield (g DM Pot^{-1})	Mean Shoot P Concentration (% P)	Mean Shoot P Uptake (mg P Pot^{-1})	Mean Shoot Mo Concentration (ppm)	Mean Shoot B Concentration (ppm)
Arrowleaf clover		2.16	0.156	3.8	0.30	6.37
Balansa clover		0.64	0.183	1.3	0.60	10.68
Caucasian clover		4.23	0.249	10.1	2.27	13.94
Gland clover		0.24	0.107	4.6	0.23	6.77
Lotus		9.62	0.233	22.5	0.49	17.09
Lucerne		5.86	0.194	12.1	0.71	16.31
Persian clover		6.12	0.191	12.1	0.92	14.77
Falcata lucerne		3.92	0.256	10.4	0.58	14.73
Subterranean clover		1.54	0.193	3.2	0.54	9.30
Strawberry clover		4.39	0.196	9.2	0.85	13.64
Tagasaste		7.05	0.203	13.2	0.59	8.32
White clover		3.94	0.243	9.6	1.27	16.14
Grand Mean		4.41	0.200	9.1	0.78	12.38
Species	SEM	0.142	0.0032	0.34	0.086	0.666
	LSD (5%)	0.395	0.0089	0.94	0.241	1.858
L Rate	0	2.16	0.184	4.0	0.10	20.84
	2	5.15	0.211	10.5	0.19	16.78
	5	5.71	0.226	13.1	0.66	10.17
	8	4.41	0.199	10.2	1.16	7.20
	15	3.28	0.182	7.5	1.78	6.70
	SEM	0.091	0.0021	0.22	0.056	0.430
	LSD (5%)	0.255	0.0057	0.61	0.155	1.199
P	Species	***	***	***	***	***
	L Rate	***	***	***	***	***
	Sp*L Rate	***	***	***	***	***

*** Significant at < 0.001 level

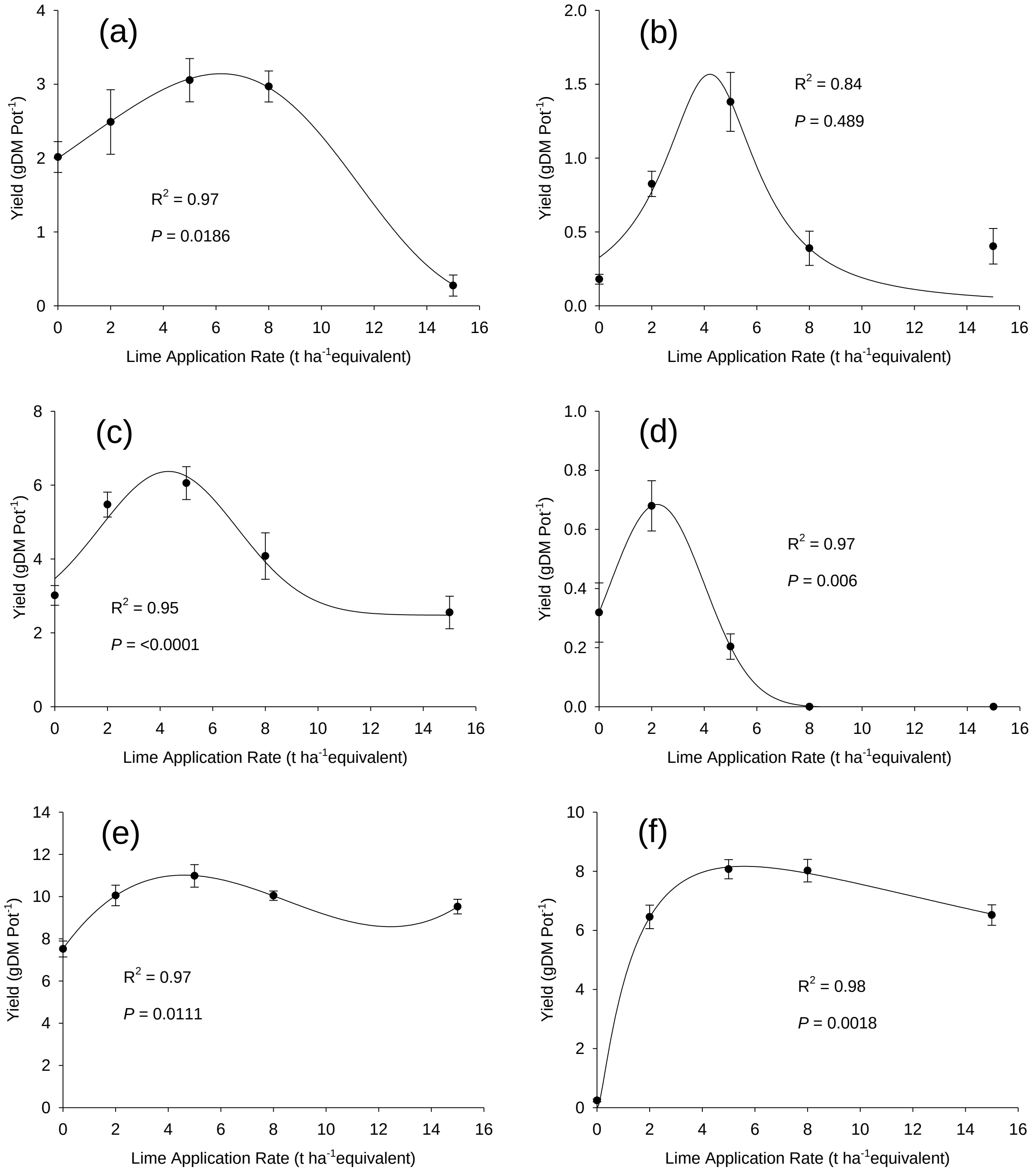

Figure 4.6: Total accumulated shoot dry matter (DM) yield response of pasture legume species (a) arrowleaf clover (*T. Vesiculosum L.*), (b) balansa clover (*T. michelianum*), (c) Caucasian clover (*T. ambiguum*), (d) gland clover (*T. glanduliferum*), (e) lotus (*L. pedunculatus*), (f) lucerne (*M. sativa*) to increasing levels of soil lime (5 levels of lime; ranging from 0 to 15 t ha^{-1} equivilant), grown in 4 mm-sieved NZ high country soil. Data are mean values ± SEM (*n*=4), with *p* and r^2 values for fitted curve showing data trend (Line equation for all graphs are in Appendix 3).

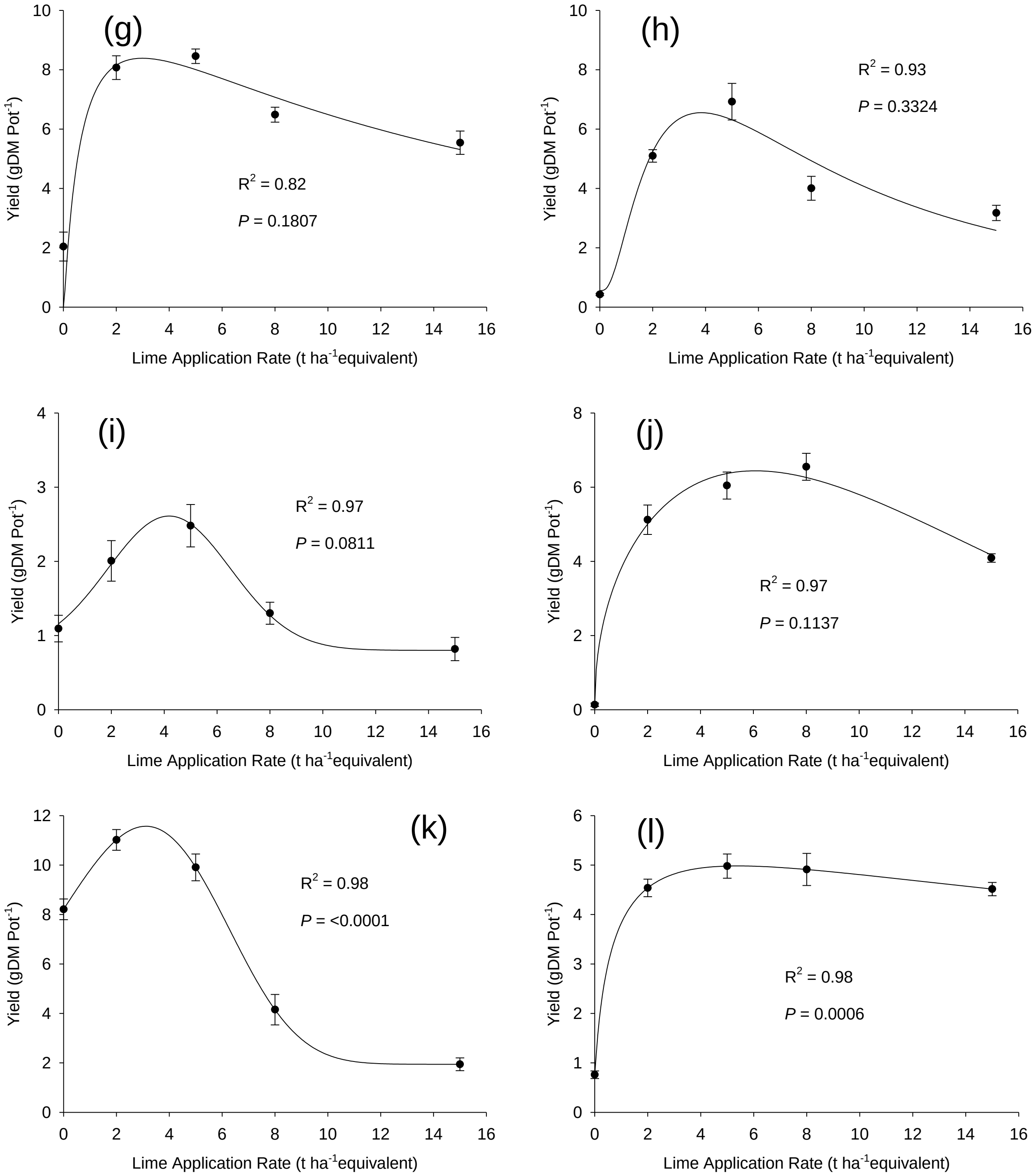

Figure 4.7: Total accumulated shoot dry matter (DM) yield response of pasture legume species (g) Persian clover (*T resupinatum*), (h) falcata lucerne (*M. falcata*), (i) subterranean clover (*T. subterranium*), (j) strawberry clover (*T. fragiferum*), (k) tagasaste (*C. proliferus*), (l) white clover (*T. Repens*) to increasing levels of soil lime (5 levels of lime; ranging from 0 to 15 t ha^{-1} equivilant), grown in 4 mm-sieved NZ high country soil. Data are mean values ± SEM (*n*=4), with *p* and r^2 values for fitted curve showing data trend (Line equation for all graphs are in Appendix 3).

Table 4.4: Rate of lime application and shoot P concentration at which maximum yield was observed for each pasture legume species, including the lime range and estimated pH range for 90% yield.

Species	Lime Rate (t Lime / ha)	Estimated pH	97% Yield (g DM pot^{-1})	Lime Range +/- 90% Yield	Estimated pH Range +/- 90% Yield
Arrowleaf clover	5.0	6.0	3.05	3.5-8.6	5.7 - 6.7
Balansa clover	3.9	5.8	1.51	3.5-4.9	5.7 - 5.9
Caucasian clover	3.5	5.7	6.17	2.8-5.9	5.6 - 6.2
Gland clover	1.8	5.4	0.66	1.4-3.0	5.3 - 5.6
Lotus	3.0	5.6	10.67	1.8-8.3	5.4 - 6.5
Lucerne	3.8	5.8	7.91	2.8-11.1	5.6 - 7.0
Persian clover	2.0	5.4	8.14	1.4-6.7	5.3 - 6.3
Falcata lucerne	3.0	5.6	6.35	2.5-6.2	5.5 - 6.2
Subterranean clover	3.5	5.7	2.53	3.0-5.5	5.6 - 6.0
Strawberry clover	4.3	5.8	6.21	3.1-10.2	5.6 - 6.8
Tagasaste	2.3	5.5	11.22	1.5-4.7	5.4 - 5.9
White clover	3.1	5.6	4.83	2.0-15.0	5.4 - 7.5

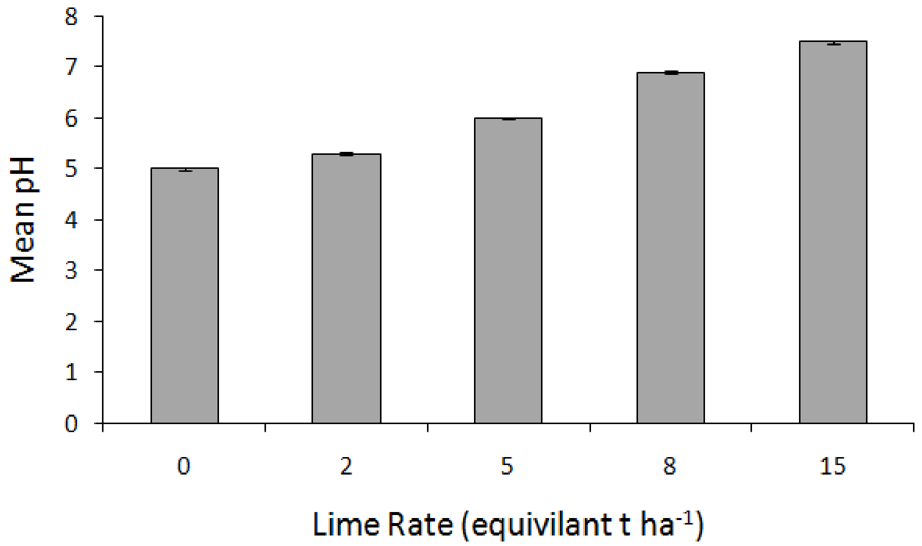

Figure 4.8: pH values of a NZ high country soil supplied with increasing levels of lime after final legume herbage harvest. Values are means ± SEM (*n*=6) of pH values from pots across all pasture legume species within each lime treatment level (lime rate) (Olsen P of all =28).

Chapter 5
Discussion

The objective of this study was to determine the yield and nutrient uptake response of 12 pasture legume species to P and lime additions when grown in an acid South Island high country soil under glasshouse conditions. The key results, and the potential implications for field conditions, are discussed below.

5.1 Phosphorus response

Increasing phosphorus inputs (P < 0.001) increased the yield of all annual and perennial legumes examined in this experiment and differences (P < 0.001) among species were also observed. Herbage P concentrations and total P uptake were also strongly influenced by P inputs.

5.1.1 Yield

The annual species yielded (P < 0.001) less than the perennial species with the exception of Persian clover due to their shorter growth period, as annual species go to seed, die and re-establish from seed each year. Persian clover had the highest total DM yield of the annual species across all phosphorus treatments (12.9 g DM pot^{-1}), while the other species were much lower yielding, producing less than 4.2 g DM pot^{-1} under optimum P conditions (16,407 - 1601 kg DM ha^{-1}) (Table 4.2). All annual species ranked in the same order across all P treatments.

Arrowleaf clover had the greatest percent increase in yield with increasing P inputs from the control treatment (0 mg P kg^{-1} soil; Olsen P = 7 µg ml^{-1}) with a 524% increase in yield when 1500 mg P kg^{-1} soil (Olsen P =181 µg ml^{-1}) was applied, but produced a relatively low yield at 3.0 g DM pot^{-1} at this P rate. Persian clover, the highest yielding annual species had a 93% increase in yield from the control (0 mg P kg soil) at 500 mg P kg soil^{-1} (Olsen P = 58 µg ml^{-1}), and yielded 13.6 g DM pot^{-1}. The P rate at which maximum yield was achieved, and no more response was evident differed between annual species, illustrated in Figures 4.1 and 4.2. This indicates that some species have better P utilization than others. This ranged from yield maximum P rates of 335 mg P kg^{-1} (subterranean clover) to 1466 mg P kg^{-1} (gland clover).

Species such as arrowleaf, balansa and gland clover only yielded once or twice before flowering and therefore dying. Gland clover failed to yield at below 250 mg P kg^{-1} (Olsen P = 31 µg ml^{-1}), and was very low yielding once it did produce herbage, with a maximum yield at just 0.3 g DM pot^{-1}. While it emerged with the other species, the seedlings remained very small throughout the experiment. The reasons for this are unknown, as other studies have shown it is tolerant of waterlogging and produced yields at a pH of 5.3 (Hayes *et al.* 2008b). This may limit the comparisons that can be made for some of these annual species. However even with limited data, strong relationships between P application rate and yield were apparent for these species ($P < 0.001$). Further experiments are required to confirm the relationships of some of these species in particular gland clover.

The perennial species yielded more ($P > 0.001$) than the annual species with the exception of Persian clover (12.9 g DM pot^{-1}) due to the longer growth period of the perennial species. Lotus had the highest mean yield across all P treatments of the species (14.3 g DM pot^{-1}). The other species were lower yielding, in the order of tagasaste > lucerne > Caucasian clover > falcata lucerne > strawberry clover > white clover (13.1, 12.1, 9.6, 9.0, 8.1, 8.2 g DM pot^{-1}) equivalent to 18168 – 9961 kg DM ha^{-1} 42 $weeks^{-1}$. Overall, white clover was the lowest yielding species from the perennials, which confirms its lack of suitability to these low fertility environments, even when soil moisture was non-limiting in this experiment.

The percent increases in yield between the control (0 mg P kg $soil^{-1}$) and maximum yield with P inputs, were much lower for the perennial species than that of the annual species. These ranged from 165% (white clover, 1500 mg P kg $soil^{-1}$) to 78% (strawberry clover, 1500 mg P kg $soil^{-1}$) with a mean increase in total DM of 103% across all perennial species between the control (0 mg kg $soil^{-1}$) and maximum P treatment (1500 mg kg $soil^{-1}$).

The P rate at which maximum yield was achieved, and yield response decreased differed among perennial species. This ranged from 132 mg P kg^{-1} (Olsen P = 21) for tagasaste to 669 mg P kg^{-1} (Olsen P = 83 µg ml^{-1}) for lucerne.

Caradus (1980) carried out a similar experiment, investigating the effect of P on ‘Treeline’ Caucasian clover, ‘Palestine’ strawberry clover, ‘Maku’ lotus, ‘Woogenallup’ subterranean clover and ‘Huia’ white clover. Comparable phosphorus rates were used (300 and 2000 mg P kg $soil^{-1}$) in pots of naturally P deficient soil (Stratford coarse sandy loam) over 24 weeks, however only the harvest three data were presented (6 weeks growth). Caucasian clover yielded 0.3 and 0.8 g DM pot^{-1}, strawberry clover yielded 1.2 and 1.7 g DM pot^{-1}, lotus yielded 3.8 and 6.0 g DM pot^{-1}, subterranean clover yielded 3.6 and 7.0 g DM pot^{-1} and white clover yielded 1.8 and 5.2 g DM pot^{-1} at 300 and 2000 mg P kg soil^{-1}. These yields were significantly less than those found in this experiment. This was because they only had one plant per pot, compared with five in this experiment. Also the plants were only grown over six weeks compared with 42 weeks in this experiment. Subterranean clover out-yielded lotus in their experiment which contrasts our result, but this was most likely due to subterranean clover being an annual species. This meant that in our trial it got to the end of its life cycle compared with lotus which was still actively growing many weeks later. The soil used in that experiment also had a very high phosphorus retention capacity. Caradus (1980) also found that lotus yield was less affected by P inputs in terms of an increase in yield, with just a 36% increase in yield, compared with a 46% increase in this experiment.

Caradus *et al.* (1995) found that there were no significant increases in yield between 400 and 500 mg P kg soil^{-1}, while the yield increased from 0 to 400 mg P kg soil^{-1} for 119 different cultivars of white clover. This contrasts the results of this experiment where 'nomad' white clover continued to increase in yield up to 1500 mg P kg soil^{-1}. This may have been due to 'nomad' white clover responding to a higher P status of the soil than the mean of the cultivars.

Hart and Jessop (1984) found that 'Maku' lotus out yielded white clover across all P application rates (50, 100, 250, 500, 1000 and 2000 mg P kg soil^{-1} in the form of H_3PO_4) which agrees with our findings. This is likely due to lotus having superior root growth compared with white clover and therefore greater opportunity to infiltrate the soil volume and take up P (Scott & Lowther 1980).

Dodd and Orr (1995a) found similar results, in a soil core experiment looking at the phosphorus response from 18 annual herbaceous legume species under low soil pH (5.4) with two rates of P fertility (Olsen P = 10 and 24 µg ml^{-1}). They found that subterranean clover, balansa clover and arrowleaf clover all yielded more with improved phosphorus status in the soil.

The magnitude of the response differed among species in this experiment, with gland clover giving the greatest response of all, yielding 1.2 g DM pot^{-1} compared with 0 g DM pot^{-1} with no P input (Figure 4.1). This was followed by arrowleaf clover, balansa clover and subterranean clover gave the greatest response from control (0 mg P kg^{-1}) to 1500 mg P kg^{-1} of 524%, 344% and 344% respectively. Of the perennial species, tagasaste and Caucasian clover gave the best response to added P (1500 mg P pot^{-1}) increasing in yield by 165% and

111%. These species gave the greatest response to high levels of P, and therefore if these species are used, a higher P status of the soil may be justified, although tagasaste still performed well under low P conditions.

Phosphorus supply was the main driver that increased yields across all species. Yield increased sharply in all annual and perennial species with increasing inputs of P to the system, with the exception of gland clover. The biological optimum yield (97%) was reached at different P rates for different species (Table 4.2). Of the annual species subterranean clover and Persian clover reached their biological optimum yields at much lower P rates (336 and 369 mg P kg^{-1} ; Olsen P 45 and 48 µg ml^{-1}) compared with gland and balansa clovers (1467 and 888 mg P kg^{-1}). Of the perennial species, tagasaste, white clover and strawberry clover achieved biological optimum yields at much lower P rates (132, 202 and 302 mg P kg^{-1}) than for lucerne and falcata lucerne (669 and 536 mg P kg^{-1}). Beyond these P rates, yield did not increase, suggesting that factors other than soil P availability began to limit yield. Of the annual species the Persian clover was the highest yielding with no P input at 7.0 g DM pot^{-1} which was higher ($P < 0.001$) than the other annual species which all yielded less than 1.0 g pot^{-1} under these conditions. Lotus, tagasaste and lucerne were the highest yielding of the perennial species with no phosphorus inputs, yielding 8.0, 6.2 and 6.1 g DM pot^{-1}, more ($P < 0.001$) than the other perennial species under these conditions. This result indicates that Persian clover, lotus, tagasaste and lucerne are able to be very productive, even in low P fertility high country soils, when compared to the other species in this experiment.

Although a growth response to P was expected, this has not been documented in scientific literature for many of the species examined in this experiment. Further, more information on optimum soil P levels for many pasture legumes does not exist. Therefore much of the data presented here is new and valuable information for species such as tagasaste, falcata lucerne and gland clover.

5.1.2 Herbage P concentration and P uptake

This increase in yield can be explained by the P uptake of the plants, driven by the P availability in the soil. Only Persian clover reduced in yield with P concentration above 0.250% P compared with the other species which increased in yield with increased herbage P concentration (Figure 4.4). An increased rate of P application to the soil increased ($P < 0.001$) the P concentration in the plant tissue, due to greater P uptake by the plants. This was shown by the grand mean across all species which increased from 6.6 to 36.2 mg P pot^{-1} when P rate increased from 0 to 1500 mg kg^{-1} soil. Phosphorus is essential for plant growth (Raven *et al.* 1992), and this was the main driving factor that increased yield. In this experiment the plants were grown under amended soil pH conditions (pH 6.0) with no other limiting nutrient due to basal lime and nutrient solution additions and no moisture limitations.

Among species there were different levels of yield responses, which occurred at different P treatment rates and at different herbage P concentrations (Figures 4.1 and 4.2, Figures 4.3 and 4.4). This was likely to have been driven by the plant species genetic adaptations to low phosphorus environments and therefore it is apparent each have different P requirements and P use efficiencies for plant growth (Figures 4.1 and 4.2). Lotus significantly ($P < 0.001$) out yielded white clover at every P treatment level (grand mean across all P treatments 11.2 vs. 6.1 g DM pot^{-1}).

The mean phosphorus content of the shoots increased ($P < 0.001$) with increasing P inputs. At 0 mg P kg^{-1} white clover and Caucasian clover had the highest P concentration at 0.220% and 0.227%, while Persian clover had the lowest of the species that yielded, at 0.129% as well as being one of the highest yielding species. At 1500 mg P kg^{-1} arrowleaf clover, balansa clover and strawberry clover all had higher P concentrations at 0.793%, 0.551% and 0.514% than gland clover, Caucasian clover and tagasaste which had the lowest P concentrations at 0.347%, 0.377% and 0.377%.

Pang (2009) found that lucerne and lotus had relatively low concentrations of shoot P with increasing P supply (max of 10 and 12 ppm P respectively). This is similar to the results of this experiment, and confirms their suitability to low phosphorus environments, indicating that the tissue has a low demand for phosphorus compared with some other species. Dodd and Orr (1995) found that white clover on average had a P content of 0.21%, which is comparable with the low P treatments of this study.

In terms of mean shoot P uptake the highest yielding species used the most phosphorus as expected, due to more DM being produced. Lotus took up the most phosphorus overall, at 66.7 mg P pot^{-1} at 1500 mg P kg^{-1} due to its higher overall yield, followed by lucerne, tagasaste and Persian clover at 53.3, 48.8, 45.2 mg P Pot^{-1} at 1500 mg P kg^{-1}. This was 7.4%, 5.9%, 5.4% and 5.0% respectively, of the P applied to the pots. At these high P rates, P was no longer limiting yield (Figures 4.1 and 4.2). At a P application rate of 100 mg P pot^{-1} the relative uptake of applied P was much greater, with lotus taking up 42.5%, tagasaste (40.9%), lucerne (33.5%), white clover (33.4%) and Persian clover (32.3%) when P was still limiting yield. This implies that some applied P had become non-labile (Section 2.2.3.1) (Mengel & Kirkby 2001).

In this soil, the Olsen P increased with increasing P inputs. It required 7.9 kg P ha^{-1} (88.3 kg Super phosphate ha^{-1} at 9% P) to increase the Olsen P by one unit once the soil had been brought to a pH of 6.0 (Table 4.5). Morton and Roberts (1999) state that to raise the Olsen P by one unit, you require between 4.0 and 7.0 kg P ha^{-1} on a sedimentary soil. Our result was only just beyond this range, which is typical of capital fertilizer inputs required when shifting P levels from a low to high status. This value was for a shift from an Olsen P of 7 µg ml^{-1} to 31 µg ml^{-1} (estimated optimum Olsen P for intensive system). This experiment has demonstrated that the P requirements for optimum growth of these pasture legume species were often at very high Olsen P values, well beyond 'typical' farmer fertiliser inputs and soil P fertility levels under field conditions.

5.2 Lime response

Lime additions also had a large effect ($P < 0.001$) on the DM yields for all the legumes in this experiment. There were also differences ($P < 0.001$) in the uptake of molybdenum, boron and phosphorus at varying soil pH's driven by the lime inputs. Importantly, differences were also found among species in this experiment. With the control soil at a pH of 5.0, lime addition to the soil was expected to reduce the exchangeable aluminium levels in the soil, while increasing the P and Mo availability to the plants up to a pH of 6.0 (Wheeler and O'Conner, 1998). Boron availability decreases with increasing pH, and in marginal B soils, this can cause plant deficiencies (Dear and Weir, 2004).

5.2.1 Yield

The annual species had ($P < 0.001$) different yields after receiving lime and were lower yielding than the perennial species, again due to the shorter growth period. Overall, Persian clover was the highest yielding annual species followed by arrowleaf clover (8.1 and 3.0 g DM pot^{-1} at pH 5.4 and 6.0) while balansa and gland clovers were the lowest (1.5 and 0.7 g DM pot^{-1} at pH 5.8 and 5.4). This was equivalent to a range of 10,364 to 845 kg DM ha^{-1} (Figures 4.6 and 4.7).

Species such as arrowleaf, balansa and gland clover again only yielded once or twice before flowering and therefore dying. This limits the comparisons that can be made for these species. However with limited data, strong ($P > 0.001$) relationships between yield and lime rate were developed for these species. Further trials are required to confirm the relationships of some of these species, particularly gland clover.

The perennial species were higher yielding than the annual species due to the longer growth period. Lotus had the highest mean yield across all lime treatments (9.6 g DM pot^{-1}), followed by tagasaste (7.1 g DM pot^{-1}), while white clover and falcata lucerne were the lowest (3.9 and 3.9 g DM pot^{-1}). The biological optimum (97%) yields, from highest to lowest, were tagasaste > lotus > lucerne > falcata lucerne > strawberry clover > Caucasian clover > white clover (11.2, 10.7, 7.9, 6.4, 6.2, 6.2, 4.8 g DM pot^{-1}) which was equivalent to 14291 – 6153 kg DM ha^{-1}. These were at a pH of 5.4, 5.6, 5.7, 5.6, 5.8, 5.7, 5.6. Beyond these values, yield decreased (Figures 4.6 and 4.7).

The pH ranges for 10% either side of maximum growth are given in Table 4.4. They provide valuable information about the optimum soil pH conditions for these species. The maximum mean yield for all species was achieved at the 5 t lime ha^{-1} rate (pH = 6.0; 5.7 g DM pot^{-1} across all species). The yields increased up to this point for most species, then decreased beyond this point.

Lime inputs increased ($P < 0.001$) the soil pH in the experiment. In this soil between 0.42 and 0.67 t lime was required to increase the soil pH by 0.1 unit suggesting this soil, has a low buffering capacity, most likely due to the low organic matter content of the soil (Figure 4.8). Morton and Roberts (1999) state that 1.00 t of quality lime ha^{-1} will raise the pH by 0.1 unit, on ash and pumice soils. Peat soils will require 0.90 quality lime ha^{-1}. This soil required much less than these recommendations, although 100% $CaCO_3$ was used in this case. Agricultural lime is more likely to be 80% $CaCO_3$. The use of 100% $CaCO_3$ allows for the easy calculation of lime rate required.

Soil pH had a large and highly significant ($P > 0.001$) effect on the yield of both perennial and annual species. The increase in yield up to pH 6.0 was strongly driven by increasing P availability in the soil, directly resulting from lime inputs. Increased molybdenum availability was apparent from increasing Mo plant tissue concentrations, resulting from increased lime inputs. Phosphorus concentrations in the plant tissue increased from a mean of 0.180% P at a pH of 5.0 (0 t lime ha^{-1}) up to 0.230% P at a pH of 6.0 (5 t lime ha^{-1}). Despite the fact that adequate P was applied the soil, P was still limiting growth as it is considered deficient at <

0.30 ppm (Morton & Roberts 1999). Molybdenum content in the shoots of the plants increased from just 0.10 mg kg^{-1} at pH 5 (0 t lime ha^{-1}) constantly and significantly (P < 0.001) up to 1.78 mg kg^{-1} at pH 7.5 (15 t lime ha^{-1}) and hence this was not limiting plant growth even at low pH, as it was not < 0.10 mg kg^{-1} (Morton & Roberts 1999). Beyond a pH of 6.0 (5 t lime ha^{-1}), the P availability in the soil decreased, which was illustrated by the P content in the shoot tissue reducing from 0.23% P down to 0.18% P at pH 7.5 (15 t lime ha^{-1}), the same P concentration as when no lime was applied (pH = 5.0). Also, with increasing pH, plant B content significantly (P < 0.001) decreased. This was illustrated when the grand mean concentrations found in plant tissue decreased from 20.8 down to 6.7 mg kg^{-1} when the pH increased from 5.0 to 7.5. The optimum ranges for plant growth are given in Table 4.4, and suggest lime would significantly increase the yields of all species in this experiment.

In a previous study, Corero and Blair (1978) found that subterranean clover had no increase in yield between a pH of 4.4 and 5.9 in a super phosphate trial on a coarse textured gravely sand soil in a glasshouse trial. This conflicts the findings of this study, most likely due to the differences in soil used. Edmeades (1991) found that while subterranean clover was not sensitive to low pH, it was moderately sensitive to Al toxicity. This soil at the control pH of 5.0 had a very high exchangeable Al content and therefore it is likely that with increasing pH, the exchangeable Al was reduced and thus increased yield.

In a field trial, Lambert and Grant (1980) found that under large (3.5 t lime ha^{-1}) liming treatments in North Island hill country, pasture legume yield decreased from the control. This was attributed to a depression in the P availability in the soil at a high pH (> 6.0) due to calcium phosphate formation (Larsen *et al.* 1965).

Even with the basal nutrients in this experiment which included phosphorus (all lime experiment pots had an Olsen P = 28), the herbage concentrations found in the highest yielding lime pots were not as high as the shoot P concentrations found in phosphorus experiment. The maximum grand mean % P was for pH 6.0 (5 t lime ha^{-1}) at 0.226% (Table 4.3) and is comparable to the 250 mg kg $soil^{-1}$ P treatment which had a grand mean of 0.226% P (Table 4.1). The shoot P concentration for the 97% of maximum yields ranged between 0.230% for tagasaste, and 0.390% for falcata lucerne. This suggests that for some species, even under optimum pH conditions P may still have been limiting plant growth, to a small extent.

The increase in yield with increasing lime inputs (increasing soil pH) were significantly (P < 0.001) different between species. Strawberry clover, lucerne and falcata lucerne had very significant increases in yield from the control (4788% increase at 8 t lime ha^{-1}, 3234% increase at 5 t lime ha^{-1} and 1536% increase at 5 t lime ha^{-1} respectively) compared with other species such as tagasaste and lotus which only had moderate increases in yield of 34% and 46% under optimum liming treatments. This may have been driven by adaptations of some species to these acidic environments, potentially making these species more suitable for acidic hill country properties than others. Species exhibiting very large lime responses are those which are particularly sensitive to low soil pH, in terms of Al toxicity, and P and Mo deficiency.

Although a growth response to lime was expected in some species, this has again not been documented in scientific literature for several of the species examined in this experiment, and further, information on optimum soil P levels for many pasture legumes does not exist. Therefore much of this is new and valuable information for many of the species examined.

5.2.2 Herbage nutrient concentrations

The proportion of soil P which was plant available was heavily affected by soil pH and this along with Mo and B deficiency drove plant yields in this experiment. In the phosphorus experiment, P content was limiting yield on average up to 500 mg kg^{-1} soil, where the P content in the tissue was 0.278%. This was close to the shoot P concentration of the biological maximum yield in the P experiment, suggesting that yield was limited by P up to this point. In the lime experiment the maximum P concentration across all species was at 5 t lime ha^{-1} (0.23%).

Legumes are considered deficient in molybdenum at levels below 0.10 mg kg^{-1} (Morton *et al.* 1999). Lucerne, lotus, falcata lucerne, subterranean clover, strawberry clover and tagasaste were all deficient in Mo at 0 t lime ha^{-1}, while lotus and tagasaste were still deficient at 2 t lime ha^{-1}. At 5 t lime ha^{-1} the plant available Mo content in the plants was sufficient for plant growth, and therefore not limiting from this point onwards. This would have affected the nitrogen fixation of these plants, and therefore decreased yields. It does not appear that Mo reached toxic levels in this experiment as they never exceeded levels of 500 mg (Gupta 1997).

Boron deficiency occurs at rates <17 – 20, 13 – 16 and 15 – 18 ppm B in plant tissue for lucerne, white clover and subterranean clover, respectively (Dear and Weir, 2004). The species in this experiment therefore became deficient beyond 5 t lime ha^{-1} for lucerne and white clover, while subterranean clover was deficient with just 2 t lime ha^{-1} applied. This suggests that this soil became boron deficient beyond a pH of just 5.3 for subterranean clover and 6.0 for the lucerne and white clover. This would have contributed to the decline in yields beyond 2 or 5 t lime ha^{-1}, depending on species. Obvious boron deficiency was visually present in subterranean, balansa, arrowleaf, Caucasian and Persian clovers, with the presence of red leaves at high liming rates.

The effect of pH on the availability of P, Mo and B was large, as sufficient nutrient was applied in the basal nutrient solution, and must have been made non-labile under acidic and or alkaline conditions. The effect of the trace elements in the lime trial may be secondary in terms of the response in yield from the increasing lime inputs. Phosphorus availability was likely the key driver for yield, although further experiments examining trace elements are required to fully examine this aspect.

5.3 Implications for field legume growth

Soil P availability appeared to be the key driver of yield in both experiments. A key factor limiting the growth and yield of these legumes was the pH of the soil, as the control yields for the lime trial were significantly lower than that of the P trial. This was likely to be the case as at a low pH (pH = 5.0) soil phosphorus is less plant available, while in the phosphorus trial the pH had been modified. In the lime experiment, phosphorus was still limiting growth, despite there being adequate P applied to the soil, P was less plant available under both acidic and alkaline soil pH's.

This experiment has identified both annual and perennial species which can perform well in low P environments, such as lotus, tagasaste and Persian clover. The P use efficiency has been identified for these species also, showing the effect on plant yield at different rates of P input. This identified the species which will perform well given relatively low P inputs provided the pH of the soil is brought up to a more favourable level (pH 5.3 – 6.0).

Optimum pH is determined by the point at which no further response to lime occurs (Edmeades *et al.* 1984), for pasture plant production. This research has identified the optimum pH ranges for the investigated species, and found that some species have wide ranges in which they can produce near maximum (90%) yields, for example white clover, lucerne, strawberry clover, lotus, Persian clover and arrowleaf clover while some have very narrow bands such as balansa clover, subterranean clover and tagasaste (Figures 4.6 and 4.7). However the relative yields of these species must be taken into account. Significant improvements in yields can be achieved with low inputs of lime in many cases, for example gland clover, lotus, Persian clover, tagasaste and white clover all producing 90% or near 90% of the maximum yield with just 2 t lime ha^{-1} on this soil.

Some species showed much higher P use efficiency than others, and as mentioned above some species performed much better than others at low P rates. This is important as these species will likely out yield the others in lower pH environments and low P conditions, leading to more pasture growth. The maximum yield must be taken into account, as some plants had strong increases or high relative yield at low P inputs, but were overall low yielding.

The implementation of some of these species may be held back by seed supply. Seed companies are reluctant to produce and stock seed in small quantities due to the low farmer demand for the seed and / or low seed production from many of these species. Because of this the seed is more expensive to buy due to the higher cost of production. For seed production and sales, the species must be significantly better than the current species marketed, hence the commercial reality of many of these species is a major barrier.

Many of these species are comparatively ‘wild’ in comparison to species such as white clover which has had a lot of research and development put into its genotype. For this reason, with more breeding, some of these species could be refined into a much stronger pasture legume.

Many of the examined species have documented agronomic or physical constraints for implementation in pastures. Caucasian clover is known to be slow to establish due to its slow secondary growth, and therefore is a poor competitor at establishment (Black *et al.* 2006) and this may limit its effectiveness in the field. Tagasaste is a herbaceous plant and therefore requires special grazing, but can be very useful for shelter and produces food for bees, which is required for the annual legumes in the sward. Lucerne and falcata lucerne have their growing points at the tip of the plant, and therefore require rotational grazing to get the most growth out of them, this is not the standard grazing method in the hill country environment so may be a limitation to the use of these species. Lotus is considered less palatable in spring, but is fine in autumn so many farmers create a feed bank, to be grazed in the autumn. The management of many of these species is often different to that of the current pastures, therefore for the maximum benefit to be gained, the management must fit the species.

It is important for the interpretation of these results that in both the lime and P experiments, other nutrient deficiencies were not limiting, as basal nutrients were applied. In the phosphorus experiment it is important to note that the pH of the soil was adjusted, so in a high country environment the pH of the soil would also have to be adjusted to replicate these results from P application.

Also, this was a glasshouse trial with the species grown as monocultures, and therefore is quite different to the environment faced by these plants in the field. The effect of inter species competition, climate, in particular drought, wind, excess rainfall, as well as physical factors such as heading date, grazing, trampling by stock, palatability and stock performance on a particular species of feed must be considered.

Chapter 6
Conclusions

The objective of this study was to determine the effects of liming and P addition on the growth and nutrient uptake of 12 novel legume species on an acidic high country soil from the Lees Valley in North Canterbury. This experiment has clearly identified the effects of these treatments on the yield and nutrient uptake of the 12 legume species in a pot trial under glasshouse conditions.

Phosphorus inputs increased (P < 0.001) the yields of all annual and perennial species. The highest yielding annual species was Persian clover at both low and high P inputs and had a 97% of maximum yield of 12.9 g DM pot^{-1} at 369 mg P kg^{-1} soil (Olsen P = 48 µg ml^{-1}). All other annual species yielded less (P < 0.001) at their respective optimum P rates. The highest yielding perennial species was lotus at both low and high phosphorus rates with a 97% of maximum yield of 14.3 g DM pot^{-1} at 439 mg P kg^{-1} soil (Olsen P = 56 µg ml^{-1}). Tagasaste and lucerne were also high yielding, while white and strawberry clovers were the lowest yielding perennial species. This increase in yield was driven by increasing P availability in the soil, and therefore increased P uptake by the plants as illustrated in the results and discussion. The P rate at which the maximum yield was achieved varied greatly between species, but the maximum yield of the species must be taken into account when interpreting this.

Lime increased (P < 0.001) the yields of all annual and perennial species up to a maximum, where yields then decreased with further lime additions. The optimum pH ranges for plant growth were identified, which varied between species. The highest yielding annual species was Persian clover across all lime treatments, with a 97% of maximum yield of 8.1 g DM pot^{-1} at a pH of 5.4 and an optimum pH range of 5.3 – 6.3. The lowest yielding annual species was gland clover under optimum pH conditions, yielding just 0.7 g DM pot^{-1}. The highest yielding perennial species was tagasaste with a 97% yield of 11.2 g DM pot^{-1} under optimum soil acidity conditions (pH 5.3). The lowest yielding perennial species was white clover which had a 97% max yield of just 4.8 g dm pot^{-1}, which occurred at a pH of 5.6 in this experiment. Yields were driven primarily by P availability in the soil, which was in turn driven by soil pH. P availability increased on average up to 5 t lime ha^{-1} (pH = 6.0), then decreased beyond this point. This was illustrated in the plant uptake of P. Trace elements also had some effect on yield, as with increasing pH, the amount of plant available Mo increased, while plant available B decreased. There were differences (P < 0.001) between species in terms of lime effects, which indicated that these species differ in their optimum pH growth conditions and also the range of pH conditions in which they perform best.

This experiment has identified the optimum phosphorus and pH soil conditions for these species, of which for many this is new and valuable information. The suitability of these species must be considered in terms of climatic and physical factors experienced in the field environment, including grazing and mixed sward competition effects. The results of this glasshouse experiment must be interpreted with these factors in mind.

Suggestions for further research:

- These findings need to be confirmed in the field, under natural climatic (rainfall, temperature, wind), topographical (aspect and altitude) and grazing (palatability, trampling) conditions. Hence this experiment should be extended to field studies.
- This experiment has been carried out on a specific soil, therefore giving the performance of these species on this soil. Further research should be carried out on a range of soils from throughout the hill country of New Zealand to strengthen these P and pH relationships for these species.
- Pasture mixes that could be used to best suit these species should be investigated in terms of inter species competition not only at establishment but also in terms of persistence of these legume species in the sward as they are the main nitrogen input for the pasture. The impact of growing in combination with other species (as mixed species swards) on plant establishment and yield is a critical aspect which requires further investigation.
- Micro element effects on these legume species should be investigated further, to separate the effects of P deficiency and trace element deficiency or toxicity on plant growth.

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Appendices

Appendix 1: Line equations for Figures 4.1, 4.2

(a) Arrowleaf clover	y=0.8446+2.0965*(1-exp(-0.0064*x))
(b) Balansa clover	y=0.6369+1.7775*x/(119.3809+x)
(c) Caucasian clover	y=4.5606+5.4190*(1-exp(-0.0064*x))
(d) Gland clover	y=-0.9621+0.9483*exp(0.0005*x)
(e) Lotus	y=7.7272+7.4684*x/(92.5860+x)
(f) Lucerne	y=6.2244+6.1492*x/(70.3233+x)
(g) Persian clover	y=if(x<=594.9726-2239.3313*((1.2506-1)/1.2506)^(1/1.2506), 0, 13.2778*((1.2506-1)/1.2506)^((1-1.2506)/1.2506) * (abs((x-594.9726)/2239.3313+((1.2506-1)/1.2506)^(1/1.2506))^(1.2506-1)) * exp(-abs((x-594.9726)/2239.3313+((1.2506-1)/1.2506)^(1/1.2506))^1.2506+(1.2506-1)/1.2506))
(h) Falcata lucerne	y=5.1746+4.2612*(1-exp(-0.0051*x))
(i) Subterranean clover	y=1.0273+3.3006*(1-exp(-0.0097*x))
(j) Strawberry clover	y=4.2536+3.8731*(1-exp(-0.0092*x))
(k) Tagasaste	y=6.1237+6.8558*(1-exp(-0.0223*x))
(l) White clover	y=3.0556+5.0153*(1-exp(-0.0159*x))

Appendix 2: Line equations for Figures 4.3, 4.4

(a) Arrowleaf clover	y=-33.7066+36.7888*(1-1.051^x)
(b) Balansa clover	y=-13.4757+15.8983*(1-4.5947^x)
(c) Caucasian clover	y=-769.7881+780.0950*(1-2.9153^x)
(d) Gland clover	y=-0.0267+0.0429*exp(9.5660*x)
(e) Lotus	y=-205.0426+219.7395*(1-1.5563^x)
(f) Lucerne	y=-69.4957+81.9296*(1-5.8777^x)
(g) Persian clover	y=13.6090*exp(-.5*((x-0.3046)/0.1653)^2)
(h) Falcata lucerne	y=-30.9923+40.4935*(1-5.4355^x)
(i) Subterranean clover	f=if(x<=0.1586-45.4095*((759.2744-1)/759.2744)^(1/759.2744),4.3604, 4.3604+-3.1661*((759.2744-1)/759.2744)^((1-759.2744)/759.2744) * (abs((x-0.1586)/45.4095+((759.2744-1)/759.2744)^(1/759.2744))^(759.2744-1)) * exp(-abs((x-0.1586)/45.4095+((759.2744-1)/759.2744)^(1/759.2744))^759.2744+(759.2744-1)/759.2744))
(j) Strawberry clover	y=-72.9979+81.2869*(1-7.1852^x)
(k) Tagasaste	y=-714.4363+727.6147*(1-exp(-30.6306*x))
(l) White clover	y=-962.3008+970.5730*(1-2.9242^x)

Appendix 3: Line equations for Figures 4.6, 4.7

(a) Arrowleaf clover	y=if(x<=6.1863-454748.43002*((83117.371-1)/83117.371)^(1/83117.371), 0, 3.1416*((83117.371-1)/83117.371)^((1-83117.371)/83117.371) * (abs((x-6.1863)/454748.43002+((83117.371-1)/83117.371)^(1/83117.371)) ^(83117.371-1)) * exp(-abs((x-6.1863)/454748.43002+((83117.371-1)/83117.371)^(1/83117.371))^83117.371+(83117.371-1)/83117.371))
(b) Balansa clover	y=1.5672*(0.9755*(1/(1+((x-4.2172)/2.1756)^2))+(1-0.9755)*exp(-0.5*((x-4.2172)/2.1756)^2))
(c) Caucasian clover	y=2.4784+3.8914*(1.5372*(1/(1+((x-4.3207)/2.6092)^2))+(1-1.5372)*exp(-0.5*((x-4.3207)/2.6092)^2))
(d) Gland clover	y=-0.0018+0.6864*exp(-.5*((x-2.2130)/1.7933)^2)
(e) Lotus	y=7.5218+1.7344*x+-0.2585*x^2+0.0101*x^3
(f) Lucerne	y= if(x<=0, 0,137.8629*exp(-0.5*(ln(x/51.0363)/1.4875)^2)/x)
(g) Persian clover	y= if(x<=0, 0,103.9862*exp(-0.5*(ln(x/51.4430)/1.6869)^2)/x)
(h) Falcata lucerne	y= if(x<=0, 0.5569,0.5569+35.3045*exp(-0.5*(ln(x/9.0358)/0.9253)^2)/x)
(i) Subterranean clover	y=0.8006+1.8103*exp(-.5*((x-4.1905)/2.3257)^2)
(j) Strawberry clover	y=if(x<=6.0808-13.6000*((1.4513-1)/1.4513)^(1/1.4513), 0, 6.4410 *((1.4513-1)/1.4513)^((1-1.4513)/1.4513) * (abs((x-6.0808)/13.6000+ ((1.4513-1)/1.4513)^(1/1.4513))^(1.4513-1)) * exp(-abs((x-6.0808) /13.6000+((1.4513-1)/1.4513)^(1/1.4513))^1.4513+(1.4513-1)/1.4513))
(k) Tagasaste	y=if(x<=3.1176-13.6158*((4.5839-1)/4.5839)^(1/4.5839),1.9432, 1.9432+9.6268*((4.5839-1)/4.5839)^((1-4.5839)/4.5839) * (abs((x-3.1176)/13.6158+((4.5839-1)/4.5839)^(1/4.5839))^(4.5839-1)) * exp(-abs((x-3.1176)/13.6158+((4.5839-1)/4.5839)^(1/4.5839))^4.5839+(4.5839-1)/4.5839))
(l) White clover	y= if(x<=0, 0.7601,0.7601+209.3019*exp(-0.5*(ln(x/454.4717)/2.1051)^2)/x)