Soil biogeochemistry and the benefits of grasses as companion plants

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Abstract

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Intercropping and other forms of mixed cropping are both widely used and beneficial in many agricultural systems. One of the best-known examples is the grass-clover production model in pastures, in which nitrogen-fixation by symbiotic bacteria (e.g. *Rhizobium* spp.) benefits the grass as well as the host species of clover (*Trifolium* spp.) or other legume species. This is one example of transgressive overyielding, which refers to situations when the yield of two species growing together exceeds the yield of either of the species growing individually on the same area of land. This doctoral study aimed to investigate the benefits of companion plants in terms of soil biogeochemistry, focusing particularly on species that are companion plants to grasses. Literature is reviewed of plant species competition and co-existence, and rhizosphere biogeochemistry, primarily in the context of pastoral grasslands in the New Zealand High Country. The research project includes an investigation of whether there are nutritional benefits to nitrogen (N) -fixing plants when they grow in combination with grasses, rather than this being simply a relationship that involves spillover of nitrogen from legumes to grasses. This is extended to studies of phosphorus (P) -mobilizing plants and grasses, to investigate whether grasses provide reciprocal nutrients to cluster-rooted plants in the Proteaceae. The hypothesis underlying this study was that rhizosphere processes in grasses may be more adept than clovers at exploiting key trace elements in soils, and that these trace elements can be exchanged for N or P, thus also providing an explanation for species coexistence.

Practical investigations consisted of pot experiments, a transplanted-soil core mesocosm experiment and field sampling, primarily concerned with nutrient deficient soils. This experimental work analysed the effects of different species combinations on (i) yield and (ii) plant and soil nutrient status (macronutrients and trace elements). Plant species combinations included both exotic and native species of grasses and legumes. The work was partly carried out on the Lincoln University Campus and at Mt. Grand Station, a university-owned high country pastoral farm in Hawea, Central Otago, from 2019 – 2022, with funding support from the Miss E.L. Hellaby Indigenous Grasslands Research Trust.

The results showed that grasses played a critical role in acquisition of soil nutrients when they were growing with companion legumes or proteas in degraded soil. I found that legumes contained more nutrients (e.g. P, potassium sulphur, molybdenum and boron) when they were growing with grasses. Both native tussock grasses and exotic pasture grasses had significant positive impacts on native and exotic legumes in terms of nutrient uptake of a range of elements including Nitrogen and Sulfur. Both legumes and grasses benefited from growing together. Cluster-rooted species of Proteacea and grasses were found to have higher foliar concentrations of nutrients when they were growing together.

The findings of this research provide evidence of facilitation between plant species with contrasting root systems when they are growing together in terms of procurement of key plant nutrients. Combinations of species can better exploit soil nutrients under conditions where fertility is constrained. This work is interpreted in the context of pasture production systems, protection of native biodiversity and land sharing by exotic and native species in the New Zealand High Country.

The thesis is presented as an introduction to the study, a broad literature review, the inclusion of three published papers[1-3], one recently-submitted manuscript[4], a chapter describing additional work carried out during the PhD study, and a chapter containing the interpretive discussion and conclusions.

[1] Zhang, W., Maxwell, T., Robinson, B., and Dickinson, N (2022) Legume nutrition is improved by neighbouring grasses. Plant and Soil 475: 443-455.

[2] Zhang, W., Maxwell, T., Robinson, B., & Dickinson, N (2022) Plant Species Complementarity in Low-Fertility Degraded Soil. Plants 11: 1370.

[3] Zhang, W., Maxwell, T., Robinson, B., and Dickinson, N (2022) Grasses procure key soil nutrients for clovers. Nature Plants 8: 923–929,

[4] Zhang, W., Maxwell, T., Robinson, B., and Dickinson, N (2022) Facilitation between species mitigates nutrient constraints in grassland. *Submitted to Plant and Soil, 29 July 2022*.

Key words: Soil nutrients, species coexistence, facilitation, legume, grass, biogeochemistry, nitrogen, phosphorus.

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Chapter 1

General Introduction

1.1 Background to Study and Rationale

In nature, the main determinant of coexistence between plants is the extent to which they compete. However, not only do plants compete with each other but they may also complement each other contemporaneously (Haynes, 1980). Intercropping is an example of plant collaboration; farmers grow two or more crops simultaneously in the same area during a growing season, which can improve yield and land use (Willey, 1980). In this system, the separate ecological niches of species enable plants to make full use of light, heat, water and soil nutrient resources (Polechová and Storch, 2019). A world-wide intercropping pattern in agriculture is the culturing of legumes and non-legumes species together e.g., in pasture, greater total herbage yields are obtained by planting a grass and a legume in association compared with only grass, particularly where no nitrogen fertilizer is applied (Haynes, 1980). Apart from obtaining better yields, growing legumes and grasses together balances the nutritional benefits of the fodder and its palatability for stock (Capstaff and Miller, 2018). In New Zealand, legume-grass associations dominate lowland and upland pasture (Brougham, *et al*., 1978), and they are also prevalent in Europe (Humphreys, 2017 and Dineen, 2018), Australia (Nichols, 2012) and elsewhere, providing farmers with higher returns and livestock with a higher food quality from more sources (Brock and Hay, 2001).

In New Zealand, indigenous species cover has decreased yearly (Stats^{nz}). Natural forest cover was replaced by pasture. Introduction of exotic pasture species and enlargement of the grazing area, threatens native species. New Zealand's unique rangeland communities are particularly associated with high country habitats that have faced the dual threats of grazing mammals and the spread of invasive plant species. Nutrient top-dressing and over-sowing of pasture grasses and legumes, particularly on

Plate 1.1 Tussock grasses dominate New Zealand high country grasslands (Photo: N Dickinson).

the lower reaches of the high country, further excludes native biota and favours more productive exotic communities of pasture plants and weeds. It could be important to develop a new and better understanding of how and why native and exotic plants can grow together and provide mutual benefits in terms of soil biogeochemistry.

It is clear that nitrogen fixed by legumes can be utilized by grasses or other companion plants. N fixation can also be substantially constrained by soil acidity and a limiting supply of key nutrients, such as phosphorus, especially when fertilizer is not applied (Nölke, *et al*., 2022). It has become clearer recently that multiple-nutrient constraints widely impact primary productivity in grasslands (Fay, *et al*., 2015). Plant species differ in their ability to acquire key nutrients; for example, to obtain P through symbiotic mycorrhizal associations and cluster roots, root adaptations, or resource partitioning (Lambers, *et al.,* 2015, Phoenix, *et al.,* 2020). Other plants, including grasses, can secrete organic acids (phytosiderophores) that mobilize the deficient chemical elements (e.g., Fe, Zn, Cu, Mn) in soil (Lambers, 2013). Competition for resources has been more extensively studied than the mutual benefits that may be derived from the coexistence of different plant species or functional groups, although the temporal and spatial advantages of plants growing together are well known (Schulze & Müller, 2005). Belowground functional traits in plants are recognized as important for nutrient uptake but are poorly understood.

Plate 1.2 White clover growing toward a tussock grass (Photo: W Zhang).

1.2 Aim and Objectives

The aim of this research was to investigate the mutual benefits of grasses and legumes growing together in terms of their exploitation of soil biogeochemistry. The work had four objectives:

Objective 1: To investigate whether there are benefit to nitrogen-fixing plants when grown in combination with grasses, rather than vice versa.

Objective 1: To investigate whether there are benefit to nitrogen-fixing plants when grown in combination with grasses, rather than vice versa.

Objective 2: To investigate transgressive overyielding and phosphorus biogeochemistry, by growing plants with cluster roots in combination with grasses.

Objective 3: To investigate whether bidirectional complementarity exists in terms of exploitation of the soil nutrient pool.

Objective 4: To investigate whether coexistence of species provides an opportunity to facilitate enhanced uptake and improved procurement of key soil nutrients by assemblages of plants in mid-altitude South Island grasslands.

1.3 Structure of thesis

This thesis consists of a general Introduction (Chapter 1), literature review (Chapter 2), three published papers (Chapter 3-5), a submitted paper (Chapter 6), additional experimental work (Chapter 7) and interpretation of the findings as Discussion and Conclusions (Chapter 8).

Chapter 2

Literature Review

2.1 Introduction

When Europeans settled in New Zealand about 150 years ago, they began to introduce legumes such as clovers, and productive grasses such as ryegrass (Charlton and Stewart, 1999). These exotic species became well established in the lowlands and gradually spread to the high country. This brought exotic grasses and weed species to these areas, many of which threaten the regeneration of indigenous species such as tussock grasses. Due to the severe impact by the dual threats of exotic and invasive mammals and weeds, native vegetation land cover has become reduced. Nowadays, about 40% of New Zealand's land is covered by exotic grasslands, and indigenous grasslands only account for approximately 13% of the country's land area, generally occupying high country areas (Stats^{nz}, 2021).

When a species invades another habitat, competition is inevitable. Intraspecific and interspecific competition between individuals and species respectively is a common phenomenon in plant populations and communities. The impact of exotic species on native species warrants attention. The main determinant of coexistence between plants is the extent to which they compete with each other. Competition among plants can be divided into interspecific competition and intraspecific competition. Interspecific competition is the phenomenon where the plants directly or indirectly inhibit each other in competition for living space, resources, nutrients, etc. Where light, heat, oxygen, water supply and nutrient supply is in excess of the needs of both plants, they will compete less with each other (Haynes, 1980). It is generally accepted that the more similar the needs of the two plants, the more intense the competition will be between them. Therefore, intraspecific competition is more intense than interspecific competition (Griffin, *et al.*, 2009).

Resources also determine the level of competition among plants. Nitrogen and phosphorus are the most vital two elements for plant growth, and are also limiting nutrients in NZ's high country, especially in the South Island (Stevens, 2014). However, more than just competition occurs between plants. A good example of plant complementarity is the intercropping system. While increasing land utilization, intercropping also brings into play the advantages of plants. For example, plants are particularly competitive for underground nutrients, legumes, as nitrogen fixers, can provide nitrogen sources for companion plants, while its ability to fix nitrogen is limited by other nutrients, such as phosphorus (Zhao, 2000). Moreover, many gramineous species have been shown to activate fixed phosphorus in the soil by releasing certain acids (Vančura, 1964, Fayun and Ziqin, 1997, Li, 2000 and

Ladouceur,2006,). This mobilized phosphorus can also be used by neighbouring plants. It is also a way for neighbouring nitrogen-fixing plants to obtain phosphorus sources under poor soil conditions (Zhang, 2022). However, not all intercropping increases the yield of plants. It is only when the complementarity between species is greater than the competition between them can the advantage of a yield increase be realized (Li, 2015).

Combinations of grasses and nitrogen-fixing legumes are ubiquitous in most natural and derived pastoral grasslands. Understanding the coexistence of native and exotic plants is the focus of our research. We investigated the mutual benefits of grasses and legumes growing together in terms of their exploitation of soil biogeochemistry, present evidence from both glasshouse experiments and field sampling.

2.2 Grasslands in New Zealand

Grasslands (including shrubland) cover 60% of New Zealand; they are dominated at high altitudes by tussock grasses (e.g. *Festuca novae-zelandiae* (hard tussock), *Poa colensoi* (blue tussock), *Poa cita* (silver tussock), and *Chionochloa rigida* (snow tussock)). Most have been modified for pastoral uses and almost all are affected by grazing feral mammals. Burning, grazing top-dressing and oversowing have depleted the tussock grasslands (McGlone, 2001). Lowland grasslands mainly consist of pasture grasses and legumes. Between 1996 and 2018, of the 429,665 hectares of exotic grassland were converted to other land uses, 64% (276,173 hectares) changed to exotic forest medium land cover class (Stats^{NZ}, 2021). The area of exotic grassland increased by 68,274 hectares in 2018 to 10,630,980 hectares (Stats^{NZ}, 2021), which is mainly distributed in the South Island.

2.2.1 Plants species associations in pasture

Legumes and non-legumes are the most common combinations in pasture, which has been an important feature of agriculture from the early days. Apart from the herbage yield obtained, this system can make a better use of ecological echo, which is why the grass-legume association has been widely used (Hayne, 1980).

Because of these added benefits in natural and agricultural pasture, the mixture generally includes one or more leguminous species and one or more species of *Graminaceae*. Lucerne (*Medicago sativa*), white clover (*Trifolium repens*) and red clover (*Trifolium pratense*) are common legumes used in pastures, and grasses include ryegrass (*Lolium perenne*), brome grass (*Bromus inermis*), tall fescue (*Festuca elata*), cocksfoot (*Dactylis glomerata*) and wild oats (*Avena sativa*). Among all legume-grass combinations, white clover-ryegrass associations dominate New Zealand pasture (Brougham, *et al.*, 1978).

2.2.2 Competition and complementarity in pasture

A community of plants is composed of a mixture of species, and a single species community rarely exists for long (Li, *et al.*, 1998). The basis of pasture science is to ensure a ‘balance’ among the species. Tothill (1978) stated that “most pasture vegetation, as opposed to natural grassland vegetation, may be considered to be in a seral nonequilibrium condition.” In other words, monocultures are unstable with time. A pasture may be in equilibrium with the environment under a certain management practice. If the pasture management procedures are altered, then the pasture begins to revert to some other form of vegetation. Hence, pasture management techniques (e.g., choice of species, date of seeding, density of seeding, fertilization, irrigation, and time and height of defoliation) are used to secure a desirable balance of grasses and legumes within pastures. There are also changes in plant community assemblages associated with seasonal climatic variables and longer-term successional changes.

2.2.2.1 Competition in a mixed species pasture

In most cases, plant-plant competition always has a stronger and weaker competitor. This is because the ability of plants to acquire resources depends on the size of the individual plant, leaf number, and root distribution, etc. (Du, *et al.*, 2004). Resource competition between plants has long been considered to exert pressure on plants and is an important determinant of the distribution and evolution of species. The main competition resources among plants are light, water and nutrition. Other factors, such as responses to temperature and $CO_{2,}$ also play a role (Craine, 2013).

Competition between plants takes place both aboveground and underground (Figure 2.2). Aboveground competition is mainly based on the competition for light, while underground competition is mainly for water and nutrients. In wet and rainy areas, light competition may become the main factor that plants compete for, conversely, water will become a limited resource for plants in arid and semi-arid areas. Many other factors including the spatial distribution of plants, association with fungi, the emergence time of seedlings and the habits of herbivores can affect competition for resources (Li and Watkinson, 1998).

2.2.2.1.1 Competition for light

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Figure 2.2 The nature of plants affects the ability to compete underground (From Casper and Jackson, 1997)

Light is one of the main competing resources among plants. When plants are large enough to shade other plants, or when one leaf shades another leaf, competition for light begins and there is an indirect influence on root development (Donald, 1963 and Weiner, 1986). Chen (1993) stated that the growth of seedlings, when the height of the grasses (*Psathyrostachys juncea, Elytrigia intermedia* and *Bromus inermis*) exceeds the legume species (*Medicago sativa* and *Onobrychis viciaefolia*), the shading effect inhibits the development of the roots of the leguminous plants. This, then, reduces their water and fertilizer absorptive capacity and ability to fix nitrogen. When water and nutrients are non-limiting, light becomes a major competitive factor among plants (Donald, 1951; Blackman and Black, 1959). In agriculture, such a situation becomes increasingly common because of the application of fertilizer (Donald, 1963). In the case of legume-grass mixtures, when soil nutrients are insufficient, especially when the nitrogen content is low, the growth of grasses is restricted. Legumes then require less nitrogen in the soil, so they grow faster than grasses; competition for light by legumes is then stronger (Chen, 1993).

2.2.2.1.2 Competition for water and nutrients

As the main organ for nutrient absorption, roots have become the main target of competitive relationships research. Root competition has been found to have a far more severe effect on plant size than aboveground competition (Weiner, 1986 and Cai, *et al*., 2000). Most competition occurs underground among plants, and it often reduces plant performance more than aboveground competition (Casper and Jackson, 1997). The water competition of plants is based on the ability to occupy the soil space, which depends on several root characteristics, including their biomass, relative growth rate, fine root density, depth of the roots and their total surface area (Casper and Jackson, 1997; Moreno, 2005). But the ability to occupy soil resources is not necessarily related to competitiveness. For example, plants increase their ability to uptake water by growing deeper root systems. Doing so may even reduce competition for water from other crops (Casper and Jackson, 1997).

Soil nutrients reach the root surface through three general processes: root interception, mass flow of water and nutrients, and diffusion (Marschner, 1995). Factors related to the physiological characteristics of roots, such as root activity, water potential and osmosis content, determine their competitiveness (Xie, *et al.*, 2013).

2.2.2.1.3 Other competition

Theophrastus and, later, Pliny recognized the "interference" between plants and pointed out their importance in agriculture (Friedman, 1985). The importance of plant-produced chemicals in plant-plant interactions was recognized by Molisch (Narwal and Jain, 1994). Rice (1984) introduced the term and definition of allelopathy; he stated that individuals may communicate by allelopathic means when they live together in communities of one or more species. Unlike resource competition, this involves the release of chemical compounds that adversely affect the growth of another plant in the same environment (Kohli, 1997).

White clover has been proven to have allelopathic effects (Grant and Sallans, 1964; Scott, 1975; Macfarlane, *et al*., 1982). Li, *et al.* (2008), and Liang (2011) and Liu, *et al.* (2015) reported that white clover can inhibit the growth of grasses by releasing allelochemicals, thereby forming single colonies of clover or invading grass lawns. Liu *et al.* (2013) showed that the amylase activity and seedling vigour of Italian ryegrass were both significantly lowered by the aqueous extracts from legumes. Liu (2015) similarly reported that white clover has an allelopathic effect on grass, which can affect the growth of grass by leaching and releasing sensitizing substances into rainwater. In other his studies, different concentrations of aqueous extracts from white clover have shown the dual effects of low concentration promote the germination rates, germination potential and seedling heights of *Poa pratensis*, *Festuca elata* and *Lolium perenne* and high concentration showed the inhibition on these. The allelopathic effect of white clover not only affects the associated plants, but also affects its own seed germination; it has self-toxicity (Liu, 2007).

2.2.2.2 Advantage of mixed species pasture

Mixed assemblages of different species of forbs and grasses can provide advantages that are mainly reflected in four factors: i) they make full use of space, increasing the utilization of light energy and land-use; for example, Tang (2018) found the net photosynthesis of grass species *Bromus inermis* and

Leymus chinensis were significantly improved when mixed with *Medicago sativa*, and this also lessened losses caused by weeds, pests, diseases and other environmental factors (Corre-Hellou, *et al*., 2011); ii) they increase the crude protein content of forage grasses compared to mono cultivation (Bork, *et al*., 2017). Mix pastures could also be used on dairy farms to help improve the nitrogen use efficiency of stock and also reduce nitrate leaching and nitrous oxide emissions (Woodward, 2013); iii) mixtures of stalked forage grass and upright grasses in pasture can prevent lodging (bending over of stems), which is conducive to hay preparation and silage; and iv) they increase soil organic matter in larger amounts, which contributes to the formation of soil aggregates, so the soil retains water and maintains fertility and enhances soil fertility, thereby improving the yield and quality (Zhang, *et al*., 2018).

2.1.3 Research on the benefits of plant associations

2.1.3.1 Nitrogen

Biological nitrogen fixation is a major focus of agricultural scientists, so improved performance and use of legumes and other N-fixing plants have become important research goals. Legumes and other N-fixers can increase N availability in soils by association with symbiotic bacteria (e.g., rhizobia) that fix N from the atmosphere by developing nodules (Spehn, 2002). Within the nodule, rhizobia draw carbohydrates, mineral salts and water from the cortical cells of the legume roots for growth and reproduction. At the same time, they fix the free nitrogen in the air through nitrogen fixation and transform it into nitrogen-containing compounds that plants can use. Thus, the rhizobia and the roots have an interdependent symbiotic relationship (Fisher, 1992). In general, legumes may be complementary to non-fixed species in nitrogen use and may also interact positively with other species (Harper, 1977). Rhizobium-fixed N can be used not only by the host, but also by the adjacent plants. For example, in legume/grass intercropping systems, when fertilizer N is limited, biological nitrogen fixation has not only become a major source of nitrogen for legumes, but also a source of N for the associated grass (Fujita, 1992). Adami (2019) used clover intercropping with corn and found the corn plant height, number of grains per row, grains per ear, and the thousand-grain weight, were all increased by N the clover provided. Research showed that both white and Caucasian (*T. ambiguum*) clovers can fix 26 kg N/t clover DM in irrigated perennial ryegrass pastures in New Zealand (Widdup, *et al*., 2001).

2.1.3.1.1 Effect of species on nitrogen fixation and transfer

Nitrogen fixed by legumes and other N-fixing species not only benefits the growth of these plants, but also benefits the growth of other species by releasing N to the soil through the loss from root exudates and decay of plant organs. Ta and Faris (1987) stated that the greatest advantage from grass-legume mixtures, with respect to the utilization of N released from the legume, and showed N mobility and transfer in species mixtures and found that N-fixation and N transfer by lucerne rated highest, red clover intermediate, and birdsfoot trefoil lowest. Meanwhile, lucerne and clover released more N to soil through root exudates than birdsfoot trefoil (Table 2.1).

Table 2.1 N-fixation, transfer and releasing ability of three legumes (Ta and Faris, 1987)

N fixation and transfer	Lucerne > Clover > Birdsfoot trefoil
N releasing	Lucerne ≈ Clover > Birdsfoot trefoil

To investigate biological nitrogen fixation, nitrogen transfer and the contribution of nitrogen derived from air to nitrogen yields in a semi-arid area of China, Wang, *et al*. (2010) used four legumes mixed with a grass, *Leymus chinensis*. They analysed the ratio of biological nitrogen fixation, fertilizer nitrogen and soil nitrogen in the mono and mixed sowing conditions of *Leymus chinensis* and legumes to calculate the contribution rate of biological nitrogen fixation on grassland nitrogen production. The results showed that biological nitrogen fixation of leguminous forage in a mixed grassland: *M. sativa* (47.82kg N/ha/yr)> *A. adsurgens* (20.42 kg N/ha/yr)> *M. ruthenica* (12.16 kg N/ha/yr)> *L. daurica* (11.98 kg N/ha/yr); The nitrogen transferred from leguminous grass to *Leymus chinensis* in mixed grassland was: *A. adsurgens* (11.79 kg N/ha/yr, 58%)> *L. daurica* (8.15 kg N/ha/yr, 68%)> *M. ruthenica* (5.47 kg N/ha/yr, 45%)> *M. sativa* (2.37 kg N/ha/yr, 5%). Clearly there are large differences in N retention and loss between different legumes.

2.1.3.1.2 Effect of species ratios on nitrogen fixation

Population density is one of the most important factors affecting the competitiveness of plant species (Watkinson, 1980). The maximum interaction between plants depends on the proportions of the combined species planted rather than the species themselves (Nyfeler, 2011; Suter, 2015).

Li (2015) showed that both grass-legume combinations, and grass-legume combinations in different ratios, have significantly different effects on soil NH_4^+-N and total N depending on the relative abundance of legumes. The grass-legume ratio (GLR) of 1:1 produced the most positive effects on soil N, which is considered to be the best ratio in mixed communities. Among four legume species (*Medicago ruthenica, Lespedeza daurica, Medicago falcate, Medicago sativa*), lucerne (*Medicago sativa*) had the greatest economic and ecological value from the application of N on mixed pasture.

Nyfeler (2011) also found that the N availability was able to be varied by manipulating the legume percentages in the sward and N fertilizer applications. The largest benefit of mixing grasses and legumes in terms of biomass yield was achieved at low to moderate levels of N fertilizer (50 kg^{-1} ha^{-1} $year^{-1}$ and 150 $kg^{-1}ha^{-1}year^{-1}$) with 40–60% legumes (white clover and red clover) in the swards. His study also showed that the positive effects on biomass production do not rely solely on the direct effect of symbiotic N_2 fixation, as it is the result of other interactions between the legume and the grass due to better N transformation and N availability.

In Europe, Suter (2015) found that across sites and years, mixtures with a one third of legumes (white clover and red clover) gained about 95% of the maximum total of N acquired by any stand and had 57% higher total N than grass (*Lolium perenne, Phleum pratense, Dactylis glomerata, Poa pratensis*) monocultures.

In the *Achnatherum inebrians* and *Medicago sativa* competitive relationships, under the conditions of constant density, the relative yield of *Achnatherum inebrians* decreased with the increase in its proportion, and the yield of *Medicago sativa* then also increased. This indicated that the inhibition ability of *Medicago sativa* increased with the increased proportion of planting. In the long term, *Achnatherum inebrians* may be replaced by *Medicago sativa* (Huang, *et al.*, 2012).

2.1.3.1.3 Effects of some nutrients on rhizobium and nitrogen fixation

Nutrient availability is one of the key abiotic factors limiting nitrogen fixation in agricultural systems. Mineral nutrients influence $N_{2,}$ both in legumes and non-legumes at various levels of symbiotic interactions (Table 2.2). Trace elements and phosphorus play an important role for rhizobium symbiosis. Each essential nutrient has specific physiological and biochemical effect, and the nutrient concentrations required for legumes and rhizobia are extremely low, so

The rate of metabolism is not limited (Weisany, 2013). The following are examples of the results of several elements on rhizobia or nitrogen fixation.

Table 2.2 Chemical elements essential for legume rhizobium symbiosis (Weisany, 2013)

Macro-elements	Carbon (C), Hydrogen (H), Oxygen (O), Nitrogen (N), Phosphorus (P), Potassium (K) Sulphur (S), Calcium (Ca)
Trace elements	Magnesium (Mg), Iron (Fe), Manganese (Mn), Copper (Cu), Zinc (Zn), Molybdenum (Mo), Boron (B), Chlorine (Cl), Nickel (Ni), tungsten (W) and Cobalt (Co)

Phosphorus

Many nitrogen-fixing plants have a strong dependence on mycorrhizae, which may be related to their value in enhancing the availability of P. Phosphorus is a synthetic raw material for ATP, an enzyme required for nitrogen-fixing bacteria to catalyse reactions. Mycorrhizae increase the contact points between plants and the soil through the formation of extracellular hyphae that enlarge the absorption area, shortening the diffusion distance of P, and increasing the absorption of soil P by plants (Zhao, 2000). This is a mutually beneficial process, as the rhizosphere provides a supply of organic C.

Sulphur

DeBoer and Duke (1982) showed that a sulphur (S) deficiency may be due to lower N_2-fixation. Based on this result, Zhao (1999) set up a pot experiment with peas in S-deficient soil to investigate the N-fixation ability influenced by S. The addition of sulphur (100 mg) increased the concentration of N in foliage and stems, and their total content in shoots of peas. It also doubled the amount of N fixed at all growth stages, which means S plays a vital role in N-fixation.

Other elements

Acidic soil is an important factor in inhibiting nodulation of rhizobia. Lime (Ca) applied in acid soil can increase nodulation and N_2 fixation has been shown by Buerkert, *et al.* (1990). Nitrogen fixation, as measured by the acetylene reduction assay, increased with the application of copper to the soil. Both total and protein nitrogen were increased by the application of cooper (Snowball, *et al*., 1980). Brenchley and Thornton (1925) reported that a boron (B) deficiency affected the vascular development in *Vicia faba*, creating ineffective N-fixing nodules. On this basis, Bolanos (1994) set up an experiment with peas (*Pisum sativum*) that showed boron is a requirement for nodule development and N_2 fixation. Ahmed and Evans (1959), and Reisenauer (1956), established that cobalt (Co) is essential for soybean and lucerne for the utilization of nitrate nitrogen in rhizobia. It is now clear that all nitrogen-fixing organisms need cobalt (Ahmed and Evans, 1960; Lowe *et al*., 1960; Taha and Elrefai, 1962; Evans and Kliewer, 1964; Koleshko, 1972). Rhizobia convert nitrogen in the air into NO_3^- (nitrate) by a biochemical process, through the action of nitrogenase; and nitrogenase is a molybdenum-(Mo)$^-$ dependent enzyme composed of two proteins: one containing iron, called ferritin, and the other containing iron and molybdenum MO_3^+, called MoFe. MoFe protein plays a catalytic and complexing role in nitrogenase catalysing nitrogen and other substrates. (Seefeldt, 2009; Bullen and LeComte, 1972). As a main component of nitrogenase, Mo is an essential element in plant nitrogen fixation. Su (1990) confirmed, by a pot experiment, that by soaking *Robinia pseudoacacia* seeds by molybdenum, cobalt and tungsten (W) solutions, these elements can improve the stem dry weight, dry weight of nodule and the activity of N-fixation. The mixing of the three elements was more obvious than in the separate treatment. Thus, phosphorus and a number of trace elements, including B, Co, Mo, Fe and W, are required for nitrogen fixation.

2.1.3.2 Phosphorus

The application of phosphate fertilizer is a very important factor for newly cultivated leguminous grass in the first year, especially on poor soil (Harris, 1961). Mulder (1954) found that the soil phosphorus content required for the growth and development of legumes was higher than for grasses and cereals. Studies have shown that under the condition of supplying organic phosphorus, legumes (chickpeas and broad beans) have stronger ability to utilize organic phosphorus than corn (Li, 2004).

Li (2000) set up an experiment to investigate the facilitation of P uptake by wheat from soybeans using wheat-soybean intercropping and found that the increase of phosphorus uptake in soybeans was caused by a rhizosphere effect due to the wheat, which was at the expense of a decrease in phosphorus uptake in wheat. However, the decrease of phosphorus uptake did not lead to a corresponding decrease in wheat yield. At the same time, the root distribution of soybeans in this experiment was biased in growth towards wheat. This study confirmed that wheat had a positive effect on soybeans. Li (2007) also studied fava bean-maize intercropping and found that maize overyielding resulted from its uptake of phosphorus mobilized by the acidification of the rhizosphere bean root release of organic acids and protons by the fava beans. Slightly different from Li's results, the effectiveness of phosphorus in the roots of cereals measured in an experiment by Betencourt *et al.* (2012) was also significantly increased (cereals, through rhizosphere alkalization, may also enhance P uptake and growth of the intercropping legume). Thus, facilitation can occur both ways, from cereal to legume and from legume to cereal.

2.1.3.3 Mycorrhizae and cluster roots

About 80%of terrestrial plants, including the majority of agricultural, horticultural and hardwood crop species, can establish a mutualistic association with arbuscular mycorrhizae (AMF) (Pozo and Azcón-Aguilar, 2007). AMF rely on their host plants to proliferate and survive. This symbiotic relationship can help AMF to obtain carbohydrates, such as glucose and sucrose, more directly (Harrison, 2005). In clay soils or extreme pH soils, nutrients are generally difficult to acquire, but AMF enhances the provision of nutrients to plants, particularly P and Fe (Li, *et al.*, 2006).

Under hostile environmental conditions such as nutrient-deficient soils, some native species may rely on mycorrhizas while other species may produce specialized roots, such as cluster roots (Smith and Read, 2010; Lamont, 1984). Instead of mycorrhizae, cluster roots species rely on their cluster roots to extract nutrients (e.g., P) from infertile soils (Figure 2.3) (Boulet and Lambers, 2005). Cluster roots are also known as proteoid roots; Purnell (1960) proposed the term proteoid root to describe the dense proliferation of rootlets of limited growth arising from the lateral roots of many members of the Proteaceae, and some members of the Casuarinaceae, Fabaceae and Myrocaceae families, are also found in some crop species (e.g., *Lupinus albus, Macadamia integrifolia and Cucurbita pepo*). It may account for 40 – 60% of the total root biomass (Shane and Lambers, 2005; Lambers, *et al*., 2006).

The availability of phosphorus in soil is the most important determinant of cluster root formation, although, N, K and Fe deficiency also enhance cluster root development (Shane and Lambers, 2005). In cluster root - forming species, carboxylates exuded from roots promote P mobilization in the soil (Gardner, *et al*., 1982). The acquisition of phosphorus by roots can be enhanced by increasing the surface area of the root system or the rate at which phosphorus reaches the root surface (Gardner, *et al*., 1983). Cluster roots species have a larger root surface area than other species. They mobilize mineral P that is bound to metal cations, such as Fe, Al, and Ca, extract P from the organic layers in soil, obtain Fe and Mn from alkaline soils, and preferentially take up organic forms of N (Dinkelaker, *et al*., 1995), all of which help plants grow in nutrient-poor soils. The advantage of the functions of cluster roots are greatly increased root surface areas to promote nutrient absorption, rhizosphere acidification, strong interception ability, improved utilization efficiency of organic phosphorus with the secretion of acid phosphatase and the release of phenols and viscose materials to bind the roots to soil particles for nutrient absorption (Dinkelaker, *et al.,* 1995 and Neumann 2000).

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Figure 2.3 Acquisition of phosphorus by roots (from Lambers, *et al.,* 2006)

Pot experiments have shown that cluster root production in white lupin (*Lupinus albus*) is stimulated by both low shoot P and low soil P concentrations (Shane *et al*., 2003a). Shoot P concentrations have a dominant impact on controlling cluster root initiation and growth (Shane *et al*., 2003b). Such phosphorus-mobilizing crop species improve P nutrition for themselves and neighbouring non-P-mobilizing species by releasing acid phosphatases, protons and/or carboxylates into the rhizosphere, which increases the concentration of soluble, inorganic P in soil (Li, *et al*., 2014). Muler (2014) also concludes that the cluster roots of *B. attenuate* facilitate the acquisition of nutrients by neighbouring shrubs through making P and Mn more available for their neighbours.

In addition to low phosphorus conditions, experiments have also shown that in poor soils, such as low nitrogen (Crocker, 1993), low iron (Waters and Blevins, 2000) and low manganese (Muler, 2014), can also stimulate the formation of cluster roots.

2.1.3.4 Transgressive overyielding

Transgressive overyielding and improvement of growth in mixed-species crops have been much discussed (Forrester, 2014). Transgressive overyielding means that when growing two or more species together there is a higher yield than is achieved by growing just a single species on the same area of land. It was found that although the so-called relative yield total (RYT) was often >1, consistent with some form of ‘complementarity’, instead, the ‘transgressive overyield’ of these species decreased with increasing species richness (Hector, 2002). Hooper and Dukes (2004) also summarized the states: “While functional differences among species led to strong complementarity and facilitation, these effects were not sufficient to cause significant transgressive overyielding or consistent increases in productivity with increased plant diversity. These results suggest that greater absolute production with greater diversity may be restricted to particular species combinations or environmental conditions.” Relative yield totals (RYT) are one of the most common metrics for assessing overyielding. RYTs measure overyielding by summing the relative yields (RY) for all species in a mixture. Transgressive overyielding is the promotion of the mixture to increase the yield of the crop, and decrease disease and pest attacks (Hauggaard-Nielsen, 2008). However, when the resource is limited, some species may have a negative influence on other species (Vandermeer, 1992). Whether positive or negative, the interaction between species is complex and influenced by many factors, such as nutrient availability, mixed density, and the relative proportions of the individual species (Connolly, 1990).

2.1.3.5 Benefits of intercropping to ecological services

In cropland, growing mixtures of routine species instead of single crops can provide better ecosystem services, such as weed control, soil erosion protection and the improvement of soil organic matter (Wendling, *et al.*, 2017). In forestry systems, mixed forests might provide better multifunctionality, e.g., higher resilience against biotic and abiotic damage (Sterba, *et al.*, 2014). Productivity has been shown to increase with the richness of species in experimental grassland communities (Schmid, 2008; Hector, 1999; Hooper and Dukes, 2004). The best examples are the well-known grass-legume mixtures. Nyfeler (2011) proved that grass-legume mixtures can yield more nitrogen than pure stands of legumes due to mutual stimulation of nitrogen uptake from symbiotic and non-symbiotic sources.

Intercropped perennial legumes can provide long-term, heterogeneous and stable habitats for insects, herbivores, predators, and microbial populations. Moreover, they contribute to the maintenance of a stable and diverse food web structure, facilitating ecosystem services (Culman, *et al*., 2010; Ratnadass, *et al*., 2012).

In root box experiments (Chaiqiang, 2005), the rhizosphere effect of intercropping on actinomycetes and the total number of microorganisms was significantly higher than that in a monoculture, and the rhizosphere microbial diversity index was significantly higher than that in the monoculture.

In agroecosystems, inclusion of trees in pasture environments improves soil quality and microbial activity over conventional systems because of the shading effect (Vallejo, 2012). The use of intercropping can provide a similar effect as from shading to increase the population of total bacteria of the soil (Menezes, 2019). The results showed that at higher levels of shading (black-nylon mesh with radiation interceptions of 55%), multivariate analysis revealed the introduction of the legume (*Clitoria ternatea* L.) intercropped with buffelgrass (*Cenchrus ciliaris* L.) pasture promoted an increase in microbial activity (respiration and microbial biomass carbon in the soil, and the activity of the acid phosphatase and β-glucosidase enzymes) (Menezes, 2019).

2.1.4 Conclusion

Competition for resources has been more extensively studied than the mutual benefits that may be derived from different plant species growing together (Craine and Dybzinski 2013; Trinder, *et al.,* 2021). Beneficial interrelationships between plants appear to have been neglected. At the beginning of this study, I observed situations where clovers were actively growing towards native tussock grasses in gardens and other habitats, raising my curiosity that the clover may be foraging for a resource within the rhizosphere of the grasses. Legumes and grasses are also naturally found together, but this is generally understood to be mainly related to the provision of spillover N from the clover to the grass.

Other possible reasons include different exploitation of different heights of the canopy in different parts of the growth season. However, the present study led me to question whether there also are any reciprocal nutritional benefits to the clover of growing together with the grass. My hypothesis was that this may be because the roots of the grasses activate or release certain trace elements, for example from organic acid root exudates that mobilize key nutrients, causing the stolons and roots of the clover to extend towards the grass. My aim was to investigate whether grasses provide benefits to the legumes in terms of nutrition. The objectives and experimental work were formulated to evaluate this hypothesis.

2.3 General Methods

This study involved a series of glasshouse experiments, an environmentally-controlled growth chamber study, a field sampling exercise and a range of additional studies. An introduction to methodology and general techniques is included in this section, with detailed methods and protocols provided in each experimental chapter.

Glasshouse experiments

The first objective (to test in this research is whether there are benefits to nitrogen-fixing plants when grown in combination with grasses, rather than vice versa) was started from 15th March 2019 and practical work was completed by December 2019 at a nursery located at the Lincoln University campus. All the plants are growing from seeds and have been transplanted into the pots, and the seeds are provided from Specialty Seeds Ltd. Soil (depth 1-20 cm) was collected from a site near Lake Lyndon in Canterbury (altitude 611 m), grown primarily by hardy shrubs and grasses with no known history of fertilization. The plants were watered daily to maintain field capacity. Glasshouse temperatures for the duration of the experiment were from range 14 - 32°C.

Plate 2.1 Legume-grass pot experiment set up in Lincoln University Glasshouse

The second objective (to investigate transgressive overyielding and phosphorus biogeochemistry, using plants with cluster roots in combination with grasses) was started from December 2019 to December 2020 at Lincoln University nursery. Protea transplants were provided from a commercial company and grasses were growing from the seeds. The plants were watered daily to maintain field capacity. The experimental soil that contains low nutrients was also collected from Lake Lyndon. Glasshouse temperatures for the duration of the experiment were from 13.6 – 34.7°C. Plants were watered sparingly every two days.

Plate 2.2 Cluster roots forming specie-grass pot experiment set up in Lincoln University Glasshouse.

Growth Chamber Experiment

The third objective (to investigate whether bidirectional complementarity also exists in terms of exploitation of the soil nutrient pool) was started in Sept 2020, the soil cores were collected from Mt Grand station and transplanted into the plastic pots and then cultivated at the Growth Chamber. The temperature was set as near as possible to the current day / night temperatures, sunlight hours and light intensity recorded at the same time of year at Mt Grand. The experiment was completed in Jan 2022.

Plate 2.3 Transplant soil core from Mt Grand set up in Lincoln University Growth Chamber.

Field Sampling

The final objective (to investigate whether coexistence of species provides an opportunity to facilitate enhanced uptake and improved procurement of key soil nutrients by assemblages of plants in these mid-altitude grasslands) was started in Sept 2021 and the plant leaf samples were collected from Mt Grand station. This trip involved three locations at Mt Grand.

Plate 2.4 Field sampling at Mt Grand station.

Additional studies

Rhizoboxes were applied for various combinations observing root system activity from April 2020 to July 2020, September 2020 to February 2021, March 2021 to July, December 2021 to July 2022 in the glasshouse of the nursery at Lincoln University. The black plastic covers are used on each side of the rihzobox glasses to prevent sun damage of the roots. The plants in rhizobox were watered daily to field capacity.

Sampling and analysis

A list of equipment used in sampling was shown as below:

Soil core sampler, the core tube contains both plants and soil (15cm depth from the ground), that is removed from ground and then transplanted into the plastic pot.

Soil auger (20cm depth) was used for soil sample collection.

pH meter, soil pH was measured in every core using a surface probe attached to this pH meter surface pH.

Soil pore water rhizon samplers were positioned diagonally at approximately 20^{o}C to sample pore water between depths of 5 - 10 cm. Soil pore water was collected after one month.

Chemical Analysis

ICP-OES (Inductively Coupled Plasma Optical Emission Spectrophotometer), plants or soil samples were dried (65 oC, 48 hrs), weighed and finely ground, prior to microwave digested using 5M nitric acid and chemical analysis using (Varian 5110) ICP-OES by Lincoln University Analytical Services.

ICP-MS (ICP-MS Inductively coupled plasma-mass spectrometry), elemental concentrations in the plant digests were analysed by ICP-MS (7500cx, Agilent Technologies) at Canterbury University.

Total Nitrogen, was analysed in plant and soil material using an Elementar Rapid Max N Elemental Analyser. The sample was combusted at 900°C in an oxygen atmosphere. The combustion process converts any elemental nitrogen into N_2 and NOx. The NOx species are subsequently reduced to N_2. These gases are then passed through a TC (thermal conductivity) cell to determine N_2 concentrations and the %N is calculated from the sample weights. Lincoln University Analytical Services provided this technical support.

Other soil analysis: R J Hill Laboratories Limited provided analysis of the soil samples used in the research. The summary of methodology is listed as follow:

Table 2.3 Soil property data

Test	Method Description
pH	1:2(v/v)soil:water slurry followed by potentiometric determination of pH. In-house
Olsen Phosphorus	Olsen extraction followed by Molybdenum Blue colorimetry. In-house method.
Sulphate Sulphur	0.02M Potasslum phosphate extraction followed by Ion Chromatography. In-house
Potassium	1M Neutral amonium acetate extraction followed by ICP-OES.
Calcium	1M Neutral amonium acetate extraction followed by ICP-OES.
Magnesium	1M Neutral amonium acetate extraction followed by ICP-OES.
Sodium	1M Neutral amonium acetate extraction followed by ICP-OES.
Organic Matter	Organic Matter is 1.72 x Total Carbon
Total Carbon	Determined by NIR, calibration based on Total Carbon by Dumas combustion
Total Nitrogen	Determined by NIR, calibration based on Total N by Dumas combustion
Phosphorus (Mehlich 3)	Mehlich 3 Extraction followed by ICP-OES
Sulphur (Mehlich 3)	Mehlich 3 Extraction followed by ICP-OES
Potassium (Mehlich 3)	Mehlich 3 Extraction followed by ICP-OES
Calcium (Mehlich 3)	Mehlich 3 Extraction followed by ICP-OES
Magnesium (Mehlich 3)	Mehlich 3 Extraction followed by ICP-OES
Sodium (Mehlich 3)	Mehlich 3 Extraction followed by ICP-OES
Iron (Mehlich 3)	Mehlich 3 Extraction followed by ICP-OES
Manganese (Mehlich 3)	Mehlich 3 Extraction followed by ICP-OES
Zinc (Mehlich 3)	Mehlich 3 Extraction followed by ICP-OES
Copper (Mehlich 3)	Mehlich 3 Extraction followed by ICP-OES
Boron (Mehlich 3)	Mehlich 3 Extraction followed by ICP-OES

Chapter 3

Legume nutrition is improved by neighbouring grasses

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REGULAR ARTICLE

Legume nutrition is improved by neighbouring grasses

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3.1 Abstract

Background Combinations of grasses and nitrogen-fixing legumes are ubiquitous in most natural and derived pastoral grasslands. This was not formerly the case in New Zealand's unique indigenous grasslands that are now frequently impacted by exotic pasture grasses and legumes. Understanding the co-existence of native and exotic plants is the broad focus of this research.

Aims Spillover of nitrogen (N) from clovers to grasses in diverse pasture is well known. We question whether grasses provide reciprocal nutritional benefits to legumes. Does the mutual exploitation of soil biogeochemistry by legumes and grasses help to explain their coexistence and, if so, does this have implications for biodiversity in NZ's novel native grassland communities?

Methods Combinations of grasses and legumes, including a native tussock grass, were grown in a nutrient-poor (low P, S, Ca, Mg, Mn, and B) high country soil in a pot experiment, quantifying the foliar acquisition of nutrients from soil. Field data were obtained by sampling foliage of clover in single- and mixed-species patches in a more fertile lowland pasture.

Results Benefits of legume and grass growing together were reflected in enhanced productivity and higher uptake of a range of key nutrients. This was most evident but not restricted to a combination of two exotic species: cocksfoot and white clover. In the nutrient-poor soil, legumes grew better in combination with different species of introduced grasses. Uptake of key elements from soil to plants significantly differed with combinations of legumes and grasses compared to individual species.

Elevated concentrations of P, K and S were recorded in clover when growing with grasses, although Ca uptake was lower. Expected reciprocal reduction of clover N or enhanced grass N were not recorded. Mass balance data (total extraction of key nutrients from the soil pool) showed that combination of grasses and legumes exploit soil nutrients (particularly P, Zn, Mn and Mo) more effectively than single species alone. In grasses, only tissue concentrations of K, S and Zn significantly increased when growing with legumes, but native tussock grass procured less nutrients when growing with the exotic legumes. Field sampling of clover from the more fertile lowland soil showed significantly higher foliar concentrations of K, Mn, Cu and B, but less Ca.

Conclusions The findings are indicative of a mutualistic relationship: legumes derive nutritional benefits from growing with grasses. Native tussock grass contained less N when growing with the exotic legumes, suggesting less compatibility and a lack of adaptation to coexistence.

3.2 Introduction

Coexistence and maintenance of diversity in plant communities are explained as a result of different species exploiting resources that are spatially and temporally variable between and within habitats (Burrows, 1990; Høgh-Jensen and Schjoerring 2001; Homulle, *et al*., 2021). Competition for resources is much more extensively studied than the mutual benefits that may be derived for different plant species growing together (Craine and Dybzinski, 2013; Trinder, *et al*., 2021). However, complementarity between species can be highly significant; agricultural intercropping of two or more species is one obvious and demonstrable example (Li, *et al*., 2014; Gliessman, 2015). This has been explained in terms of the abilities of each species to exploit different niches in the above-ground environment and root zone (Zhang et al. 2015). In terms of plant nutrition, the best-known example of complementarity is symbiotic nitrogen fixation in legumes that provides incidental spillover of nitrogen to soil, for example from clovers to grasses in mixed-species grasslands (Fornara and Tilman, 2008; Pirhofer-Walzl, 2012). A greater total herbage yield is obtained by the two functional types growing together rather than individually (Schipanski and Drinkwater, 2012; Sturludóttir, *et al*., 2014); this also balances palatability and improves nutritional benefits to stock. In this example, and in view of widespread and naturally occurring grass-legume associations, we question whether this relationship and its association with soil fertility operates only in one direction, or if grasses provide other elements to clovers as reciprocal nutrition. Our hypothesis is that spillover of N from clovers is an intentional strategy to procure limiting nutrients from neighbouring grasses. This is based on an assumption that there would be no selective advantage to legumes of fixing N in excess of their own requirements. Conversely, there would be a clear advantage to be able to acquire limiting soil nutrients without direct metabolic cost (Lynch, *et al*., 2021).

This topic has contemporary interest in New Zealand where unique indigenous grasslands existed largely without herbaceous legumes and entirely without grazing mammals, thus providing a near unique location to study this particular type of plant functional complementarity. Pastoral farming systems first arrived with European settlers in the 19th Century, and clovers were introduced less than 100 years ago (Caradus, 1996). Indigenous tussock grasslands currently account for about 13% of land cover, with a further 34% of land converted to sheep, beef and dairy pasture that is largely exotic (Stats^{NZ} 2021). Native grassland plant communities that are associated particularly with higher altitude (High Country) habitats now co-exist with grazing mammals and invasive plants species, and they are frequently impacted by nutrient top-dressing and over-sowing of exotic pasture grasses and legumes. This means they now exist as novel native grassland communities (*idem* Wilsey, *et al*., 2018). Pasture containing exotic fodder species is far more responsive to enhanced fertility. Exotic legumes (including *Trifolium* spp. and *Lotus* spp.) have become widely naturalised, including in high country native tussock grasslands, potentially threatening more than one-third of the country's native plant species, of which 93% are endemic in the High Country (Mark and Galloway, 2012). Herbaceous legumes are virtually absent from native flora and this family is restricted to only a relatively small number of species: 34 species that are mostly trees and shrubs, compared to at least 113 species of fully naturalized exotic legumes (Wardle, 1991; Allen and Lee, 2006; Dollery, *et al*., 2019).

Graminaceous species are known to release phytosiderophores, which promote absorption of Fe and Zn (Erenoglu, *et al*., 2000; Banakar, *et al*., 2017). There are only a few examples where this has been shown to benefit adjacent species; for example, both Fe and Zn acquisition are increased in peanuts when they are grown with maize (Inal, *et al*., 2007; Zuo and Zhang 2008; Xiong, *et al*., 2013) and in guava growing with grasses (Kamal, *et al*., 2000). However, there appear to be few insights into whether legumes similarly receive nutritional benefits from grasses in grassland pastures.

The aim of the present study was simply to investigate whether native and exotic grasses provide nutritional benefits to invasive legumes, rather than this relationship being a one-way provision of nitrogen to grasses. This is investigated using glasshouse experiments and field sampling.

3.3 Materials and methods

3.3.1 Plants and soils

The legumes selected for this study were white clover (*Trifolium repens* L.) and birdsfoot trefoil (*Lotus pedunculatus* Cav.), both of which have been widely seeded in New Zealand and elsewhere. White clover is the main legume of temperate pastures and meadows (Rochon, *et al*., 2004), planted in New Zealand since the late 1920s alongside several other species of annual and perennial clovers (Rattray, 2005). *Lotus* is also commonly sown on hill country in New Zealand, particularly at wetter, more acid, or more infertile sites (Armstrong 1974). Two common exotic pasture species of grasses and one native tussock grass were selected: perennial ryegrass (*Lolium perenne*), cocksfoot (*Dactylis glomerata*) and silver tussock (*Poa cita*). Ryegrass is the most productive, palatable and digestible grass species under fertile conditions and is also the most widely planted species in New Zealand but is less suited to drier locations and the high country. Cocksfoot is more hardy and drought-tolerant (Sanada et al. 2010). *Poa cita* is a common tussock grass species particularly suited to exposed, windy locations and dry soil (Hunter and Scott, 1997) and is widespread in natural grasslands and less developed grazed pasture. Ryegrass and cocksfoot were grown from seed and *Poa* tussock seedlings were collected from the Lincoln University campus where the species has been widely planted.

A previously-identified nutritionally-poor soil, vegetated largely by more hardy shrubs and grasses with no known history of fertilization, was collected (1-20 cm depth) from a site near Lake Lyndon in Canterbury (altitude 611m) described by Gutierrez-Gines, *et al*. (2019). The soil was thoroughly mixed, then air-dried and sieved (2mm). Samples were analysed using standard methods by Analytical Services, Soils and Physical Sciences Department, Lincoln University (Table 3.1), showing a range of key determinants (pH, calcium, sulphate-sulphur, soluble phosphorus, copper, magnesium, manganese) were less than optimum for plant growth. Available phosphorus, calcium and boron were extremely deficient.

In a smaller sampling exercise, white clover was also sampled from a planted lowland pasture on a more fertile soil on the university campus (Table 3.1). This was an attempt to investigate whether differences in foliar nutrient concentrations of clover differ if the plants are established in single-species patches of clover, compared to when it is growing in assemblages with other species.

Table 3.1 Physico-chemistry of the two soils

Indicators	Units	Pot Experiment	Field Soil	Typical Range*
pH [1]	pH Units	5.70	5.90	5.7-6.2
Total Nitrogen [2]	%	0.46	0.29	0.3-0.6
Total Carbon [3]	%	5.80	3.3	-
Organic Matter [4]	%	10	5.6	7-17.0
Potassium [5]	me/100g	0.49	0.91	0.3-0.6
Calcium [5]	me/100g	2.03	5.7	5-12.0
Magnesium [6]	me/100g	0.60	1.31	0.6-1.2
Sodium [6]	me/100g	0.05	0.15	0.00-0.3
Sulphate Sulphur [7]	mg/kg	6.43	2	10.0-20.0
Olsen Phosphorus [8]	mg l^{-1}	4.33	21	20-30
Iron [6]	mg l^{-1}	84	382	-

Manganese[6]	mg l^{-1}	3.40	27.4	8.0-65.0
Copper[6]	mg l^{-1}	0.37	1.3	0.4-2.0
Zinc[6]	mg l^{-1}	1.73	5.4	0.80-4.00
Boron[6]	mg l^{-1}	0.19	0.48	0.60-1.20

*Typical range for agricultural soils in New Zealand. Method: [1] 1:2 (v/v) soil:water slurry followed by potentiometric determination of pH. *[2]* Determined by NIR, calibration based on Total N by Dumas combustion. [3] Determined by NIR, calibration based on Total Carbon by Dumas combustion. [4] Organic Matter is 1.72 x Total Carbon. [5] 1M Neutral ammonium acetate extraction followed by ICP-OES. [6] Mehlich 3 Extraction followed by ICP-OES. [7] 0.02M Potassium phosphate extraction followed by Ion Chromatography. [8] Olsen extraction followed by Molybdenum Blue colorimetry.

3.3.1.1 Pot experiment

A pot experiment was located in a glasshouse at the Lincoln University Nursery. All legumes and grasses were initially propagated and established in seed trays, then transplanted, singly or in combination with each other (Figure 3.1 and figure 3.2), into (15cm diameter, 20cm height, 3.5L) plastic pots. Eleven experimental treatments consisted of three grasses and two legumes growing either singly (5 species) or in six paired combinations (Clover & Ryegrass; Clover & Cocksfoot; Clover & Tussock; *Lotus* & Ryegrass; *Lotus* & Cocksfoot; *Lotus* & Tussock). Each treatment had 5 replicates (11 x 5 = 55 pots), arranged in a randomized single block design on a glasshouse bench with natural daylight. Pots were watered each day to maintain field capacity. Mean glasshouse temperatures for the duration of the experiment were 18.2°C (range 14 - 32°C) and. After four months' growth, above-ground plant biomass was harvested, sorted into separate species, then dried (65 °C, 48 hrs), weighed and finely ground, prior to microwave digested using 5M nitric acid and chemical analysis using ICP-OES following standard methods.

Figure 3.1 Legumes and grasses were grown as either single or mixed species.

3.2.1.2 Field sampling

White clover foliage was sampled from the lowland pasture on the university campus which was a fenced enclosure containing ryegrass, cocksfoot, white clover and few other species apart from plantain (*Plantago lanceolata* L.). The site had been planted about 5 years previously on a Wakanui soil type consisting of silt/sandy alluviums that are widespread in the Canterbury lowlands on South Island (Molloy 1998). The paddock had no recent fertilizer application or grazing. Clover foliage was sampled where it was growing in patches alone, or else in patches with cocksfoot, ryegrass or multiple assemblages of species. Each sample was excised at the ground surface and consisted of approximately 20 leaves and petioles, without attached stolons. Soil contamination of the samples was carefully avoided. Bulked samples each consisting of 2 separate samples were replicated 5 times. Samples were analysed as described above.

3.2.1.3 Statistical analysis

Data not normally distributed were log-transformed before analysis. Differences between means were determined using one-way ANOVA, with a post-hoc Fisher LSD test. All analyses were conducted using Minitab 19.

3.4 Results

3.4.1 Pot experiment

Clover grew better in combination with cocksfoot, and *Lotus* grew better in combination with ryegrass (Figure 3.2). None of the grasses had significantly different yields with or without legumes; yields of grasses were in the range of 1.36 – 1.96 g pot $^{-1}$ (c.f. 4.0 – 4.9 g pot $^{-1}$ for legumes). There were significant differences in nutrient concentrations in the foliage of the two functional types (legumes, grasses) according to whether they were growing singly or in combination. In the legumes (Table 3.2), this was mostly reflected in higher concentrations of P, K and S, and lower concentrations of Ca, when growing with at least one of the three grasses. Manganese concentrations in foliage were also lower in the legumes when they were growing with cocksfoot. Nitrogen concentrations in legume foliage were not significantly different, whether or not the legumes were growing with grasses.

When grasses were growing with or without legumes (Table 3.3), there was no evidence that N concentrations increased in grass foliage, although P concentrations were frequently lower (Table 3.3). Otherwise, elemental concentrations in the foliage of the three grasses showed few consistent significant differences when growing with legumes. In ryegrass, only K and S concentrations were significantly higher when this species was growing with one of the legumes, but five of the 13 elements were in lower concentrations. In cocksfoot, only Ni and Zn were elevated when it was growing with clover. In tussock, six elements (N, P, K, Ca, B, Zn) were in lower foliage concentrations in grasses when growing together with one of the legumes.

The data were also analysed to show total uptake of each element into grasses or legumes, calculated as *biomass x element concentration* after 4 months' growth (Fig 3.3). As well as having higher tissue concentrations of P, significantly more P was taken up by clover foliage when it was growing with cockfoot or tussock, and seven other elements were taken up in larger amounts when it was growing with cocksfoot. Total uptake of both P and K into *Lotus* was higher when this legume grew with ryegrass. When growing with clover, K and S uptake into ryegrass was higher, and Zn and Ni uptake were higher in cocksfoot. In tussock, P, B and Zn uptake into cocksfoot and tussock were lower when they were growing with legumes.

An estimate of the total acquisition of nutrients from the soil pool was calculated by adding together data for all of foliage contained in each pot. Total offtake of nutrients often was not always simply explained by higher biomass yields or higher tissue concentrations, but by a combination of both (Fig. 3.4). When clover grew with cocksfoot, a 30% larger harvest of biomass corresponded to nearly 50% greater extraction of P from soil, and substantially more Mn, Zn and Mo.

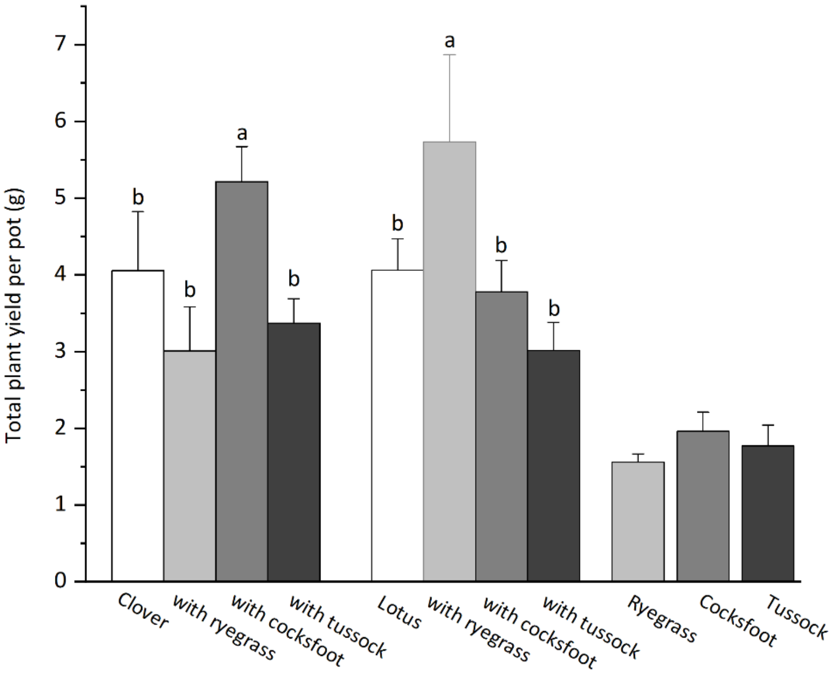

Figure 3.2 Yield (g. dry wt. pot $^{-1}$) of each species growing alone or in combination in the pot experiment after 4 months growth (Open bars show species growing alone. Shaded bars show clover and lotus yield when growing together with ryegrass (grey), cocksfoot (dark grey) or tussock (black)). Statistical differences (*, $P < 0.05$) are shown for growth of clover and Lotus grown alone or in combination.

Table 3.2 Foliar concentrations of nutrients in each legume growing alone or with different companion species of grasses. Values are means (Clover: n = 5; Lotus: n=3). Letter in superscript indicate significant differences ($P < 0.05$); bold values indicate differences to when the legumes were growing alone.

Treatments	N (%)	P	K	Ca	Mg	S	Fe	Mn	Zn	Cu	Mo	B	Ni
		mg kg $^{-1}$											
Clover growing alone	2.7 ^{a}	1023 ^{a}	13700 ^{a}	18300 ^{a}	2920 ^{a}	1630 ^{a}	1300 ^{ab}	137 ^{ab}	28.4 ^{a}	7.31 ^{a}	0.24 ^{a}	31.9 ^{ab}	2.91 ^{a}
growing with ryegrass	2.9 ^{a}	1350 ^{ab}	16100 ^{a}	15700 ^{a}	3370 ^{a}	2590 ^{a}	607 ^{a}	158 ^{a}	31.4 ^{a}	8.34 ^{a}	0.43 ^{a}	39.1 ^{a}	3.35 ^{a}
growing with cocksfoot	2.7 ^{a}	1060 ^{a}	13400 ^{a}	17600 ^{a}	2830 ^{a}	2090 ^{a}	542 ^{a}	**75.3** ^{c}	27.8 ^{a}	7.47 ^{a}	0.27 ^{a}	28.9 ^{ab}	2.53 ^{a}
growing with tussock	3.1 ^{a}	**1410** ^{b}	16900 ^{a}	16500 ^{a}	3520 ^{a}	2610 ^{a}	1750 ^{b}	87.0 ^{bc}	29.6 ^{a}	8.22 ^{a}	0.27 ^{a}	27.2 ^{b}	3.62 ^{a}
Lotus growing alone	2.4 ^{a}	660 ^{a}	8210 ^{a}	20000 ^{a}	4920 ^{a}	1220 ^{a}	316 ^{a}	136 ^{a}	53.9 ^{ab}	5.19 ^{a}	0.08	61.2 ^{a}	3.60 ^{a}
growing with ryegrass	2.4 ^{a}	663 ^{a}	**14300** ^{b}	**14200** ^{b}	**3740** ^{b}	**2990** ^{b}	407 ^{a}	103 ^{ab}	42.5 ^{ab}	**3.41** ^{b}	-	**46.0** ^{b}	2.30 ^{ab}
growing with cocksfoot	2.8 ^{a}	997 ^{b}	**16900** ^{b}	**11500** ^{b}	**3580** ^{b}	**3500** ^{b}	480 ^{a}	**92.1** ^{b}	39.1 ^{a}	4.81 ^{ab}	-	47.0 ^{ab}	**1.90** ^{b}
growing with tussock	2.9 ^{a}	874 ^{ab}	**18700** ^{b}	**12400** ^{b}	5180 ^{a}	**3590** ^{b}	318 ^{a}	133 ^{a}	58.0 ^{b}	5.04 ^{ab}	-	55.1 ^{ab}	2.61 ^{ab}

Table 3.3 Foliar concentrations of nutrients in grasses growing with and without the two legumes. Values are means ± s.e. (n = 3). Superscripts indicate significant differences ($P < 0.05$); **bold** values indicate differences to when the grasses were growing alone.

Treatments	N (%)	P	K	Ca	Mg	S	Fe	Mn	Zn	Cu	Mo	B	Ni
		mg kg^{-1}											
Ryegrass growing alone	1.5^{a}	1610^{a}	9880^{a}	9030^{a}	4100^{a}	2420^{a}	239^{a}	194^{a}	50.8^{a}	7.35^{a}	3.63^{a}	16.4^{a}	0.92^{a}
growing with clover	1.6^{a}	1330^{a}	**24900**^{b}	5770^{a}	3060^{ab}	**5640**^{b}	169^{a}	195^{a}	26.4^{a}	6.85^{ab}	1.82^{a}	10.4^{ab}	1.19^{a}
growing with lotus	1.3^{a}	**565**^{b}	**16000**^{b}	6100^{a}	**2140**^{b}	2740^{a}	366^{a}	104^{a}	31.2^{a}	**4.18**^{b}	2.7^{b}	**8.3**^{b}	0.54^{a}
Cocksfoot growing alone	1.2^{a}	1030^{a}	24800^{a}	6780^{ab}	2280^{a}	2690^{a}	254^{a}	376^{a}	24.6^{a}	4.85^{a}	2.7^{ab}	8.26^{a}	0.99^{a}
growing with clover	1.3^{a}	918^{a}	**17300**^{b}	7700^{a}	2350^{a}	2980^{a}	672^{a}	485^{a}	**68.6**^{b}	7.34^{a}	5.32^{a}	7.44^{a}	**1.27**^{b}
growing with lotus	1.1^{a}	782^{a}	17700^{ab}	5340^{b}	2770^{a}	4790^{a}	129^{a}	277^{a}	42.6^{a}	4.69^{a}	2.51^{b}	5.67^{a}	0.67^{a}
Tussock growing alone	1.1^{a}	1330^{a}	12400^{a}	3670^{a}	1350^{a}	3030^{a}	99.5^{a}	89.6^{a}	30.1^{a}	4.15^{a}	1.92^{a}	6.76^{a}	0.72^{a}
growing with clover	1.0ab	**847**^{b}	16500^{a}	2940^{ab}	1220^{a}	3790^{a}	72.2^{a}	71.6^{a}	**19.9**^{b}	4.58^{a}	1.63^{a}	**4.98**^{b}	0.51^{a}
growing with lotus	**0.7b**	**458**^{c}	**6930**^{b}	**2590**^{b}	1160^{a}	2540^{a}	111^{a}	111^{a}	**20.7**^{b}	2.71^{a}	2.35^{a}	**5.13**^{b}	0.45^{a}

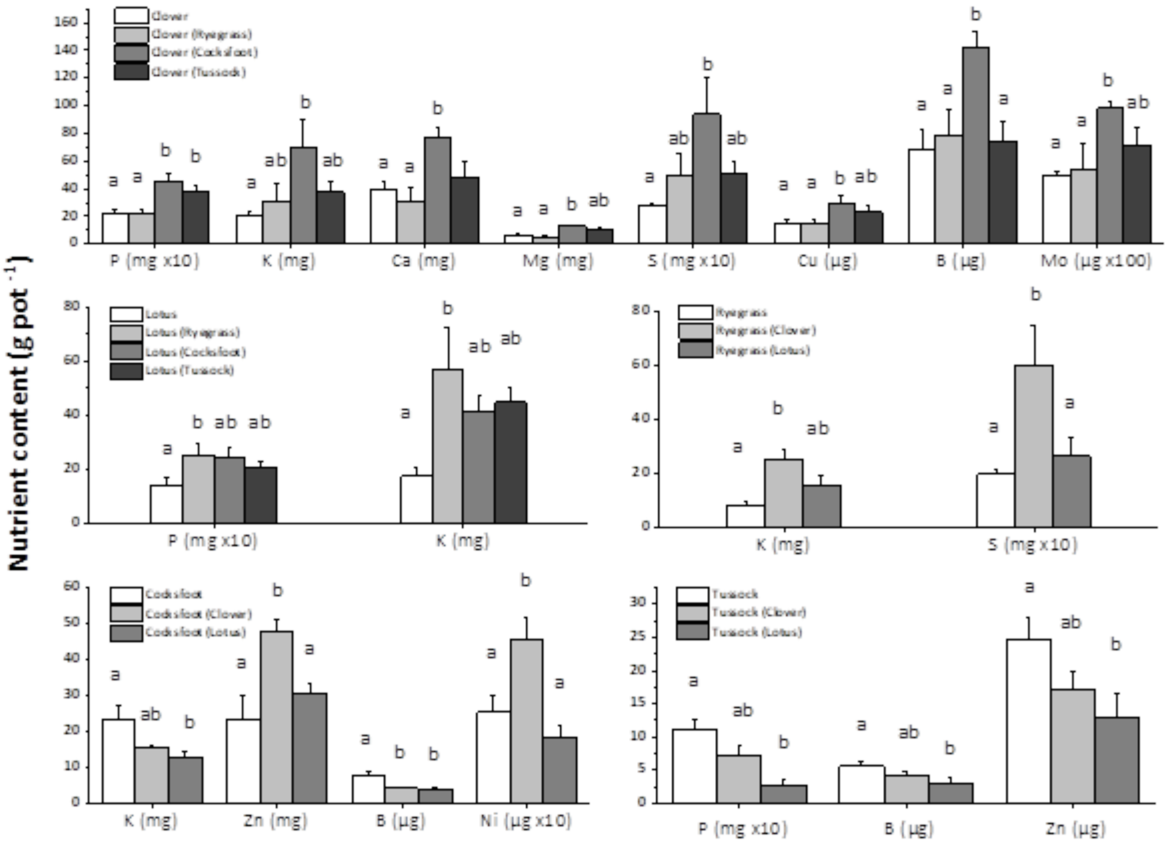

Figure 3.3 Total foliar content of selected nutrients in legumes and grasses, growing either alone or with each other (companion species are shown in the keys in brackets).

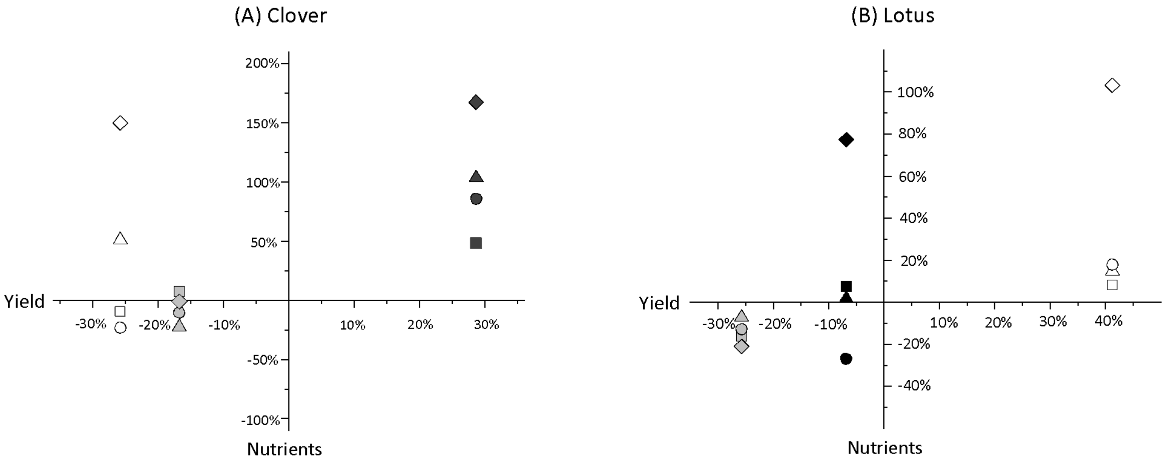

Figure 3.4 Differences in the total harvest of vegetation and nutrients from pots when clover (A) or lotus (B)

were growing with companion species. Symbols descriptions: squares = P; triangles = Mn; circles = Zn; diamonds = Mo. Open symbols are the legume with ryegrass; shaded symbols are the legume with native tussock; black symbols are the legume with cocksfoot. Percentage change is the difference to when the legumes grew alone.

3.4.2 Field sampling

When clover grew with any of the companion plant species in a field situation, K and Mn were consistently in higher concentration in its foliage (Table 3.4). In some cases, Ca, B and Cu were also higher, but foliar concentrations of P and Mo were lower in clover when growing with cocksfoot.

Table 3.4 Comparison of foliar concentrations (mg kg $^{-1}$) of nutrients in clover growing in patches by itself or with different companion plants in a mixed-species paddock

Treatments	P	K	Ca	Mg	S	Fe	Mn	Zn	Cu	Mo	B	Ni
	mg kg $^{-1}$											
growing alone	2980 ab	7660 a	7890 a	2950 a	1890 ab	342 ab	37.6 a	31.6 ab	7.30 a	0.47 a	15.81 a	1.76 a
growing with ryegrass	3380 a	**9100** **b**	9400 ^{a}	2830 a	2030 a	464 a	**66.8** **b**	38.2 a	**10.7** **b**	0.50 a	17.86 ab	2.45 a
growing with cocksfoot	**2500** **c**	**8570** **b**	**12000** b	3300 a	1790 b	279 b	**70.2** **b**	27.4 b	8.77 ab	**0.19** **b**	**18.69** **b**	1.18 a
growing with muti-plants	2510 bc	**9000** **b**	8570 ^{a}	2950 a	1690 b	363 ab	**61.1** **b**	30.1 ab	7.67 a	0.11 b	17.52 ab	2.37 a

3.5 Discussion

Legume-grass assemblages are usually more productive than either of these plant functional types in monoculture (Sleugh, 2000; Sturludóttir, *et al.*, 2014). This is an example of transgressive overyielding in which functional differences between species lead to a strong complementarity (Schmid, 2008; Hooper and Dukes 2004). It is generally understood that productivity is enhanced by better spatial and temporal capture of light in the mixed-species canopy, and of soil resources below-ground (Gliessman, 2015, Homulle, *et al.*, 2021). In particular, nitrogen fixation in legumes, spillover of this nutrient to soil and its exploitation by adjacent species has provided an explanation for overyielding in grassland pastures (Høgh-Jensen and Schjoerring, 2001, Gylfadóttir *et al.*, 2007; Scott *et al.*, 2018). Little attention appears to have been given to the possibility that grasses may also improve the nutritional status of legumes through modification of soil biogeochemistry, but the findings of the present study suggest this is the case.

Clover and *Lotus* had higher yields when growing with grasses than when growing alone. No more or less N was measured in foliage of any of the species apart from the native tussock grass that actually acquired less nitrogen when growing with legumes. Otherwise, there were no significant differences in the total foliar content of N. However, as was also indicated by the routine soil analysis, N did not appear to be an important nutrient limiting plant growth in this soil. The experimental conditions provided adequate soil moisture for growth which is likely to have favoured the growth of legumes over grasses, and there may have been little investment in root nodules and reliance on N from this source.

At least six elements (P, S, Ca, Mg, Mn, and B) were deficient in the pot experiment soil. There was evidence from the present study that all these elements, and also Mo, were extracted in higher concentrations, quantities or total amounts from the total soil pool by a combination of legumes and grasses, compared to either plant type alone. The best example was of clover growing with cocksfoot; eight elements were taken up in larger amounts into clover foliage. Field sampling of a more fertile soil similarly showed significantly higher uptake of K, Mn, Cu and B, which suggests compatibility is a real phenomenon in the field situation in grasslands.

Phosphorus, K and S concentrations in the foliage of legumes were higher in the presence of grasses. Foliar concentrations of K and S were also higher in ryegrass when it was growing with legumes. Otherwise, key elements were in lower foliar concentrations in grasses when they were growing with clovers. This was most evident in the native tussock where N, P, K, Ca, B and Zn were in lower foliage concentrations when growing with legumes. Grasses consistently extracted lesser amounts of the total soil pool of nutrients when growing alone. The native tussock grass did not benefit nutritionally from the presence of legumes, but higher P concentrations were found in tussock foliage and, as a companion plant, clover had a higher concentration of P when it was growing with tussock. It appears that tussock grass has some ability to mobilise P in its rhizosphere.

Possible mechanistic explanations for some of these findings are provided in the scientific literature. Phosphorus is a particularly critical element due to its low solubility and mobility in soil (Scott and Condron, 2003; Saleem, *et al*., 2020); P availability in the rhizosphere is increased by secretion of organic exudates and also by the release of protons, the latter particularly in acid soil (Cu, *et al*., 2005). Evidence of partner plants being complementary to one another in the context of exploiting this element has been found previously (Homulle, *et al*. 2021, Lambers, *et al*., 2021). Potassium was not deficient in the experimental soil, but it is known that legumes have a higher K absorption efficiency than grasses (Wang, *et al*., 2014), which would suggest legumes have an advantage in competition for K when growing with grasses. Root exudates containing glutamic acid, tyrosine, and leucine increase K uptake from the soil. In the pot experiment of the present study, K concentrations in *Lotus* and grass foliage were both higher when they were growing together rather than alone. In the field sampling, clover had higher foliar K concentrations when it was growing with grasses. This may be due to K mobility being increased in the grass rhizosphere soil, but then shared with the legumes.

Of course, there are also functional interactions between physico-chemical variables. The interaction of S with other elements through its modification of rhizosphere conditions generally exceed its own direct nutritional value, but legumes require more S than grasses and microbial activity associated with grass rhizospheres accelerate the oxidation and mineralization of organic S into S^{2-} or SO_4^{2-} (Wainwright 1984). When soil pH is lower, soil redox potential decreases and solubility of Fe and Mn increases. Grasses also respond to Fe, Zn, Mn and Cu deficiency with enhanced siderophore release from roots (Marschner and Römheld, 1994; Erenoglu, *et al*., 2000). Uptake of Zn, Mn and Cu is poorly controlled in plants (Lambers, *et al*., 2021). In the present study, clover had higher foliar concentrations of these elements when it grew with ryegrass and tussock. Clover-cocksfoot and *Lotus*-ryegrass assemblages procured more Mn in the present study. Also, in the field sampling of the present study, when clover grew with any of the grass species or multi-species assemblages, foliage concentrations of Mn were higher.

Phytosiderophores may have played a key role in higher Cu and B concentrations in clover foliage when growing with cocksfoot, but mechanistic explanations are likely to be more complex. Understanding Zn deficiency provides a good example (Hafeez, *et al*., 2013). Each essential nutrient has specific physiological and biochemical roles; Zn influences the nitrogen content of legumes (Buerkert, *et al*., 1990; Bolanos, *et al*., 1994) and rhizobia can mobilise Zn in soil to some extent (Chen, 2003). The results of the present study indicate that cocksfoot mobilized Zn and its uptake when growing by itself. Clover may then have consumed mobile Zn, limiting availability of this element to cocksfoot, in turn stimulating cocksfoot to synthesize and secrete mugineic acids. Molybdenum is known to be an important trigger for photosynthesis and N metabolism (Imran, *et al*., 2021), and this element was better exploited from the soil pool by mixtures of legumes and grasses in the pot experiment of the present study. Similarly, Mg is known to improve N-use efficiency (Tian, *et al*., 2021); foliar concentrations of Mg were lower in grasses in the presence of legumes.

It is recognised that it is difficult to identify the specific plant traits that explain species complementarity and transgressive overyielding (Hooper and Dukes, 2004; von Felten and Schmid, 2008), and clearly nutrient mobility in soil and acquisition by plants is particularly complex. The present study lacks any investigation or analysis of the root systems in the pot experiment or field site, but it is known that there is a substantial rhizophere priming effect on nutrient cycling (Lu 2020). Variations between plant species in root exudation pattern and efficiency of acquiring nutrients has long been discussed (Bardgett, *et al*., 1999), and combinations of clovers and grasses have been shown to increase microbial enzyme activity in soil and release of nutrients from organic matter (Sekaran, *et al*., 2020). Findings in the present study relating to each of the elements that were analysed are hardly definitive, but the body of evidence presented clearly illustrates benefits to legumes through partnership with grasses. Productivity is increased and nutrient acquisition from the soil is enhanced by neighbouring grasses that have a complimentary role. Increases of both productivity and foliar concentrations showed a greater total offtake of P, Zn, Mo and Mn from the soil nutrient pool.

In a broader practical context, increasing the legume component of pasture has been a goal of high-country farming in New Zealand for several decades by attempting to introduce a wide range of exotic clovers and other herbaceous legumes to increase vegetation yields and stock productivity. The findings of the present study indicate that cocksfoot and other grasses, including native tussock, play a mutually beneficial role that previously has been largely overlooked. Native tussock grass contained lesser amounts of N and five other nutrients when it was growing with the exotic legumes, suggesting less compatibility, a lack of adaptation to coexistence and perhaps to competition for soil N. Exotic herbaceous legumes appear to offer little benefit to tussock grasses, but sustaining residual assemblages of indigenous species within the productive farming landscape is hugely important to biodiversity conservation in New Zealand. This study suggests it would be worthwhile to elucidate functionality of plant diversity in a wider range of native plants in terms of biodiversity protection, soil biogeochemistry and efficient exploitation of nutrient resources.

3.6 Conclusions

Legume nutrition is improved by neighbouring grasses, and coexistence of these functional groups provides more efficient soil biogeochemistry. This manuscript appears to be a first report that spillover of N from clovers to grasses is an adaptation and strategy to procure *quid pro quo* other limiting nutrients from neighbouring grasses. In a nutrient-poor soil, productivity increased and the mobility of key elements in plants and soils significantly differed with combinations of legumes and grasses, compared to either functional group alone. Foliar concentrations differed and mass balance results demonstrated that mixed species assemblages improved the efficiency of exploitation of the soil pool of nutrients. White clover and *Lotus* grew better in combination with different species of introduced grasses. The results provide a demonstration of transgressive overyielding. Sampling of clover in a more fertile lowland pastoral grassland provided results that suggested mutual compatibility between legumes and other species is a more widespread and frequent phenomenon. Mechanistic explanations for these responses were not sought in this experimental work, but numerous potential explanations exist in the scientific literature; it is likely that modification of Ca and S and subsequently pH in the rhizosphere, which is further modified by different root structures and root exudates play key roles. These findings have obvious significance towards understanding the value of plant diversity in pastoral farming, but they also advance knowledge of native and exotic plant co-existence that is highly relevant to conservation and management of New Zealand's native biodiversity. The indigenous tussock grass contained less N and no other nutritional benefits when growing with exotic legumes but did improve the overall offtake of nutrients. This suggests less compatibility, a lack of adaptation to coexistence and a limited ability to compete for soil N. Further research is justified.

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Chapter 4

Plant Species Complementarity in Low-Fertility Degraded Soil

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Plant Species Complementarity in Low-Fertility Degraded Soil

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Abstract: The aim of this study was to investigate the compatibility of plants with contrasting root systems, in terms of procurement of limiting soil nutrients. Paired combinations of species of proteas and grasses were grown in a pot experiment using soil from a site with impoverished vegetation and degraded soil. The soil contained sufficient N but was low to deficient in P, Mn, S, Fe, and B. The uptake of chemical elements into the foliage differed significantly according to whether the plants were growing as single or mixed species. When two species of *Grevillea* and grasses with evolutionary origins in low fertility soils were growing together, there was an enhanced uptake of P and Mn, in one or both species, in addition to other elements that were in low concentrations in the experimental soil. In contrast to this, *Protea neriifolia* that probably originated from a more fertile soil procured lesser amounts of the six elements from the soil when growing together with grasses. Two grasses tolerant of less fertile soils (*Dactylis glomerata* and *Poa cita*) obtained more nutrients when they grew together with proteas; this was a much stronger neighbour effect than was measured in *Lolium perenne* which is better adapted to high fertility soils. The findings illustrate both the functional compatibility and competition for plant nutrients in mixed-species rhizospheres. Species combinations substantially increased the acquisition of key elements from the soil nutrient pool.

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Keywords: soil nutrients; plant nutrition; co-existence; rhizosphere; phosphorus; manganese

4.1 Abstract

This paper investigates species complementarity and soil nutrient acquisition by plants. Paired combinations of species of proteas and grasses were grown in a degraded soil with sufficient N but low to deficient P, Mn, S, Fe and B. Uptake of chemical elements into foliage differed significantly according to whether plants were growing as single or mixed species. When growing together, proteas and grasses with evolutionary origins on low fertility soils provided mutually enhanced uptake of key soil nutrients. This was reflected in elevated uptake of P and Mn, as well as other elements that were in low concentrations in the experimental soil. In contrast to this, a species of protea originating from a more fertile soil transferred lesser amounts of six elements from soil to foliage when growing together with grasses. Two grasses tolerant of less fertile soils (cocksfoot and silver tussock) obtained more of a similar range of limited nutrients when they grew together with proteas; this was a much stronger neighbour effect than was measured in ryegrass which is more suited to high fertility soils. The findings illustrate both functional compatibility and competition for plant nutrients in mixed-species rhizospheres. Species combinations substantially increased procurement of key elements from the soil nutrient pool.

4.2 Introduction

There is wide variability in the mobility of chemical elements in the rhizospheres of different species that is reflected in exploitation of the soil nutrient pool and uptake of nutrients by plants [1-2]. This would suggest that combinations of plant species may be more effective than single species at modifying mobility and management of chemical elements in soils [3]. The hypothesis underlying this study is that plants naturally adapted to low fertility or degraded soils are likely to benefit by growing in combination with other species that possess different functional traits in the rhizosphere, since most plant species have similar fundamental metabolic demands for the same range of key nutrients [4]. A strategy of sharing different capabilities to procure key soil nutrients might prevail over competition for accessibility to a limited resource. Contrary to this, plants adapted to more fertile soils may be more likely to employ a competitive strategy to rapidly acquire a majority share of nutrients from a more plentiful pool of available soil nutrients. We test this hypothesis by growing a combination of plants that do not naturally occur together, but that are known to have different root functional traits that are adapted to either fertile or infertile soils; combinations readily found in both Proteaceae (proteas) and Poaceae (grasses).

There are often added benefits to plant productivity from two or more plant species growing together. Intercropping in agriculture and horticulture provides increased yields, often referred to as transgressive overyielding [5-9], for example when legumes are grown together with other crops [10, 11]. In this example, the fortuitous spillover of N fixed by rhizobial symbionts from the legume to neighbouring plants is generally viewed as incidental, even though it seems unlikely that evolution has favoured an adaptation in plants that expends metabolic energy and resources towards obtaining N that is then readily shared with competitors. We have recently shown that grasses reciprocate in this relationship by procuring key trace elements in the rhizosphere that are then passed on to legumes [3]; grass - clover assemblages enhanced overall productivity and uptake of P, K, S Mn, Cu, Mo and B. The present paper investigates whether sharing of phosphorus and key trace elements can also be identified between proteas and grasses of different origins when they grow together in soil with sufficient N for healthy growth, but with deficiencies of other key nutrients. We grew a selection of species that do not share a common biogeographical origin.

Uptake of chemical elements is known to differ between species and according to whether plants are grown in monoculture or in mixed species assemblages [12], or naturally found in the same location and habitat [3]. However, investigations of the physiological traits associated with uptake seldom extend to a consideration of two-way sharing of soil nutrients. One of the few more detailed examples of complementarity is with plants that produce cluster roots, found within a few plant families, including the Proteaceae and in a few crops such as *Lupinus albus* [13, 14]. Cluster roots primarily enable plants to exploit less labile pools of soil phosphorus (P) in P-deficient soils, by releasing organic acids to mobilise mineral P that is bound to metal cations and organic complexes in soil [15]. There is also some evidence that cluster roots can also facilitate the acquisition of nutrients by neighbouring plants [16]. Different strategies to acquire soil nutrients are employed by grasses, for example through different root structures [17], using mycorrhizal associations [18], or by secreting organic acids (phytosiderophores) [19]. Complementarity has previously been found to explain coexistence between different functional groups of grasses [20]. Our assumption was that acquisition of P would play a definitive role in our findings.

The aim of this study was to investigate species complementarity in the context of P and key trace elements, by measuring the uptake of nutrients into foliage of species of different origins growing together artificially in a nutrient-depleted and degraded soil. The work is particularly relevant to management of species diversity in low-fertility production systems, but also has potential significance for phytoremediation science and practice, where exotic species are introduced to contaminated and degraded soils to manipulate chemical elements [21-23].

4.3 Results

Elevated concentrations of P and Mn were particularly notable in the *Grevillea* spp. when growing with grasses (Figure 4.1), in contrast to *P. nerifolia* foliage which had lower Mn concentrations when it was growing with the grasses. On at least one of the two sampling occasions, *Grevillea barklyana* had higher foliar concentrations of most chemical elements when growing together with grasses, particularly with cocksfoot (Figure 4.2; Appendix, Table 4.2). Five of the elements with higher concentrations (P, Mg, S, Mn, Zn, B) were nutrients known to be deficient in the soil, in addition to elevated K and Mo. In contrast, lower concentrations of 5 elements in addition to P, were often recorded in *P. neriifolia* foliage when it had grown with grasses (Figure 4.3). Only 3 elements in *G.* Robin Hood foliage were in significantly different concentrations when it was growing with grasses (Figure 4.2).

There were fewer changes of elemental concentrations of the two pasture grasses when they were growing with proteas, relating largely to higher concentrations of P, K and Ca (Figure 4.4). Tussock grass was different, with significantly elevated foliar P, K, Ca, S, Mn and Zn when it was growing with proteas (Appendix, Table 4.3). Focusing on cocksfoot, as the grass with most evident neighbour effects (Figure 4.5), there was a shift in foliar nutrient concentrations of both proteas and grasses when they were growing with a neighbouring species, that was associated with the differential of uptake of nutrients.

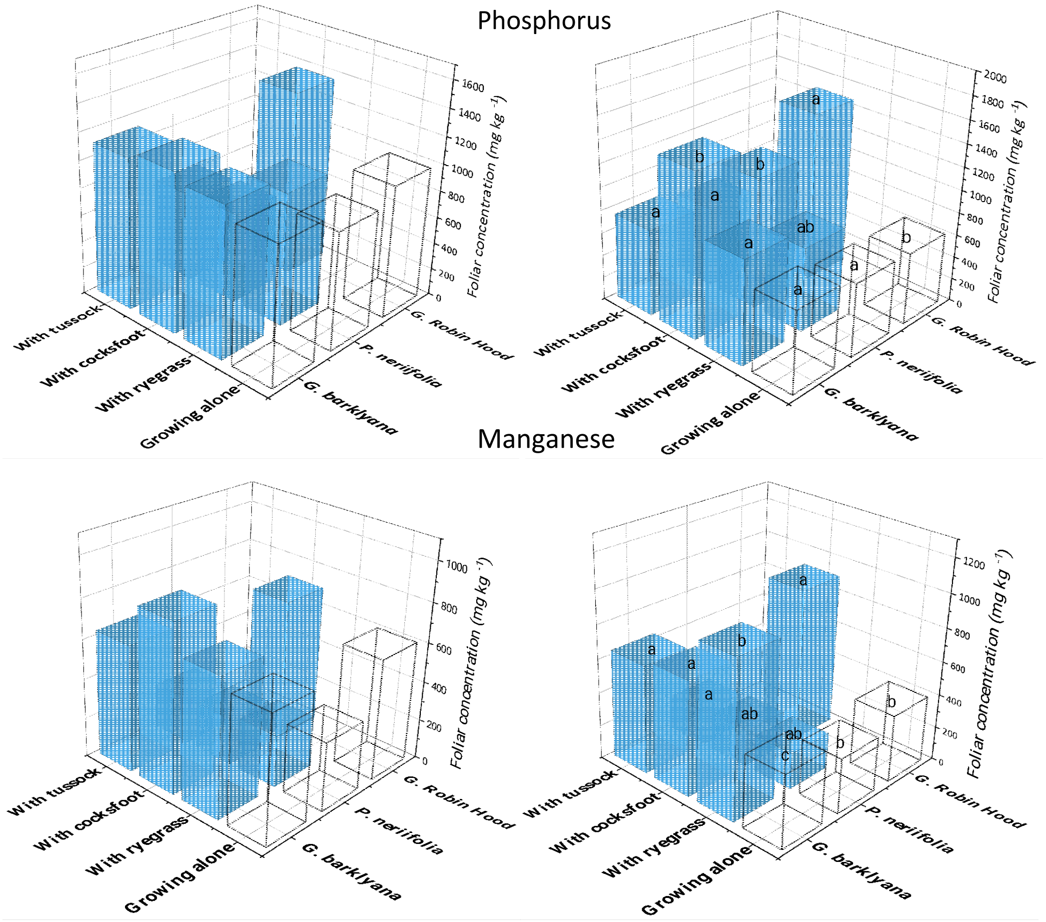


Figure 4.1 Phosphorus and manganese concentrations in the foliage of the three species of proteas when they were growing alone (open bars) or with one of three species of grass (coloured bars). Charts show results of 1st (LHS) and 2nd (RHS) sampling. Different letters separately indicate significant differences ($p < 0.05$) for each protea (full results in Appendix, Table 4.2).

In terms of plant productivity, there were few significant differences in the biomass of proteas or grasses after they had grown with neighbouring plants, but variability was high within each treatment (Figure 4.6). Exploitation of the soil pool of chemical elements may be more accurately represented by mass balance calculations (multiplication of dry wt. of foliage x nutrient concentration). This calculation was performed using data separately for each species (Figure 4.7; Appendix, Table 4.4). More P, Mn and Zn were procured by the *Grevillea* spp. but less by *P. nerifolia* when these species had grown in the same pots as grasses. In percentage terms, neighbouring grasses only marginally reduced biomass of protea foliage, but higher foliage concentrations of elements led to significantly increased the total offtake of nutrients by as much as 100% in the proteas (Figure 4.8). There was a much lower biomass of grass foliage than of protea foliage, on average amounting to 11% of the latter, which meant that the total amount of each element extracted from the soil by grasses was much less than the amount extracted by proteas (Appendix, Table 4.4). Nonetheless, these calculations illustrated clear differences between *P. neriifolia* and the *Grevillea* spp., and between the different grass species (Figure 4.8). When the proteas and grasses were growing in combination with each other, compared to growing as monocultures, they extracted substantially increased amounts of nutrients from the soil.

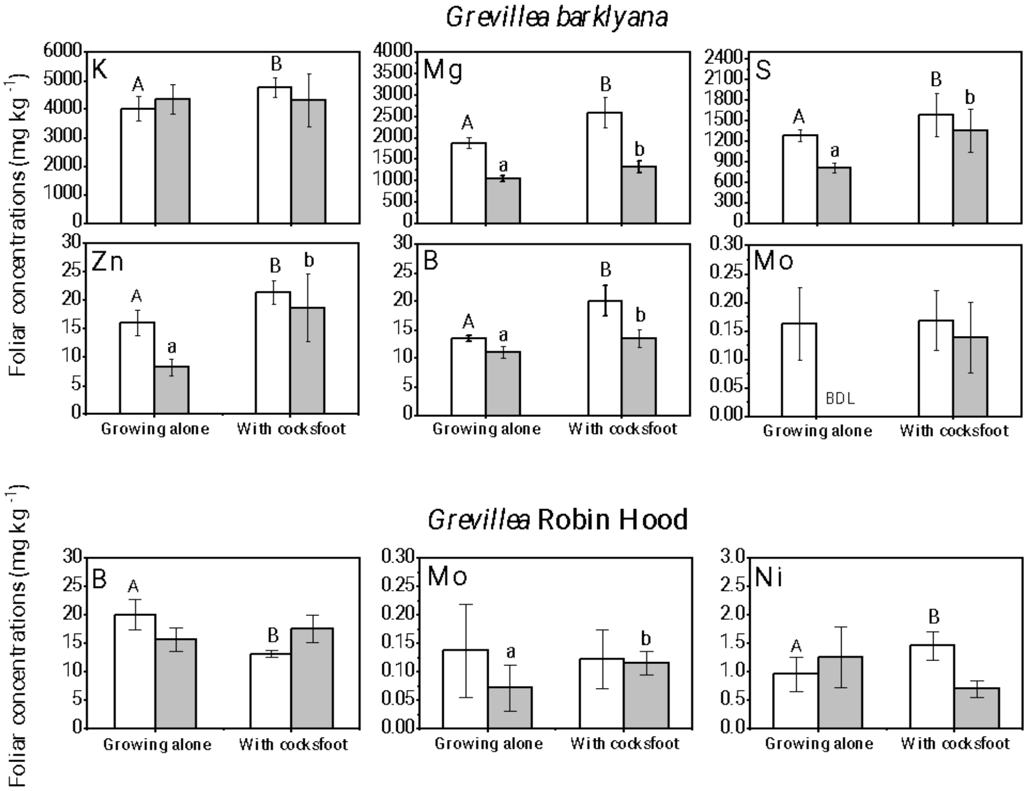


Figure 4.2 Nutrient concentrations in the foliage of the two *Grevillea* spp. when they were growing alone or together with cocksfoot (Open bars, first sampling; Shaded bars, final sampling). Different letters separately indicate significant differences ($p < 0.05$) within each sampling event. Elements without significant differences are not shown (full results in Appendix, Table 4.2).

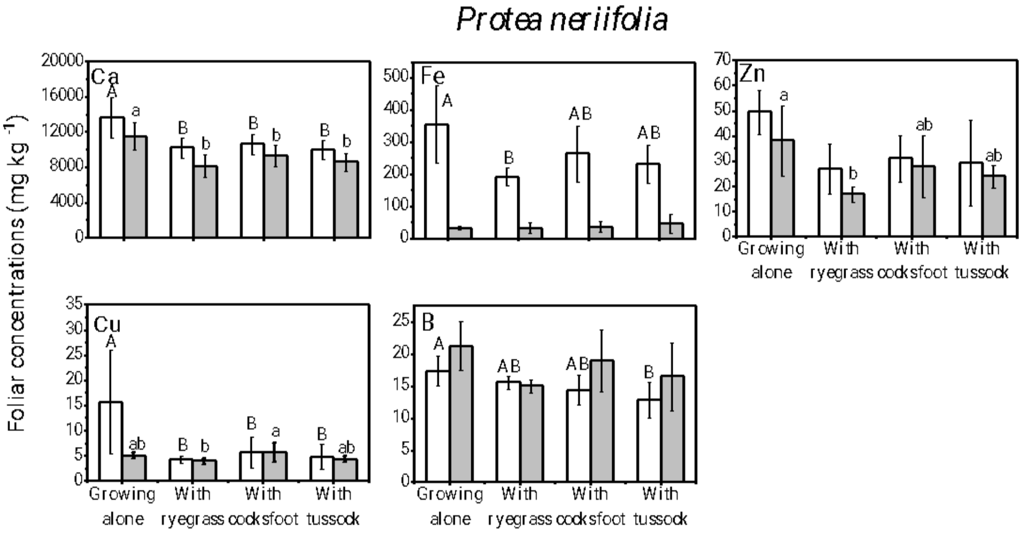
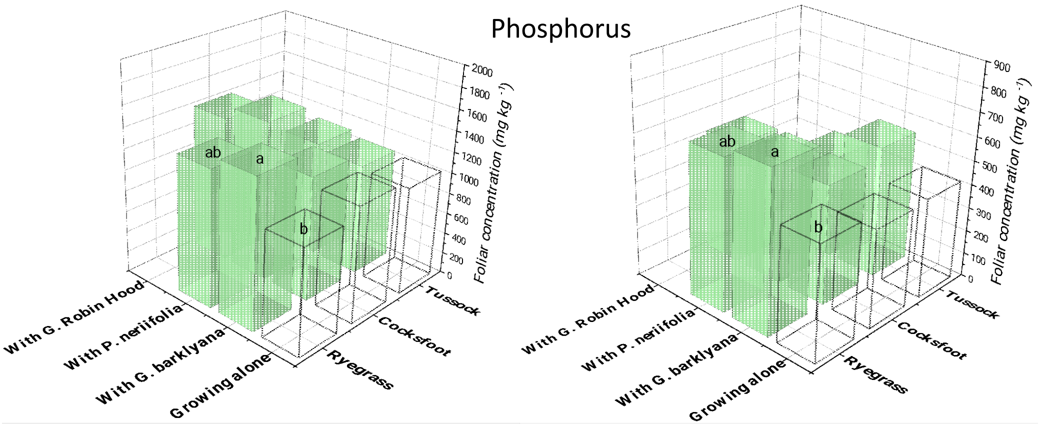
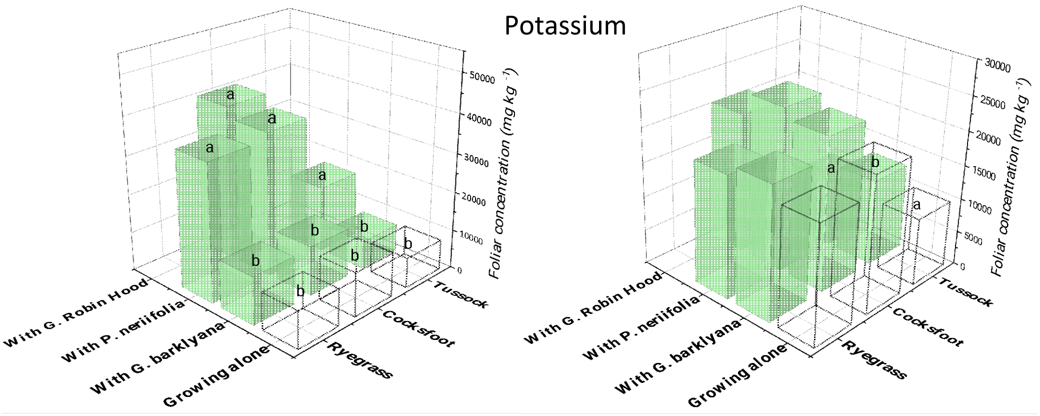


Figure 4.3 Nutrient concentrations in the foliage of the *Protea neriifolia* when growing alone or together with the three species of grass (Open bars, first sampling; Shaded bars, final sampling). Different letters separately indicate significant differences ($p < 0.05$) within each sampling event. Elements without significant differences are not shown (full results in Appendix, Table 4.2).





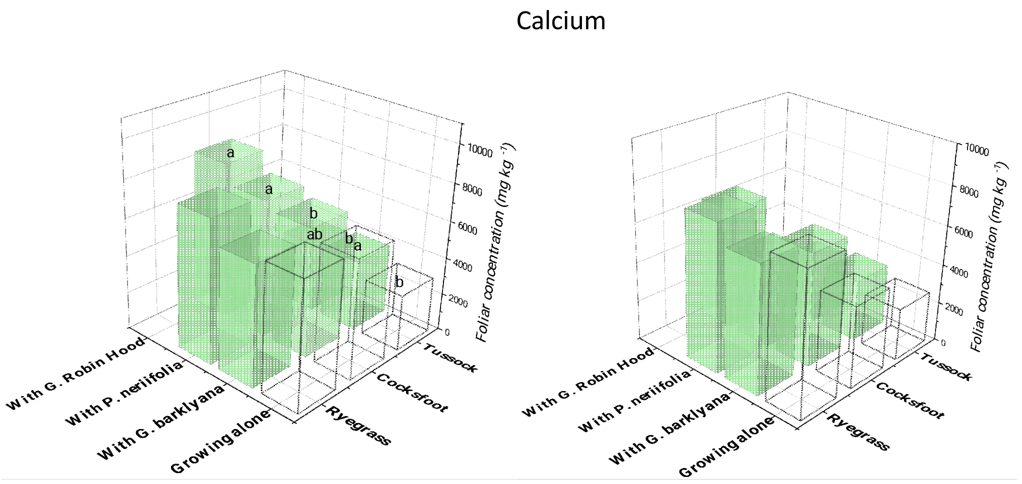


Figure 4.4 Phosphorus, Ca and Mn concentrations in the foliage of the three species of grass when they were growing alone (open bars) or with one of three species of proteas (coloured bars). Charts show 1st (LHS) and 2nd (RHS) sampling. Different letters separately indicate significant differences ($p < 0.05$) separately for each species of grass (full data in Appendix, Table 4.3).

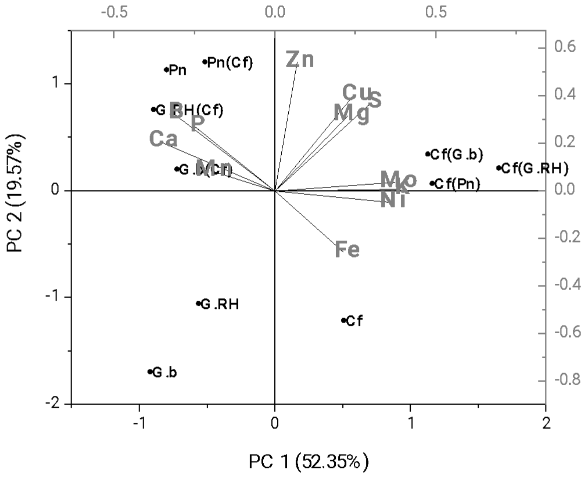


Figure 4.5 Principal Components Analysis describing foliar nutrient concentration data for each of the protea species (*Grevillea barklyana,* Gb; *Protea neriifolia*, Pn; *Grevillea* Robin Hood, G.RH) growing alone or with cocksfoot (Cf), and for cocksfoot growing alone or with each of the proteas. Abbreviations in brackets indicate the companion species.

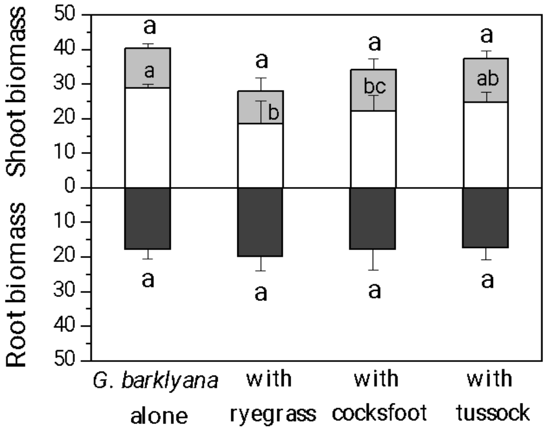


Figure 4.6 Harvested biomass of *Grevillea barklyana* (dry wt., g pot^{-1}) when it was growing alone or with one of the three species of grass. Shoot biomass shows stems (open bars) and foliage (shaded bars). Different letters indicate significant differences ($p < 0.05$) within separate plant components.

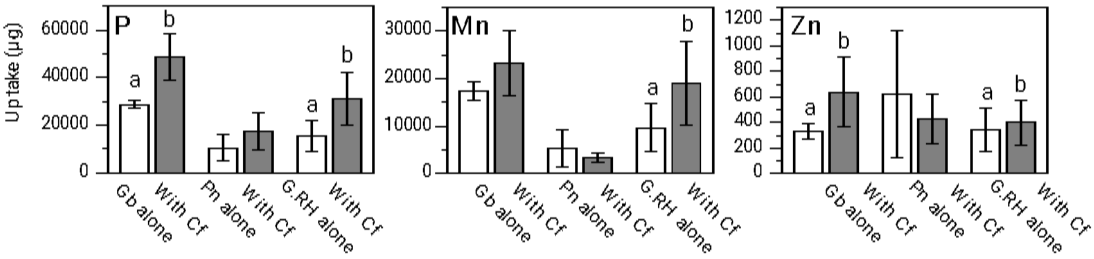


Figure 4.7 Total uptake of P, Mn and Zn into foliage of each of the three species of Protea, when they were growing alone or with cocksfoot (Gb: *Grevillea barklyana*, Pn: *Protea neriifolia*, G.RH: *Grevillea* Robin Hood, Cf: Cocksfoot). Open bars, first sampling; Shaded bars, final sampling. Different letters separately indicate significant differences ($p < 0.05$) within each of the sampling events. Elements without significant differences are not shown

(full data in Appendix, Table 4.4).

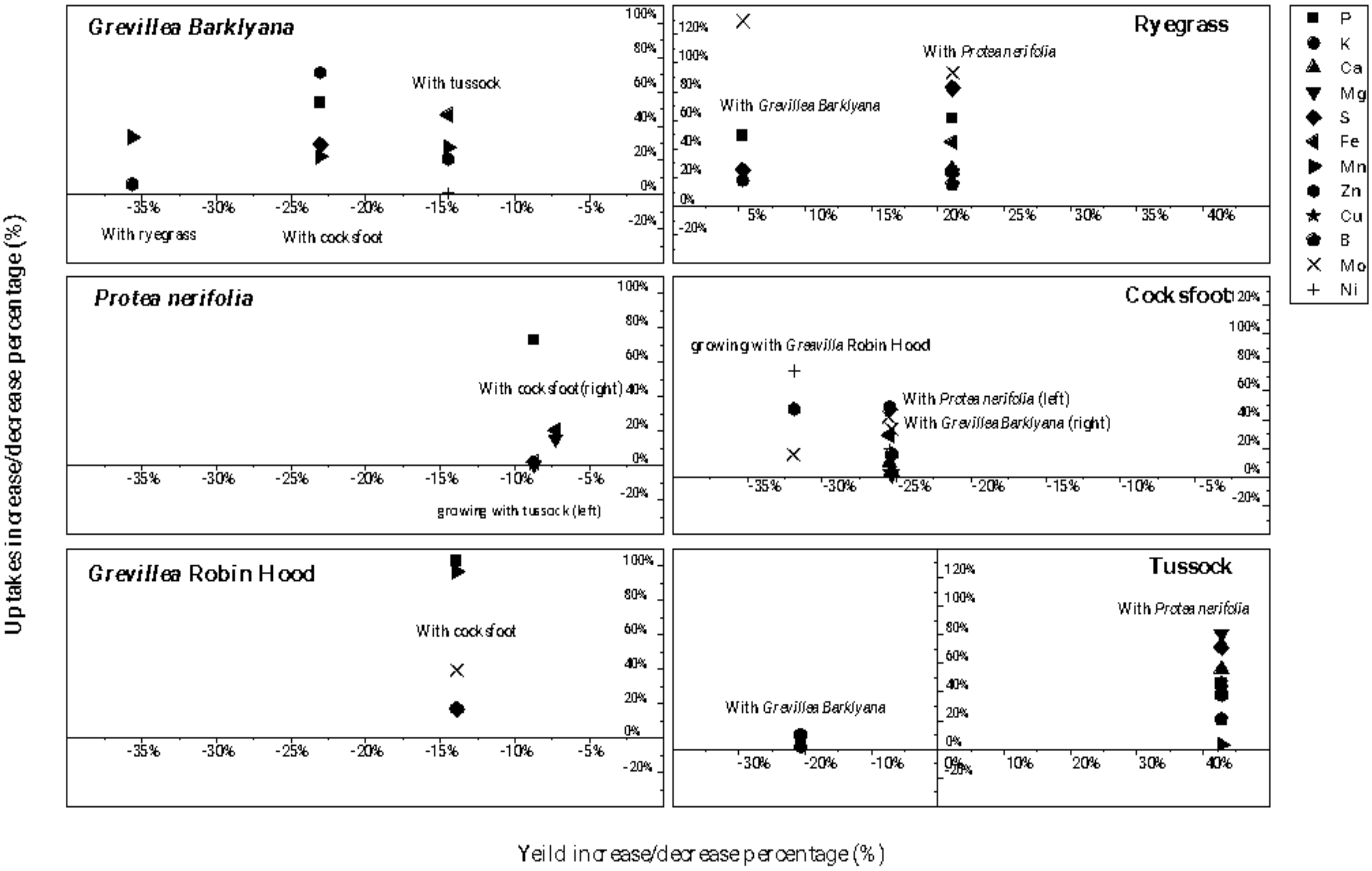


Figure 4.8 The percentage change in yield (horizontal axes) and total foliar uptake of key nutrients (vertical axes), when each of the protea and grass species was growing with a companion species. Percentage change is the difference to when each species grew alone.

4.4 Discussion

Measuring nutrient concentrations in foliage yields provided a surrogate but arguably the most realistic measure of exploitation of the soil nutrient pool. No direct attention was given to processes in the soil that were responsible for different plant uptake. Interactions between plants that take place belowground are often overlooked, even though roots of different species are frequently intermingled, with growth, root exudates [24, 25], root turnover [26], death and decay [27] occurring in mixed species rhizospheres. Furthermore, the main input of nutrients to soil is from root decomposition [28]. Otherwise, nutrients are largely bound to solid phase constituents and generally only small proportions enter the in the soil solution and become available for uptake. Clearly, complex interactions in the rhizosphere affect soil biogeochemistry and nutrient acquisition by plants [24, 29] but were not part of the experimental work in the present study.

The results showed that foliar concentrations of P and Mn uptake in proteas and grasses were consistently modified when growing in species combinations, compared to growing alone, but this also extended to elevated foliar concentrations of up to nine other elements (K, Ca, S, Fe, Mg, Zn, Cu, B, Mo). Our assumption that P acquisition by the cluster-rooted proteas would play a definitive role is evident in the results, in terms of its increased concentrations in the foliage of grasses, together with elevated K and Ca. The tussock grass had enhanced amounts of at least five elements (K, Ca, S, Mn, Zn), but not of P, when growing with proteas. There appeared to be little reciprocation from tussock grass to proteas. Low-fertility soil would have probably provided more natural conditions for this native grass, which is likely to explain its competitive abilities a procuring deficient nutrient from the soil when it was growing with proteas.

Cocksfoot provided a better demonstration of species complementary than ryegrass, probably because if grew better and had more biomass. Combinations of cocksfoot with the proteas provided the most significant impacts on nutrient uptake in the proteas. The two *Grevillea* species with origins in the ancient, fertility-depleted soils of Australia, benefited substantially through coexistence and the presence of neighbouring grasses in terms of elevated uptake of most of the range of nutrients. In comparison, less uptake of key nutrients (including Mn, Fe, Zn and B) by the South African protea when growing together with grasses implies some combination of competitive losses and less sharing, perhaps reflecting an evolutionary history on soils with more adequate fertility.

There were clear differences between the *Protea* and the *Grevillea* spp. in terms of foliar nutrient concentrations when growing with grasses. Grevillea spp. and grasses clearly benefited by growing in combination with each other, with both obtaining more P, providing evidence of transgressive overyielding for this element, especially for *G. barklyana* - cocksfoot combinations. Otherwise, Grevillea also secured more of S, Mg, Mn, Zn and B, and the grasses obtained more K and Ca. *Protea nerifolia* - grass combinations were competitive rather than complementary, with the protea less able to procure key deficient elements in the presence of grasses, with higher foliar uptake of six elements when it was growing alone. There was no obvious benefit to *P. neriifolia* of growing with neighbouring grasses.

Likely explanations that would describe the processes responsible for different patterns of uptake are to be widely found in the scientific literature. In broad terms, two of the most important ways that root exudates influence nutrient availability and uptake are through organic acid and phytosiderophore secretion [25]. In proteas, availability of phosphorus in soil is the most important determinant of cluster roots formation, and carboxylates exuded from roots promote P mobilization in soil [13]. Deficiencies of other elements, including N, K, Mn and Fe also enhance cluster root development. Graminoid-secreted phytosiderophores release chelators to form complexes with soil metals, increasing metal solubility and mobility, particularly of Fe that is often in abundant but insoluble Fe (III) precipitates in soil. Many phenolics produced in the rhizosphere of dicots can form complexes with metals that may also increase their availability. In low-nutrient environments, plants can produce root exudates as symbiotic signals to soil microbes involved in nutrient procurement, to use extracellular enzymes to release P from organic compounds, and organic acids to solubilize soil Ca, Fe and Al phosphates [30]. There is increasing evidence that plants can be complementary to one another to procure nutrients more efficiently [31] and at reduced metabolic costs [32]. However, mechanistic explanations are complex; for example, many phytohormones are involved in interactions between roots, soil and microbial communities [33]. Clearly, rhizosphere processes are complex and insufficiently understood [34], and there remains a paucity of studies that provide mechanistic evidence from soil-based systems [35].

The importance of considering multiple nutrient constraints on plant productivity has been stressed elsewhere [36]. The requirements of plants for similar base concentrations but differing amounts of particular nutrients is likely to be specific to the plant species, and this is probably reflected in differing foliar concentrations recorded in the present study. The most likely capacity-based approach to nutrient acquisition [29] assumes that plants expend metabolic energy to acquire nutrients by exploiting gradients of nutrient molecules inside and outside the root, using specific nutrient-acquiring proteins (pumps, transporters, and channels) [29].

There was some evidence from the present study of transgressive overyielding in the context of increased proportion of key nutrients being removed from the total soil pool by combinations of species compared to monocultures. No account was taken of nutrient uptake into woody or green stems of the proteas in terms of total offtake. Nutrient concentrations in these plant components would be expected to be much lower than in foliage, but the amount of additional nutrients in these fractions could have much relevance to production systems and phytoremediation technologies. Competition for nutrients, facilitation and complementarity are all major driving forces of ecosystem productivity [34].

The present paper has drawn attention to the lack of fundamental and applied research knowledge of functional biodiversity and plant species complementarity in the context of soil biogeochemistry, particularly in low-fertility and degraded soils. The findings illustrate functional compatibility as well as competition between plant rhizospheres for plant nutrients; concepts that could have wider application to management of plant species diversity in low-fertility production systems. Beneficial coexistence appears to be explained by differences between plant rhizospheres of different species to exploit different components of the soil nutrient pool [37, 38]. In particular, enhancing species diversity, beyond simply focusing on legumes and grasses [3], may be a better way to manage low-fertility or degraded soils. We suggest it would be worthwhile to extend the experimental approach used in the present study to a wider range of species combination that have a direct practical application to less-intensive grazing systems, phytotechnologies, and to conservation and restoration of biodiversity.

4.5 Materials and Methods

Soil (1-20 cm depth) was collected from a site in Canterbury (altitude 611m), South Island, New Zealand (S 43° 20´35´´, E 171° 36´59´´), that has been described in detail in earlier papers [3, 39]. The site was originally forested, probably until the mid-19th century. Since then, the land has been extensively grazed by sheep and wild ruminants but otherwise has been largely undisturbed. A patchy vegetation cover, probably for a century or more, has meant soils have been exposed and eroded. The collected soil was thoroughly mixed, then air-dried and sieved (2mm) prior to being used in the experimental work. Samples were analysed using standard methods by Analytical Services, Soils and Physical Sciences Department, Lincoln University (Table 4.1), showing a range of key determinants (pH, Ca, sulphate-S, soluble P, Cu, Mg, Mn, B) were less than optimum for plant growth. Available P, Ca and B were extremely deficient, although there was adequate N for healthy plant growth at yields that could be achieved in the landscape of its origin.

We grew three species from the Proteaceae (proteas): *Grevillea barklyana* F. Muell. Ex Benth. (Gully- or large- leaf grevillea) endemic to south-western Australia, *Grevillea* Robin Hood (a hybrid cultivar of *G. hookeriana* Meisn.), endemic to south-eastern Australia, and *Protea neriifolia* R. Br. (narrow-leaf sugarbush), endemic to South Africa. South African soils are not characterised by high soil fertility [40], but the inherent fertility of soils is often much lower in the more ancient and strongly weathered soils of Australia [41]. All three species only grow ornamentally in New Zealand. These proteas were grown either alone or in combination with single species of grasses; one of two widespread and common grasses, *Lolium perenne* L. (perennial ryegrass) and *Dactylis glomerata* L. (cocksfoot), or *Poa cita* Edgar (silver tussock) which is an endemic New Zealand tussock grass [42].

A pot trial was set up in a glasshouse at Lincoln University. The cluster root forming species were taken from cuttings of single plants, rooted in seed trays then transplanted into 3.5 L plastic pots (15cm diameter, 20cm height). Thirteen experimental treatments consisted of three cluster roots forming species and three grasses growing either singly or in combinations, with 5 replicates per treatment. Pots were arranged in a randomized single block design on a glasshouse bench. Glasshouse temperatures for the duration of the experiment were 19.0°C (mean); 13.6°C (min) - 34.7°C (max). Plants were watered sparingly every two days. Survivorship was generally good, but one Gb and one Pn died after transplanting, one Pn (Rg) died after first harvesting, and one Pn (Tg) died a few days before the final harvest.

After 6 months' growth, plant material was sampled for chemical analysis; 5 leaves were harvested from each of the proteas, and the grasses were harvested to 2 cm above the soil surface. This was repeated 6 months later when the plants were completely harvested, and root systems were separated by careful washing. Aboveground plant material was sorted into separate species, dried (65° C, 48 hrs.), weighed and finely ground, microwave digested in 5M HNO_3, and then chemically analysed by ICP-OES using standard methodology.

Table 4.1 Physico-chemical determinants of the experimental soil.

Indicators	*Units*	*Concentration*	*Typical Range**
pH [1]	pH Units	5.70	5.70-6.20
Total Nitrogen [2]	%	0.46	0.30-0.60
Total Carbon [3]	%	5.80	-
Organic Matter [4]	%	10.0	7.00-17.0
Total Phosphorus	mg kg^{-1}	464	700-1600
Olsen Phosphorus [5]	mg l^{-1}	4.33	20.0-30.0
Potassium [6]	me/100g	0.49	0.30-0.60
Calcium [6]	me/100g	2.03	5.00-12.0
Magnesium [7]	me/100g	0.60	0.60-1.20
Sodium [7]	me/100g	0.05	0.00-0.30
Sulphate Sulphur [8]	mg kg^{-1}	6.43	10.0-20.0
Iron [7]	mg l^{-1}	84.0	500-1000
Manganese [7]	mg l^{-1}	3.20	8.00-65.0
Zinc [7]	mg l^{-1}	1.75	0.80-4.00
Copper [7]	mg l^{-1}	0.37	0.40-2.00
Boron [7]	mg l^{-1}	0.19	0.60-1.20

*Typical range for agricultural soils in New Zealand. Method: [1] 1:2 (v/v) soil:water slurry followed by potentiometric determination of pH. [2] Determined by NIR, calibration based on Total N by Dumas combustion. [3] Determined by NIR, calibration based on Total Carbon by Dumas combustion. [4] Organic Matter is 1.72 x Total Carbon. [5] Olsen extraction followed by Molybdenum Blue colorimetry.[6] 1M Neutral ammonium acetate extraction followed by ICP-OES. [7] Mehlich 3 Extraction followed by ICP-OES. [7] 0.02M Potassium phosphate extraction followed by Ion Chromatography.

4.6 Appendix

Table 4.2 Nutrient concentrations in protea foliage when each species was growing with grasses (Cf, Cocksfoot; Rg, Ryegrass; Tg, Tussock grass) at 1st and 2nd sampling events. Different letters separately indicate significant differences ($p < 0.05$) within each treatment. Bold alphanumericals indicate significant differences to when the proteas were growing alone.

Elements		***Grevillea barklyana***				***Protea neriifolia***				***Grevillea* Robin Hood**	
		Alone	**With Rg**	**With Cf**	**With Tg**	**Alone**	**With Rg**	**With Cf**	**With Tg**	**Alone**	**With Cf**
P	1st	1010a	1100a	1170a	1120a	870a	977a	667a	650a	985a	1370a
	2nd	710a	910a	**1430b**	754a	642a	739ab	**1140b**	696a	630a	**1490b**
K	1st	4020a	4450ab	**4790b**	4240a	4900a	4630a	6170a	5050a	7600a	7570a
	2nd	4350a	3950a	4320a	4270a	7920a	5700a	8270a	7340a	6070a	5640a
Ca	1st	10100a	10300a	10800a	10900a	**13600a**	10200b	10700b	10000b	11400a	11800a
	2nd	9270a	11200a	10200a	10300a	**11590a**	8180b	9370b	8650b	9210a	10610a
Mg	1st	1880a	2030a	**2590b**	2040a	2110a	1660a	1750a	1610a	1780a	1910a

	2nd	1050a	1220ab	**1320b**	1150a	1130a	1380a	1310a	1290a	1170a	1320a
S	1st	1290a	1360ab	**1590b**	1330ab	1620a	1150a	946a	901a	1620a	1830a
	2nd	812a	1000a	**1360b**	854a	1300ab	923b	1350a	1020ab	940a	1280a
Fe	1st	115ab	110ab	151a	80.1b	356a	192b	265ab	233ab	98.6a	112a
	2nd	54.9a	58.3a	52.4a	93.8b	33.3a	32.5a	36.7a	46.1a	68.3a	49.5a
Mn	1st	637a	694a	843a	636a	347a	262a	235a	231a	611a	708a
	2nd	429a	**926b**	**679c**	**643c**	325a	216ab	219ab	207b	398a	**910b**
Zn	1st	16.1a	15.0ac	**21.4b**	13.1c	49.6a	27.1a	31.1a	29.4a	16.2a	15.0a
	2nd	8.26a	**14.0b**	**18.7c**	11.6ab	38.2a	17.0b	28.1ab	24.1ab	13.9a	18.9a
Cu	1st	17.9a	16.6ab	19.8a	8.53b	**15.8a**	4.27b	5.79b	4.89b	12.3a	10.2a
	2nd	4.15a	4.27a	4.32a	3.82a	5.11ab	4.03b	5.74a	4.47ab	4.55a	5.17a
B	1st	13.5a	14.3a	**20.2b**	13.7a	17.4a	15.6ab	14.4ab	12.9b	20.1a	13.2b
	2nd	11.1a	12.1ab	**13.5b**	11.5a	21.3a	15.1a	19.1a	16.5a	15.8a	17.6a
Mo	1st	0.16a	0.16a	0.17a	0.13a	0.13	<0.02	0.13	0.12	0.14a	0.12a
	2nd	<0.02	0.06	0.14	0.08	0.03a	0.11a	0.02a	0.03a	0.07a	**0.12b**
Ni	1st	1.49a	1.48a	1.08a	1.41a	1.86a	1.26a	1.76a	1.06a	0.97a	**1.47b**
	2nd	0.87a	0.73a	0.89a	1.07a	0.72a	0.73a	0.67a	0.71a	1.27a	0.70a

Table 4.3 Nutrient concentration in grass foliage when they were growing alone or with proteas (Gb: *Grevillea barklyana*, Pn; *Protea neriifolia*, GRH; *Grevillea* Robin Hood) at 1st and 2nd sampling events. Different letters separately indicate significant differences ($p < 0.05$) within each treatment. Bold alphanumericals indicate significant differences to when the species was growing alone.

Element		**………. Ryegrass ………**			**………. Cocksfoot ……….**				**………. Tussock grass ……….**		
		Alone	**With Gb**	**With Pn**	**Alone**	**With Gb**	**With Pn**	**With GRH**	**Alone**	**With Gb**	**With Pn**
P	1st	1010a	1430b	1310ab	1110a	1190a	1390a	1510a	1030a	1060a	1110a
	2nd	477a	**675b**	635ab	412a	501ab	**514b**	**516b**	421a	**552b**	430ab
K	1st	9570a	**12300b**	35400b	11300a	12700a	**39900b**	**37100b**	7770a	7160a	**17100b**
	2nd	17300a	19400a	17900a	19900a	22300a	20500a	23800a	9840a	**12600b**	9900a
Ca	1st	6770a	6400a	7630a	6340a	6860a	**8330b**	**7300b**	2990a	**3770b**	3620ab
	2nd	7170a	6460a	7460a	4140a	5760a	6110a	4450a	2640a	3120a	2890a
Mg	1st	3460a	3370a	3660a	2830a	2920a	2590a	2990a	865a	1000a	1250a
	2nd	2870a	2700a	2920a	1170a	1600a	1280a	1390a	684a	660a	866a
S	1st	3190ab	2760b	4030a	2870a	3280a	3310a	3130a	2230a	1600a	2320a
	2nd	2140a	2540a	3220a	1350a	1560a	1440a	1760a	1040a	**1290b**	1250ab
Fe	1st	222a	247a	265a	128a	97.0a	248a	124a	74.9a	83.2a	82.4a
	2nd	108ab	82.3b	129a	46.6a	52.7a	80.8a	67.2a	138a	50.7a	46.1a
Mn	1st	94.1a	103a	90.1a	287a	351a	364a	320a	105a	**141b**	**121b**
	2nd	197a	125a	147a	201a	263a	396a	270a	108a	116a	77.9a
Zn	1st	50.0a	34.2ab	28.1b	39.4a	34.1a	90.4a	43.3a	22.2a	39.2a	24.1a
	2nd	43.1a	17.3b	24.4b	12.9a	20.1a	25.8a	27.9a	11.0a	**15.3b**	10.7a
Cu	1st	8.23a	10.3a	9.23a	11.8a	11.9a	16.7a	16.9a	8.22a	8.16a	9.83a
	2nd	6.11a	5.63a	6.07a	4.52a	5.59ab	**6.28b**	**5.67b**	4.31a	4.22a	4.42a
B	1st	8.7a	8.07a	7.42a	5.79a	6.71a	6.67a	7.10a	6.27a	5.44a	8.56a
	2nd	10.7a	9.03a	10.1a	7.42a	8.20a	9.51a	5.92a	7.07a	7.81a	6.01a
Mo	1st	0.41a	0.46a	0.43a	0.69a	0.58a	0.66a	0.89a	0.47a	0.53a	0.33a
	2nd	0.20a	0.44a	0.32a	0.21a	0.38a	0.41a	0.36a	0.44a	0.33ab	0.27b
Ni	1st	2.03a	2.05a	2.31a	3.01a	3.36a	3.66a	2.77a	1.88a	1.43a	2.12a
	2nd	2.04a	1.75a	1.59a	1.56a	1.73a	2.50a	3.99a	5.36a	1.18b	1.51ab

Table 4.4 The total foliar uptake (µg) of each nutrient by each of the six species according to whether they were growing alone or with a companion species. (Gb: *Grevillea barklyana*, Pn; *Protea neriifolia*, G.RH; *Grevillea* Robin Hood, Cf, Cocksfoot; Rg, Ryegrass; Tg, Tussock grass). Different letters separately indicate significant differences ($p < 0.05$) within each treatment. Bold alphanumericals indicate significant differences to when the species was growing alone. Minimum detectable limits of Mo (0.02 mg kg^{-1}) were used for uptake calculations of this element.

Species	N	P	K	Ca	Mg	S	Fe	Mn	Zn	Cu	B	Mo	Ni
Gb	**51.3a**	20600a	127000a	268000a	30500a	23600ab	1600ab	12500a	240a	121a	322a	0.58a	25.3a
Gb(Rg)		16900a	73900b	206000a	22700b	18900a	1130a	16600a	255a	79.5b	226b	0.72a	14.1a
Gb(Cf)	40.0b	**31600b**	94600ab	227000a	29200ab	30400b	1150a	15200a	**410b**	95.0ab	302ab	3.03b	20.3a
Gb(Tg)		18700a	106000a	256000a	28500ab	21200ab	2340b	15900a	289a	95.2ab	286ab	2.10b	25.6a
Pn	11.2a	6930ab	77700a	126000a	12000a	15200a	358a	3780a	412a	55.3a	240a	0.23a	8.59a
Pn(Rg)		3570a	25100a	32800b	5390a	3840b	131a	853b	76.1b	18.8a	63.5b	0.27a	2.93a
Pn(Cf)	7.30a	12000b	79200a	92900ab	12100a	12600ab	366a	2120ab	277ab	55.6a	175ab	0.2a	6.48a
Pn(Tg)		6100ab	66100a	84500ab	13800a	10000ab	432a	2350ab	220ab	45.3a	150ab	0.23a	7.74a
G.RH	29.8a	10800a	112000a	155000a	20500a	16200a	1200a	6610a	257a	80.9a	279a	0.82a	23.3a
G.RH(Cf)	26.3a	**22400b**	90200a	159000a	20800a	19500a	752a	**13400b**	287a	83.5a	275a	**1.80b**	11.2a
Rg		517a	18000a	7100a	2850a	2230a	106ab	221a	47.2a	6.36a	10.7a	0.21a	2.15a
Rg(Gb)		691a	20500a	6950a	2810a	2690a	85.7a	150a	18.7b	5.73a	9.59a	0.62a	1.89a
Rg(Pn)		752a	21700a	9260a	3580a	3760a	157b	192a	30.1ab	7.33a	12.8a	0.41a	2.00a
Cf		1050a	49100a	10300a	2970a	3450a	115a	488a	33.8a	11.1a	19.1a	0.55a	3.96a
Cf (Gb)		899a	38800a	11300a	2680a	2600a	92.7a	451a	31.6a	9.56a	16.1a	0.72a	3.20a
CF(Pn)		867a	33900a	10300a	2240a	2500a	133a	625a	43.5a	10.6a	16.6a	0.70a	4.43a
Cf (GRH)		913a	44900a	7410a	2510a	3310a	111a	494a	60.8a	10.5a	10.2a	0.70a	6.84a
Tg		554a	13400a	3710a	1020ab	1430a	**140a**	152a	15.2a	5.8ab	9.96a	0.57a	**5.36a**
Tg(Gb)		596a	13600a	3490a	715a	1400a	51.5b	125a	17.0a	4.5b	7.87a	0.38a	1.35b
Tg(Pn)		767a	19200a	4410a	1460a	2390a	76b	125a	19.0a	7.19a	11.1a	0.53a	2.36b

Table 4.5 The total uptake of each nutrient (µg) by all of the foliage contained in the pots (species combined), at final sampling, according to whether the six species were growing alone or with a companion species. (Gb: *Grevillea barklyana*, Pn; *Protea neriifolia*, G.RH; *Grevillea* Robin Hood, Cf, Cocksfoot; Rg, Ryegrass; Tg, Tussock grass). Different letters separately indicate significant differences ($p < 0.05$) within each block of treatments. Bold alphanumericals indicate significant differences to when the species was growing alone. Minimum detectable limits of Mo (0.02 mg kg^{-1}) were used for uptake calculations of this element.

Species	P	K	Ca	Mg	S	Fe	Mn	Zn	Cu	B	Mo	Ni
Gb	20600b	127000a	268000a	30500ab	23600b	1600b	12500b	240b	121a	322a	0.58cd	25.3a
Gb&Rg	17600b	94400b	213000a	25500b	21600b	1210b	16800a	273b	85.2b	236b	1.34c	16.0ab
Gb&Cf	**32500a**	133000a	238000a	31900a	**33000a**	1240b	15600ab	**442a**	105ab	318a	**3.75a**	23.5a
Gb&Tg	19300b	120000ab	260000a	29200ab	22600b	2390a	16000ab	306b	99.7ab	294ab	2.48b	27.0a
Rg	517c	18000d	7100b	2850c	2230c	106c	221c	47.2c	6.36c	10.7c	0.21d	2.15c
Cf	1050c	49100c	10300b	2970c	3450c	115c	488c	33.8c	11.1c	19.1c	0.55c	3.96bc

											d	
Tg	554c	13400d	3700b	1020c	1430c	140c	152c	15.2c	5.80c	10.0c	0.57cd	5.36bc
Pn	6930b	77700ab	126000a	12000a	15200ab	359ab	3780a	412a	55.3ab	240a	0.23b	8.59abc
Pn&Rg	4320bc	46800bc	42000bc	8970ab	7600bc	287ab	1050bc	106bc	26.2bc	76.3bc	**0.68a**	4.94bcd
Pn&Cf	**12800a**	113000a	103000a	14400a	15100a	499a	2740ab	320a	66.3a	192a	**0.90a**	10.9a
Pn&Tg	6950b	85300ab	88900ab	15300a	12400ab	524a	2500ab	214ab	53.8ab	161ab	**0.76a**	10.8ab
Rg	517c	18000c	7100c	2850bc	2230c	106b	221c	47.2bc	6.36c	10.7c	0.21b	2.15d
Cf	1050c	49100bc	10300c	2970bc	3450c	115b	488c	33.8bc	11.1c	19.1c	0.55ab	3.96cd
Tg	554c	13400c	3700c	1020c	1430c	140b	152c	15.2c	5.80c	10.0c	0.57ab	5.36bcd
G.RH	10800b	112000ab	155000a	20500a	16200a	1200a	6610b	257a	80.9a	279a	0.82b	23.3a
G.RH&Cf	**23300a**	135000a	167000a	23300a	22800a	863a	**13900a**	348a	94.0a	285a	**2.50a**	18.0ab
Cf	1050c	49100b	10300b	2970b	3450b	115b	488c	33.8b	11.1b	19.1b	0.55b	3.96b

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Chapter 5

Grasses procure key soil nutrients for clovers

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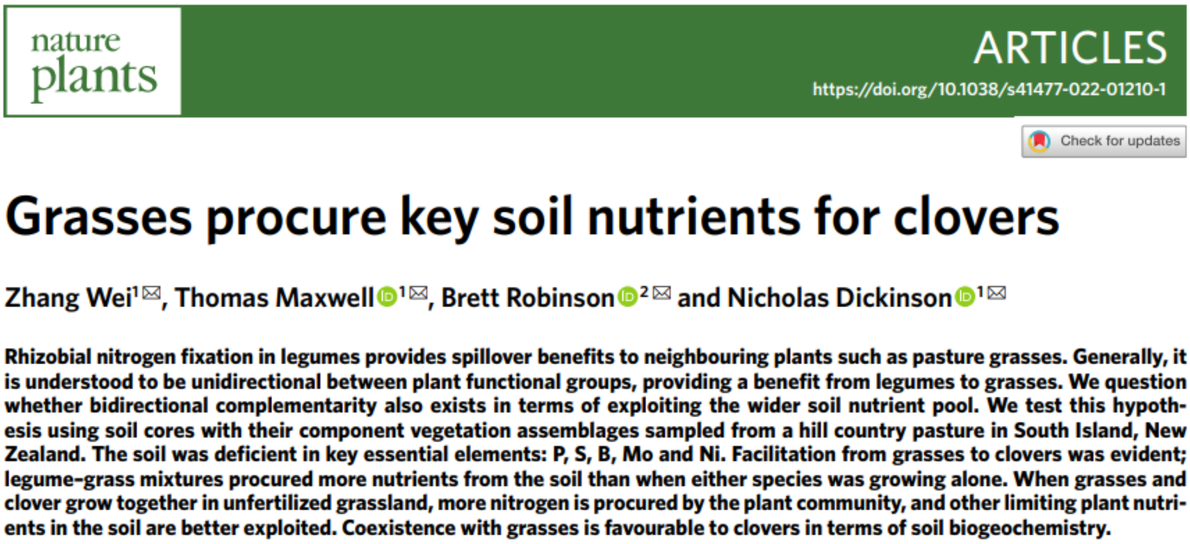

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Grasses procure key soil nutrients for clovers

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Rhizobial nitrogen fixation in legumes provides spillover benefits to neighbouring plants such as pasture grasses. Generally, it is understood to be unidirectional between plant functional groups, providing a benefit from legumes to grasses. We question whether bidirectional complementarity also exists in terms of exploiting the wider soil nutrient pool. We test this hypothesis using soil cores with their component vegetation assemblages sampled from a hill country pasture in South Island, New Zealand. The soil was deficient in key essential elements: P, S, B, Mo and Ni. Facilitation from grasses to clovers was evident; legume–grass mixtures procured more nutrients from the soil than when either species was growing alone. When grasses and clover grow together in unfertilized grassland, more nitrogen is procured by the plant community, and other limiting plant nutrients in the soil are better exploited. Coexistence with grasses is favourable to clovers in terms of soil biogeochemistry.

5.1 Abstract

Rhizobial nitrogen fixation in legumes provides spillover benefits to neighbouring plants such as pasture grasses. Generally, it is understood to be unidirectional between plant functional groups, providing a benefit from legumes to grasses. We question whether bidirectional complementarity also exists in terms of exploiting the wider soil nutrient pool. We test this hypothesis using soil cores with their component vegetation assemblages sampled from a hill country pasture in South Island, New Zealand. The soil was deficient in key essential elements: P, S, B, Mo and Ni. Facilitation from grasses to clovers was evident; legume–grass mixtures procured more nutrients from the soil than when either species was growing alone. When grasses and clover grow together in unfertilized grassland, more nitrogen is procured by the plant community, and other limiting plant nutrients in the soil are better exploited. Coexistence with grasses is favourable to clovers in terms of soil biogeochemistry.

5.2 Introduction

Mid-altitude grasslands (approx. 400 – 1,000 m asl., with slopes >15°) account for around 18% of the total land area of New Zealand[1]. This pastoral hill country farmland was converted from forests, mostly in the last few centuries, and contributes substantially to the economy[2]. Vegetation productivity and stocking rates are constrained by a hot and dry summer climate combined with nutrient-poor, low-pH, shallow soils. Steeper slopes are prone to erosion and typically only about 10-20% of this land is sufficiently flat to be cultivated for crops. Grazing herbage consists largely of introduced exotic species with some remnants of native grasses and shrubs. Native species are less productive and nitrogen-fixing forbs are almost entirely absent from the indigenous flora[3]. Pastoral farming is reliant on over-sowing with seeds of more productive introduced grasses and legumes and top-dressing with fertilisers and lime. The latter is prohibitively expensive due to the large expanse of this landscape and its difficult trafficability that requires the use of aircraft. Opportunities to establish productive pasture, such as ryegrass-white clover swards, are restricted; more hardy drought-tolerant perennial grasses tend to proliferate. Pasture management aims to improve forage quality by increasing the prevalence of annual and perennial legumes, rather than relying on vegetation dominantly consisting of more hardy grasses of less forage value[4,5]. Stock (mostly sheep) productivity and health are markedly improved by increasing the establishment, productivity and persistence of naturalised nitrogen-fixing plants. The hill country environment as described provides the template for this study.

We question whether we properly understand the mutualism between grasses and clovers (*Trifolium* spp.) in terms of soil biogeochemistry. It is well known that nitrogen fixed by legumes can be utilized by grasses or other companion plants[6]. Nitrogen fixation can be substantially constrained by soil acidity and a limiting supply of key nutrients such as phosphorus, especially without fertiliser intervention. It has become clearer recently that multiple-nutrient constraints widely impact primary productivity in grasslands[7,8]. Plant species differ in their ability to acquire key nutrients; for example, P is obtained through symbiotic mycorrhizal associations[9] or through root adaptations to resource partitioning of this element[10]. Other plants, including grasses, can secrete organic acids (phytosiderophores) that mobilise deficient chemical elements (e.g., Fe, Zn, Cu, Mn) in soil[11]. Competition for resources is more extensively studied than are the mutual benefits that may be derived from coexistence of different plant species or functional groups, although the temporal and spatial advantages of plants growing together are well known[12-14]. Belowground functional traits in plants are recognised to be important for nutrient uptake, but interactions between root systems and possible complementarity between species are poorly understood[15]. However, there would be an obvious advantage to a plant of sharing limiting soil resources with a neighbouring species if there is reduced metabolic cost to both[16].

In the present study, we hypothesize that clovers benefit from the presence of companion grasses in terms of procurement of essential nutrients, and this relationship is not limited to spillover of nitrogen from clovers that is subsequently exploited by grasses. We aim to determine whether complementarity between legume and grass rhizospheres is bidirectional in nutritional terms and more significant than we currently realise. We extracted 200 soil cores with their component plant assemblages from a hill country sheep station to investigate how the soil pool of nutrients was exploited. The cores were maintained as experimental microcosms in a controlled environment growth chamber that simulated historic ambient temperature and sunlight conditions without limiting water availability. The study had three objectives: (i), to evaluate existing small-scale variability of nutrient mobility and uptake, (ii), to investigate whether grasses modify the nutrient content of clovers, and (iii), to explore the extent to which manipulation of key soil nutrients modifies the nutrition of clover/grass assemblages. Three separate experiments were designed to pursue these objectives

5.3 Results and discussion

Clovers produced more above-ground biomass than grasses in all soils below pH 7, growing significantly better in the more acid soils where yields were double those of grasses (Supplementary Data, Figure 5.7). There was an effect of soil pH on mobility and uptake of nutrients (Figure 5.1). Optimal pH for uptake of most nutrients was in the range of 5 - 6.5, but analyses of these cores indicated there is considerable small-scale spatial variability across the sampling site both below and above this pH range (Figure 5.2). Nutrient concentrations in pore water tended to be higher in lower pH soil cores for six elements (P, S, Fe, Ni, B, Zn). Except for B, this was reflected in foliar concentrations of the same elements in either clover or grasses, or in both. Foliar concentrations of Mo were unique in this dataset, substantially declining in lower pH cores. Three elements (P, Mo and Ni) were at significantly higher foliar concentrations in grasses than in clovers, and five elements (K, Ca, Mg, Zn, B) were in much higher concentrations in clover foliage. Manganese concentrations in soil pore water varied in the opposite direction to concentrations in foliage which may be a result of the plants readily taking up this element through non-specific transporters[17] and depleting mobile forms of this element in the soil. We have found previously that pore water data collected from rhizon samplers can be variable and unreliable, depending on pathways of water flow through the soil which can produce a rapid flush into the sampler or a slow seepage, thus providing differing amounts of time to accumulate solutes. A range of critical but complex interactions occur in the rhizosphere that are likely to be critical to mobilisation of nutrients and to accessing discrete resources from the same soil volume[13], which undoubtedly play an important role in grassland productivity[7,18].

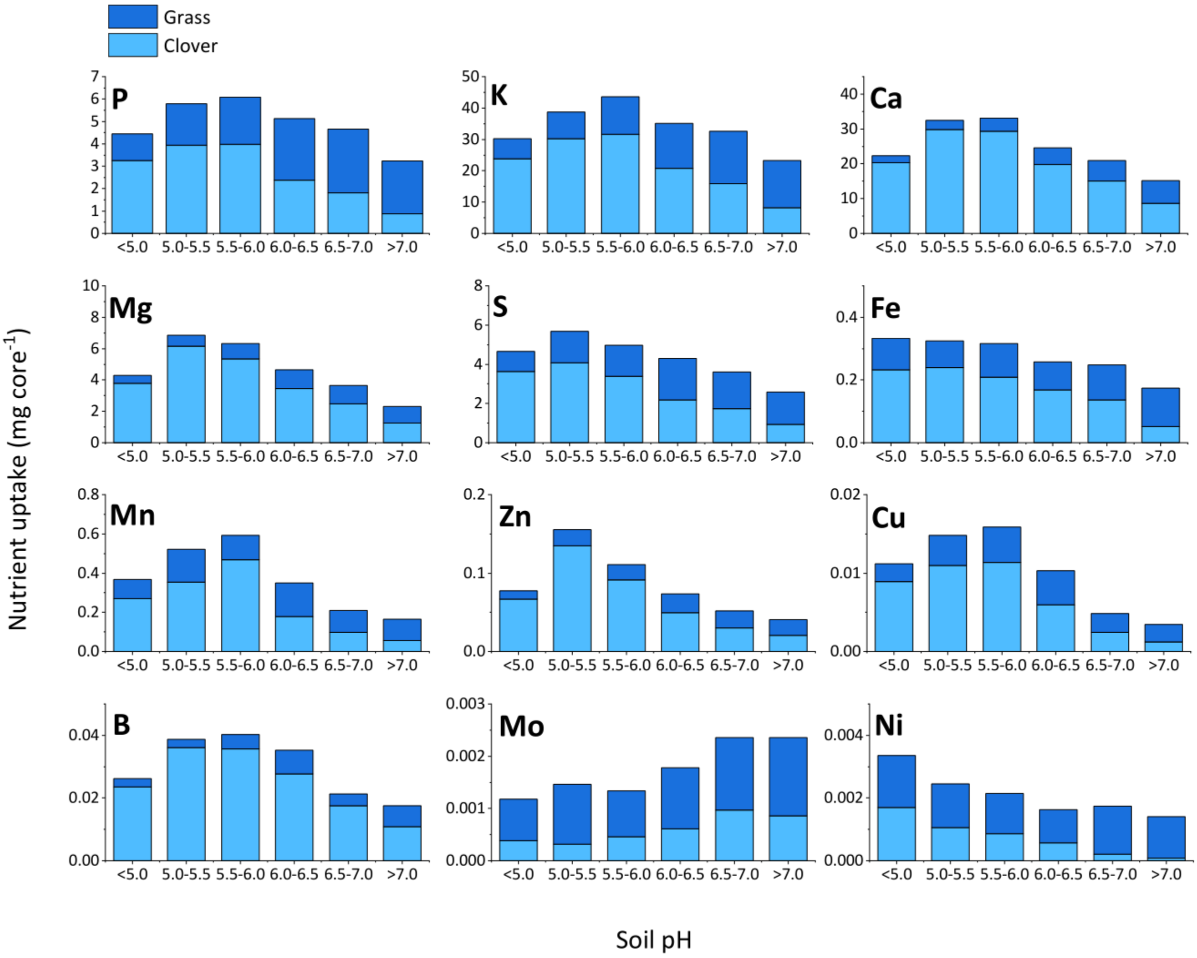


Figure 5.1 Mass uptake of each nutrient from soil into foliage of clovers and grasses growing in cores across the soil pH range (means, n=5).

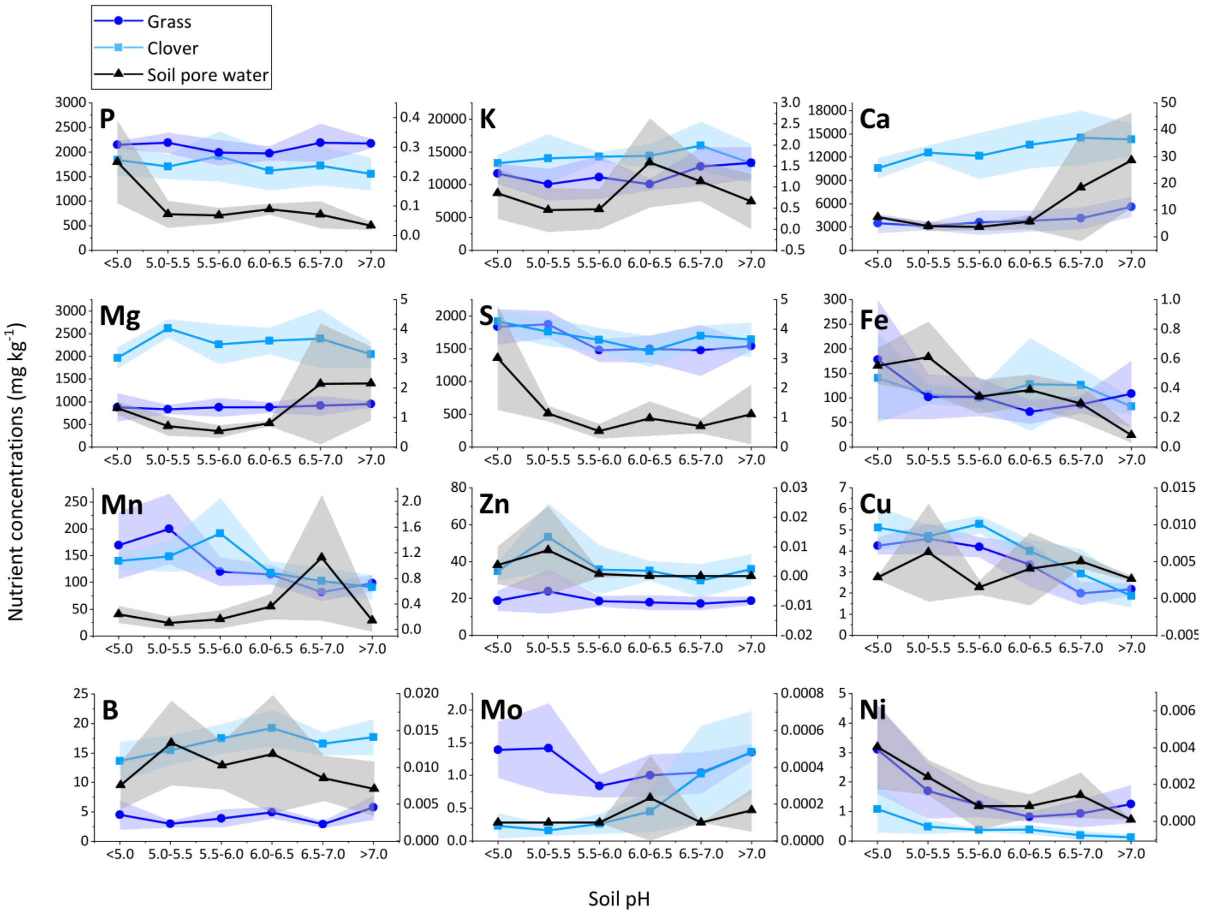


Figure 5.2 Nutrient concentrations in the foliage of clovers (mg kg $^{-1}$, n=5) and grasses (mg kg $^{-1}$, n=5) and in soil pore water (mg L $^{-1}$, n=3) across the pH range of the soil cores. RHS y axis is for pore water. Values are means.

In a second experiment, clovers were competitively dominant in terms of yield when growing in mixed species assemblages with grasses and appeared to be the main beneficiary of coexistence. Growing as single species, yield of clovers was approximately double that of grasses and did not significantly differ in the presence of grasses (Figure 5.3). Conversely, grass yield was reduced by approximately half when growing in mixtures. Clover had higher foliar concentrations of the major nutrients N, P, K, S and Ni when growing in combination with grasses (Figure 5.4). Clovers but not grasses acquired more N when growing in combination, and there was no evidence of N spillover to grasses. Grasses benefited by growing together with clovers through higher foliar concentrations only of Cu and Ni. Potassium, Ca, Mn and B concentrations in grasses were significantly lower when they grew with clovers. Soil pore water contained higher Fe concentrations in cores with only grasses, and higher Zn concentrations in cores with clover-grass mixtures (Supplementary Data, Figure 5.8). When these data are analysed using mass balance (Figure 5.5), mixed species assemblages extracted significantly larger quantities of 4 elements (P, S, and Fe) from soil than when either grasses or clovers were growing alone. This is largely explained by the increased combined biomass in mixed species assemblages, and by less yield of grasses in mixture. However, differences in foliar nutrient concentrations between clovers and grasses also contribute substantially to quite different exploitation of the nutrient content of the soil cores. When growing as single species, clovers captured more of 9 of the 13 elements than grasses. Grasses were more efficient than clovers at procuring only Mo from the soil, which is a particularly critical element for N fixation in legumes[19].

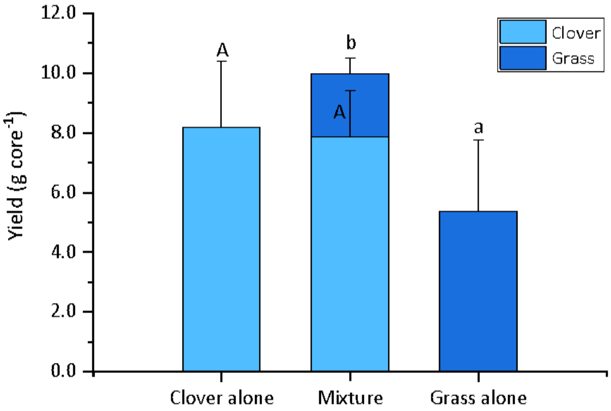


Figure 5.3 Biomass yields of clovers and grasses growing either alone or in a mixed species assemblage (mean ± s.d., n=10). Upper case letters show significant differences (p< 0.05) between clovers, lower case letters between grasses. Data were analyses using one-way ANOVA following log transformation where data were not normally distributed.

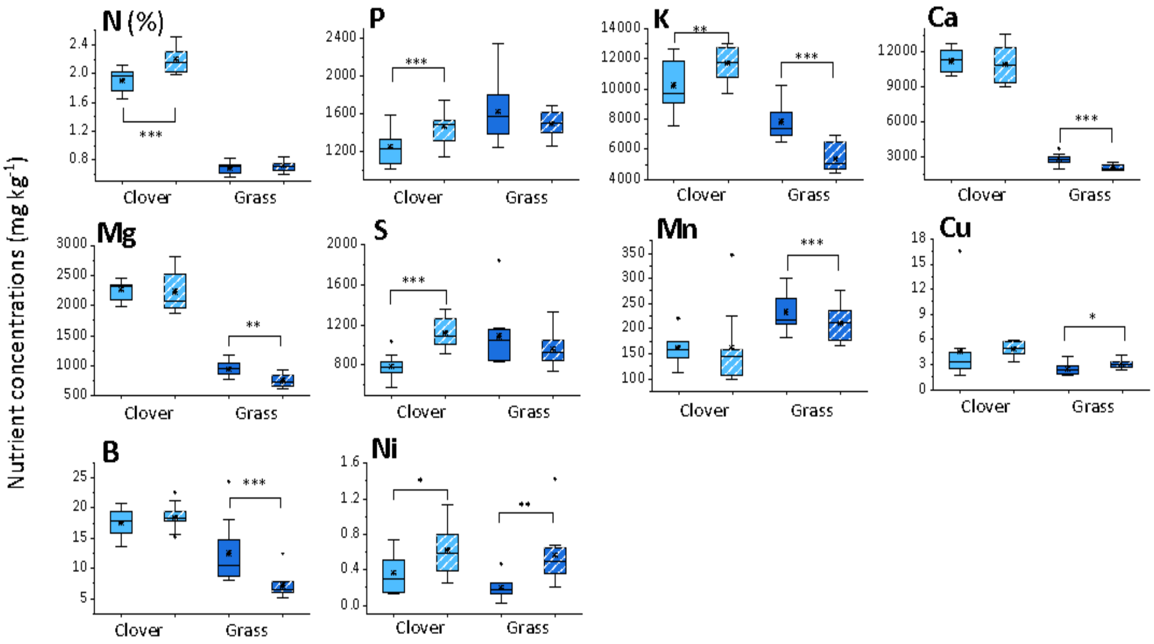


Figure 5.4 Nutrient concentrations in foliage of clovers and grasses according to whether they were growing alone (solid bars) or in mixed species assemblages (striped bars), n=10. Asterisks show significant differences (* p < 0.05; ** p < 0.01; ***p < 0.001). Box plots show the upper whisker, upper quartile, median, lower quartile and lower whisker from top to bottom. Data were analyses using one-way ANOVA following log transformation

where data were not normally distributed.

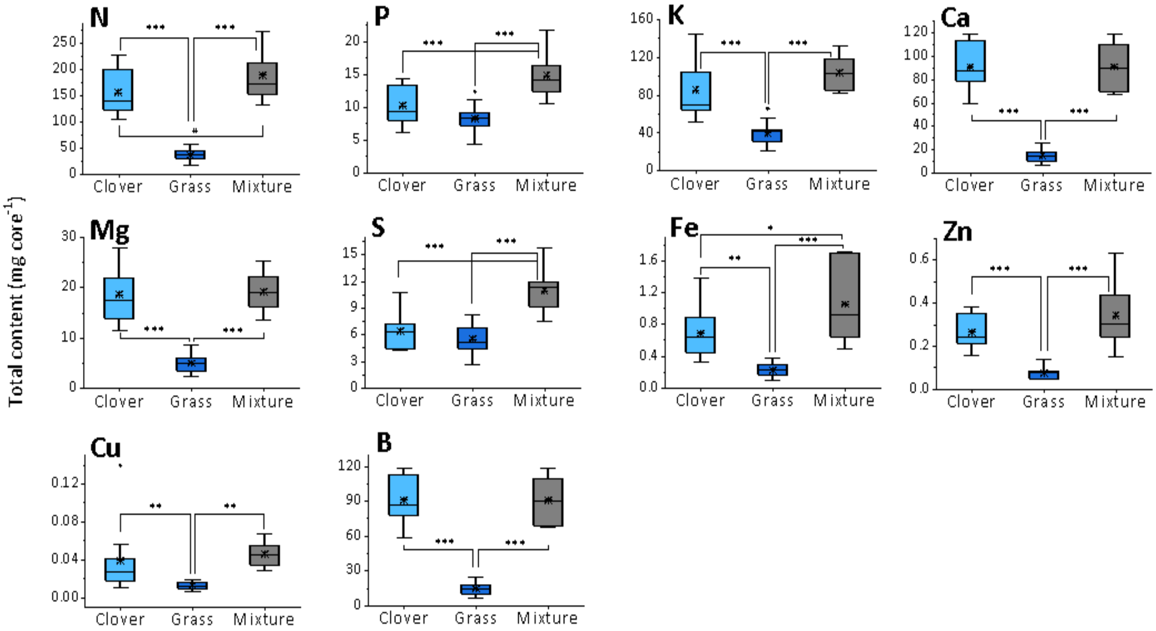


Figure 5.5 Total uptake of nutrients from soil cores that supported clovers or grasses growing either alone or in mixed species assemblages (n=10). Asterisks show significant differences (* $p < 0.05$; ** $p < 0.01$; ***$p < 0.001$). Box plots show the upper whisker, upper quartile, median, lower quartile and lower whisker from top to bottom. Data were analyses using one-way ANOVA following log transformation where data were not normally distributed.

In a third experiment, adding nutrients to the soil cores had a negligible effect on plant yield, with the exception of adding P (Figure 5.6). We interpret this finding as a failure to apply the fertilisers early enough in the growth season when plants were entering their most productive phase of the growth cycle. Nevertheless, manipulation of key soil nutrients using fertiliser to improve the productivity of clover/grass assemblages has been well described previously in the field situation[6,7,20,21] and may have added little to the present findings in relation to unfertilised pasture.

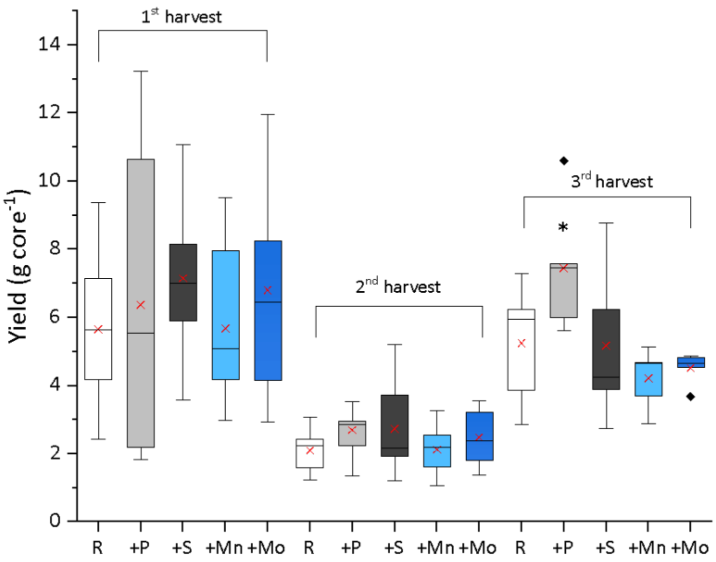


Figure 5.6 Biomass yield of mixed clover- grass assemblages after fertilising the soil cores with either sulphur (+S), molybdenum (+Mo), manganese (+Mn) or phosphorus (+P). Fertiliser were applied twice, after the 1st and 2nd harvest (n=10; 3rd harvest, n=5). Open bars (R) are for the reference cores (n = 10; 3rd harvest, n=5) without fertiliser amendment (* P<0.05). Asterisk show significant difference (* $p < 0.05$). [Red x= means, ♦ = data points excluded from the statistical analysis as outliers]. Box plots show the upper whisker, upper quartile, median, lower quartile and lower whisker from top to bottom. Data were analyses using one-way ANOVA following log transformation where data were not normally distributed.

Contrary to the field situation in the high country, clovers were significantly more productive than grasses in the microcosms (apart from cores with soil pH > 7). In the field there is no doubt that grasses generally tend to become dominant without proactive management of grazing and fertility. This is largely a consequence of low rates of naturalization of clovers and the inability of both annual and perennial clovers to withstand summer drought without going to seed. Moisture conditions that were maintained in the soil cores favoured the clovers more than the more drought-tolerant perennial grasses. The present experimental work did not realistically match the field situation in terms of simulating typical moisture deficits, but it was considered more important to maintain suitable conditions for plant growth. Modelling the effect of soil moisture deficit on nutrient cycling requires more work.

Whilst soil nutrient analysis data has drawn attention to deficiencies of P, S, B, Mo and Ni in these acid soils, the biogeochemical complexities of nutrient mobility within the rhizosphere makes the prediction of key deficient elements less straightforward[22]. Nevertheless, many of the findings of the present study are explained by existing knowledge of nutrient mobility in soils and plants. For example, S, Fe and Zn were at higher concentrations in pore water at lower pH, and this was reflected in plant tissue concentrations. This would be expected for the two metals that are more soluble in acid conditions and enter plants through passive flow. Low concentrations in pore water may simply reflect plant uptake and depletion of the labile soil pool; for example, the lowest concentrations of Mn in pore water occurred alongside high concentrations in foliage. Foliar Mn is known to be closely associated with P acquisition in the rhizosphere[17]. Other pore water concentrations are less easy to explain, and the reliability of the pore water data is likely to be questionable. For example, soluble phosphate and availability of this element to plants is highest at pH 6-7[23], but this is not reflected in the data in the present study. Boron is known to be most available under neutral pH conditions in soils and is readily leached at low pH but, like Ni, its apparent lack of correlation with soil pH may be indicative of the very low soil concentrations of these elements. At higher pH, pore water concentrations of five other elements (K, Ca, Mg, Mn, and Mo) appeared to be generally more labile.

Our data do not elucidate the exact processes of the interaction between clovers and grasses, and more detailed mechanistic studies are required to guide further thinking and experimentation. Interactions between different nutrients within the rhizosphere undoubtedly played a large role in our findings. The main reason for adding lime to hill country soils is to increase the mobility and availability of Mo to clovers to increase N fixation[23]. Hydrogen ions and organic acids released by roots acidify rhizosphere soil. One effect of this is to decrease soil redox potential, reducing oxidized materials and increasing the solubility of Fe and Mn[24]. Phytosiderophore exudation from grass roots are part of a rhizosphere priming effect that stimulates organic matter decomposition and nutrient cycling[25,26]. Uptake of K is mainly determined by the characteristics of the root system and root exudates[27], but also involves Ca^{2+} and other secondary messengers[18]. Production of root exudates can be a response to environmental stress, including nutrient stress[26]. Magnesium improves nitrogen use efficiency, although basic research on Mg nutrition is lacking[28]. Exudates released by grasses are known to enhance the activity of soil microorganisms which accelerate oxidation of S, allowing more available forms of S to be utilized by plants. Microorganisms play a major role in sulphur oxidation in soil, for example by mineralising organic S^{2-}[29]. Clearly, mechanistic explanations are complex and require more detailed study.

In terms of complementarity of species, there is an obvious advantage to a plant of acquiring a limiting soil resource at reduced metabolic cost[16]. Clovers acquired higher concentrations and quantities of all nutrients (apart from Ni and Mo) than grasses. Grasses were better than clovers at procuring Mo from soil, even though the Mo requirement of clovers is likely to be greater[22]. Molybdate is the predominant form of Mo available to plants, but there is little knowledge of how plants access molybdate from the soil solution and redistribute it within the plant. We know its availability is strongly dependent on soil pH and organic compounds in soil colloids[30]. Furthermore, phytosiderophore release by grass roots that occurs under Fe, Zn, Mn and Cu deficiencies is thought to be an adaptive response to enhance the acquisition of micronutrient metals[31,32]. We speculate that there are important mutually beneficial rhizosphere interactions between grasses and clovers within which Mo is likely to play a key role. More nitrogen was procured by clovers, but not grasses, when clovers were growing in mixture. An explanation for this may be that the growth cycle of grasses was already advanced at the onset of the experimental work. The grasses were not in an early stage of seasonal growth and may have had limited demand for N. This was also our best explanation for the failure of a selective herbicide to kill the grasses.

Our hypothesis that clovers benefit from the presence of grasses is supported by the fact that clover had higher foliage concentrations of major nutrients N, P, K, and S when growing in combination with grasses, procuring significantly larger quantities of P, S and Fe from soil than when either were growing alone. The latter is a combination of improved plant nutrition and higher yields. Many earlier studies have shown that mixed plant assemblages result in overall higher plants biomass[33] and extract more nutrients from the soil than monocultures[34]. This is generally explained by better spatial exploitation of the soil by different root architectures[35,36]. The present findings are indicative of more positive mutually beneficial interactions most likely involving a rhizosphere priming effect[26,37] of grasses that benefits clovers. This is a first report of the nutritional benefits of mixed communities of grasses and clovers in a field situation, showing that grasses enhance the ability of clovers to procure limiting plant nutrients. Chemical communication that has evolved in the rhizosphere of mixed plant communities is poorly understood, even in the context of the nitrogen cycle[38]. The findings of the present study show that grasses provide an advantage by enhancing N-fixation by clovers, also increasing the mobility and uptake of other major nutrients and trace elements. Recognised deficiencies of P, S, Mg, B, Mo and Ni in these soils, to a significant extent, are mitigated by rhizosphere interactions that occur between grasses and clovers. This plays a key role in the interplay of key elements that are modified by and modify the physicochemistry of soil, nutrient lability and plant uptake.

Enhanced exploitation of soil chemistry probably has wider relevance to other low-input forms of agriculture[22] and to provide research pathways to build science-based evidence relating to regenerative agriculture[39]. We conclude that more attention is required towards enhancing diversity rather than attempting to simplify species assemblages in grassland pastures. Clovers and grasses have different functional roles that are complementary. Advancing this knowledge through further research could also help optimise the use of fertiliser amendments in pasture farming systems. We suggest future work could identify root exudates of mono- and dicot combinations using rhizoboxes fitted with suction cups; deployment of planar optodes in such rhizoboxes may reveal the spatial locations of root interactions. This knowledge may be used to determine optimal plant species combinations for pasture management. Our findings in this paper support other studies that have argued that maintaining and re-establishing plant diversity could be a way to sustainably manage temperate grasslands[40] towards sustainable intensification[41] as well as to combat grassland degradation[42].

5.4 Methods

5.4.1 Study site

Soil cores were extracted from a 1607 ha high country sheep farm (Mt Grand Station) situated in Hawea, Central Otago on South Island which has a continental-type climate of hot dry summers and cold winters10. Soils are derived primarily from metamorphic schists and loess, and are defined as a Pallic Yellow Grey Earths. They are acidic (typically pH 5.5) with low concentrations of P, S, B, Mo and Ni compared to typical soils elsewhere in New Zealand (Supplementary Data, Table 1). They are also deficient in Na which is an essential element for stock but not for plants. Annual rainfall is 703 mm with high annual and seasonal variability 5. The station supports < 2 stock units of sheep ha -1 that probably harvest < 3 t DM ha -1 across the whole landscape (Lucas, D., pers comm.).

The sampling location was an approximately 400 m2 area of mixed grass-legume pasture on a mid-altitude (700m) north-facing (sunny aspect) moderately steep (20 – 30 o) hillside slope within a 65-ha grazed pasture block. White clover (Trifolium repens L.) and subterranean clover (T. subterraneum L.) have been periodically sown at this location, but the vegetation has a larger component of adventive and naturalized grasses and forbs; in an earlier study of the same sampling area, Maxwell et al., 5 identified 4 other species of clovers, 11 species of grasses and 4 other forb species. There is also a very small component of native grasses (Rytidosperma spp.). The sampling area is largely below the altitude and too heavily grazed for native tussocks to form a significant component of the vegetation, although remnants of one lower-altitude tussock grass (Poa cita) and a native woody shrub (Discaria toumatou) are established in patches adjacent to the sampling location.

5.4.2 Experimental

A total of 202 cores were randomly collected (10 cm diameter, 15 cm depth, 1.2L) using a soil corer in the Spring month of September when sward height was approximately 3-5 cm. The core samples were inserted into polythene sleeves of slightly larger diameter (15 cm diameter, 20 cm depth, 3.5L). The gap surrounding the cores was packed with perlite and they were then transferred into a growth chamber set as near as possible to the current day / night temperatures, sunlight hours and light intensity recorded at the same time of year at Mt Grand. Environmental conditions in the chamber were modified with time accordingly. Soil moisture was carefully maintained in the microcosms by regular watering in equal amounts to prevent the cores either drying out or becoming saturated: approximately 20ml of water was added twice weekly during three-month period. We considered this deviation from field conditions was justified to maintain plant growth and nutrient mobility in soil and plants during the experimental period.

Cores selected for the experimental work were those contained an approximately equal amounts of grasses and clovers. The experimental cores were then further selected and grouped in accordance with the objectives of three experiments. At the outset, vegetation in all cores was clipped to 2cm above the soil surface. Three separate experiments were designed as follows:

(i) pH and nutrient mobility. Soil pH was measured in every core using a surface probe attached to a HANNA HI 99121 pH meter surface pH. Cores were then divided into a range of six pH intervals: 4.5 – 4.99 (n=10), 5.0 - 5.49 (n=61), 5.5 - 5.99 (n=52), 6.0 - 6.49 (n=33), 6.5 - 6.99 (n=34), > 7.0 (n=12). Five replicates from each of the 6 pH ranges.

Soil pore water rhizon samplers (5cm porous samplers with female luers, Rhizosphere Research Products) were inserted into 3 cores randomly selected at each pH interval. They were positioned diagonally at approximately 20 o to sample pore water between depths of 5 – 10 cm. Soil pore water was then collected after one month in the growth chamber. Above-ground vegetation was harvested from the cores by hand clipping to 2 cm after 3 months in January, then dried and weighed. Nutrients were analysed separately in foliage of grasses and clovers, each bulk sampled from each core.

(ii) Grasses and the nutrient content of clovers. A further 30 cores were selected within the pH range 5.2 – 5.7. Either grasses or clovers were selectively removed from 10 cores each. Both a selective herbicide and careful hand removal were tested in a preliminary trial, before settling on the latter. The additional ten cores were left with a mixture of grasses and clovers, selecting those with an approximately equal ratio of clovers and grass. Five soil pore water rhizon samplers were inserted as above into cores of each treatment. Harvest and analyses followed those described above.

(iii) Amendment of soil fertility. One of four nutrients (P, S, Mn and Mo) were added to the microcosms at approximately standard fertilizer rates of application that would typically be used in the hill country. Salts of each element (0.58g (NH_4) H_2PO_4, 513 kg·ha^{-1}, 0.0037g $Na_2MoO_4 \cdot 2H_2O$, 3.3kg·ha-1, 0.6g $MnSO_4 \cdot 3H_2O$, 530kg·ha^{-1} and 0.15g S, 135.4kg·ha^{-1}) were dissolved in 10ml volume of water. Pots with similar pH value (range 5.7 - 6.7) and a comparable ratio of legume and grass were selected, and herbage was clipped at 2cm before adding nutrients. Yield was measured twice, at two-month intervals.

Analytical. Soil and plant samples were dried at 25^{o}C/65 ^{o}C and ground (2 mm) then analysed by

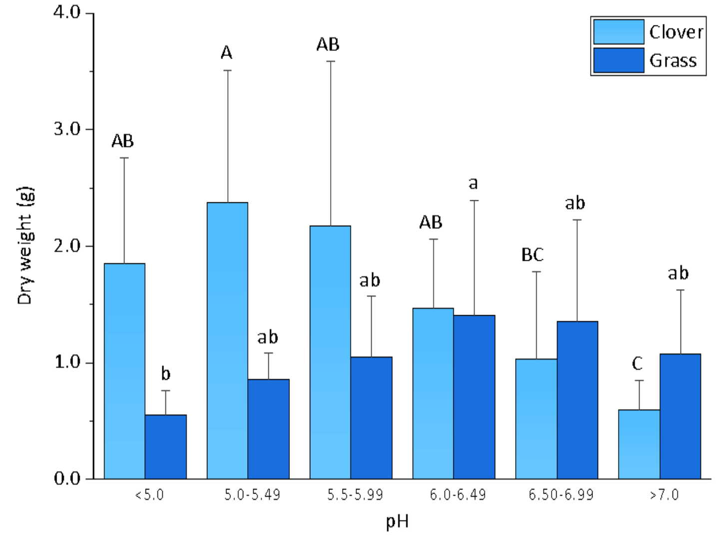
Analytical Services at Lincoln University following standard procedures. N was determined by Elementar Rapid Max N Elemental Analyser. Other elements were determined using ICP-OES. Soil pore water was analysed using an ICP-MS facility at Canterbury University. Nutrients in foliage are expressed both in terms of foliage concentrations (mg kg $^{-1}$) and mass balance (concentration x dry weight of foliage). For statistical analysis, data not normally distributed were log-transformed before analysis. Differences between means were determined using one-way ANOVA, with a post-hoc Fisher LSD test. All analyses were conducted using Minitab 19.

5.5 Supplementary Data

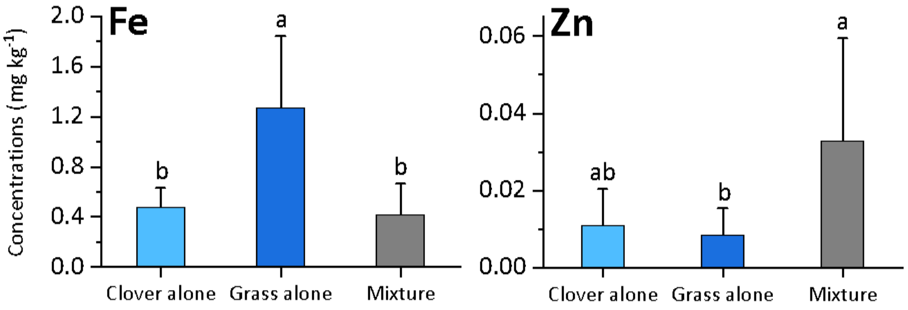
Table 5.1 Soil properties of the experimental soil, with typical range for agricultural soils in New Zealand.

Determinant	Unit of Measurement	Concentration	Typical Range
pH		5.6-7.4	5.7-6.2
Organic Matter [1]	%	**5.0**	7.0-17.0
Total Nitrogen [2]	%	0.26	0.3-0.6
Olsen Phosphorus [3]	mg L^{-1}	**6.3**	20-30
Phosphorus[7]*	mg kg^{-1}	551	200-1,500
Potassium [4]	me 100g^{-1}	0.29	0.3-0.6
Calcium [4]	me 100g^{-1}	7.3	5.0-12.0
Magnesium [5]	me 100g^{-1}	0.7	0.6-1.2
Sulphate-Sulphur [6]	mg L^{-1}	**2.3**	10.0-20.0
Sulphur [7]*	mg kg^{-1}	364	300-3,000
Iron[7]*	mg kg^{-1}	12,170	5,000-10,000
Manganese [7]*	mg kg^{-1}	377	200-3,000
Copper [5]	mg L^{-1}	0.4	0.4-2.0
Zinc [5]	mg L^{-1}	2.6	0.8-4.0
Boron [5]	mg L^{-1}	**0.2**	0.6-1.2
Molybdenum [7]*	mg kg^{-1}	**0.05**	0.5-10.0
Nickel [7]*	mg kg^{-1}	**4.6**	20-30

Analyses follow standard methodology from a commercial laboratory and also Lincoln University Analytical Services*. Analyses by the commercial laboratory are routinely carried out on defined volume rather than mass of soil. Analytical methods were: Loss-On-Ignition [1]; NIR [2], Total N by Dumas Combustion; Olsen extraction [3] followed by Molybdenum Blue colorimetry; 1M Neutral ammonium acetate extraction followed by ICP-OES determination [4]; Mehlich 3 Extraction followed by ICP-OES [5]; 0.02M Potassium phosphate extraction followed by Ion Chromatography [6]; HNO_3 – $HClO_4$ microwave digestion followed by ICP-OES determination [7]



Supplementary Figure 5.7. Clover and grass yields from cores supporting mixed plant species assemblages across the soil pH range (means ± s.d., n=5). Upper case letters show significant differences ($p < 0.05$) between clovers, lower case letters between grasses. Data were analyses using one-way ANOVA following log transformation where data were not normally distributed.



Supplementary Figure 5.8. Iron and zinc concentrations in soil pore water from soil cores supporting clover or grasses growing alone and in mixed species assemblages. Values are means (± s.d.). Data were analyses using one-way ANOVA following log transformation where data were not normally distributed. Different letters indicate significant differences ($p < 0.05$).

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Chapter 6

Facilitation between species mitigates nutrient constraints in grassland

Plant Soil
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RESEARCH ARTICLE

Companion species mitigate nutrient constraints in high country grasslands in New Zealand

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Brett Robinson · Nicholas Dickinson

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6.1 Abstract

Background Experimental work using pot trials and mesocosm studies has indicated that species combinations are more effective in mitigating the soil nutrient constraints that limit pasture productivity in New Zealand's hill country, but there is little field evidence to support this.

Aim We question whether coexistence of species provides an opportunity to facilitate enhanced uptake and improved procurement of key soil nutrients by assemblages of plants in these mid-altitude grasslands.

Methods Native and exotic legumes and co-occurring plant species were sampled according to whether they were growing together in close proximity or in single species patches. Foliar concentrations of nutrients were compared.

Results Uptake of nutrients by a native broom, *Carmichaelia petriei*, was enhanced when it was growing in combination with native tussock grasses. Enhanced uptake of eight nutrients was recorded in foliage of an exotic legume, *Lotus pedunculatus*, when it was growing with native grasses or within the acuminate foliage of *Aciphylla aurea* (golden spaniard). Foliar concentrations of only P and Mn were elevated in white clover (*Trifolium repens*) foliage when it was growing in combination with grasses. Overall, mutual facilitation of nutrient uptake by combinations of species growing together was in evidence.

Conclusions Some species that are less desirable from an agricultural perspective may improve acquisition of soil nutrients by the plant community. Novel native species assemblages represent an

opportunity to refine pasture management. Exploiting combinations of plant species that facilitate optimal exploitation of nutrients could reduce fertiliser requirements, enhancing and protecting biodiversity in pastoral grasslands.

6.2 Introduction

Steep hill country at altitudes of about 400 - 1000 m accounts for 37% of New Zealand's land area, with approximately half of this being pastoral farmland (Thom 2016, Stats^{NZ} 2021). Future environmental and economic resilience of this landscape is considered to be critical, although this is a multi-faceted and complex management issue (Brower, *et al*., 2020, Rissman, *et al*., 2021, Tozer, *et al*., 2021). Our thesis is that sustainable agricultural development requires more knowledge of nutrient dynamics in the context of biodiversity of both pastoral and conservation grasslands.

Earlier forest clearance, combined with historic overgrazing and associated soil erosion, has provided scope for rebuilding hill country soils through improved pasture management, potentially combined with restoration of native vegetation. Native plant species are likely to be better adapted to the natural environment, but they are low yielding and of much lesser forage value. Agricultural management has focused on conversion of native vegetation and replacement with more productive exotic species. However, pasture and stock production in the hill country are constrained by low soil pH and fertility, particularly in terms of phosphorus, sulphur and trace elements that include molybdenum and boron (Hendrie, *et al*., 2021). Mt Grand Station, the location of the present study, provides a typical example of hill country conditions in these respects (Maxwell, 2010; 2016; Zhang, *et al*., 2022a).

Top dressing with lime and fertilisers is largely impractical and too costly due to topography and the large area of land that will only support limited yields of herbage and stock in the prevailing climate and environment. During little more than 150 years since conversion of this landscape to sheep farming, oversowing with exotic species of grasses and legumes has improved productivity (Bork, *et al*., 2017). Nevertheless, both establishment and sustainability of improved pasture with a suitable component of annual and perennial legumes remains a challenge; seasonal resilience is difficult to achieve and there is encroachment by less desirable invasive grasses and shrubs.

A better understanding of the coexistence of native vegetation with introduced grasses and legumes in the hill country environment may be of benefit to both agriculture and conservation. We have previously reported the results of experimental work of plant uptake of nutrients from hill country soils to investigate the compatibility of both exotic and native plants with contrasting root systems.

Pot trials provided evidence of functional compatibility of mixed-species rhizospheres that facilitate and improve the procurement of limiting soil nutrients (Zhang, *et al*., 2022b). We identified the existence of a mutualistic relationship between legumes and grasses that provided nutritional benefits not just to grasses, but also to legumes (Zhang, *et al*., 2022c). However, in that study, a native tussock grass had lower nitrogen concentrations when growing with the exotic legumes that may reflect a lack of adaptation to coexistence. More recently published mesocosm studies used soil cores with component vegetation assemblages that were extracted from unfertilised grassland in the hill country, and then transferred to a controlled environment growth chamber (Zhang, *et al*., 2022a). Once again, species co-existence was beneficial in terms of uptake of key soil nutrients; facilitation from grasses to clovers was evident.

The aim of the research reported in the present paper was to investigate whether the same type of facilitation between species could be demonstrated *in situ* in a hill country grassland (Figure 1). This sampling exercise was an attempt to validate earlier findings that legumes derive nutritional benefits from growing with grasses in terms of procurement of trace elements in limited supply in the hill country soils. Our hypothesis was that plants growing with companion species would have demonstrably different foliar concentrations of key nutrient elements compared to the same species growing alone.

Plate 6.1 Hill country grassland at Mt Grand Station in Hawea, South Island, New Zealand. *Aciphylla aurea* (Golden spaniard) in the centre foreground amongst *Chionochloa rigida* (snow tussocks), *Festuca novae-zelandiae* tussocks within exotic pasture grass vegetation in the middle distance, and more heavily grazed pasture in the background.

6.3 Methods

This study simply involved sampling and analysing foliage of a range of exotic and native legumes at different altitudes at Mt Grand Station, a Lincoln University owned hill country pastoral farm situated in Hawea, Central Otago on South Island. Sampling locations were semi-randomly selected between altitudes of 700 – 1,000 m asl. in a walkover of the site on a single day by one individual (ZW); where species could be found both growing in close proximity and also in single species patches 0.5 – 3.0 m apart. Our assumption was that both above- and below-ground interactions between species would be markedly less when they were growing further apart. Visually comparable environments and similar soil were important selection criteria; the reason why paired comparisons were always recorded within 3 m of each other. Above-ground plant biomass was sampled of legumes growing either in single species patches or in combination with exotic pasture grasses, a native tussock grass or a native acuminate umbellifer (*Aciphylla aurea*), all of which were widely established across the sampling site.

Table 6.1 Deficient soil nutrients of the experimental soil, with typical ranges for agricultural soils in New Zealand (full details in Zhang, *et al.*, 2022a).

Determinant	Unit of Measurement	Concentration	Typical Range
Olsen Phosphorus [1]	mg L^{-1}	6.3	20-30
Phosphorus[2]*	mg kg^{-1}	551	200-1,500
Potassium [3]	me $100g^{-1}$	0.29	0.3-0.6
Boron [4]	mg L^{-1}	0.2	0.6-1.2
Molybdenum [2]*	mg kg^{-1}	0.05	0.5-10.0
Nickel [2]*	mg kg^{-1}	4.6	20-30

Analyses follow standard methodology from a commercial laboratory and also Lincoln University Analytical Services*. Analyses by the commercial laboratory are routinely carried out on defined volume rather than mass of soil. Analytical methods were: Olsen extraction [1] followed by Molybdenum Blue colorimetry; HNO_3 – $HClO_4$ microwave digestion followed by ICP-OES determination [2]; 1M Neutral ammonium acetate extraction followed by ICP-OES determination [3]; Mehlich 3 Extraction followed by ICP-OES [4].

Vegetation across the sampling site varied with altitude and aspect, consisting of mixed communities of native tussock grassland species with oversown pasture grasses and legumes (Figure 2), with scattered assemblages of woody shrubs (mostly *Discaria toumatou*, *Kunzea robusta* and *Coprosma propinqua*) (*see* DOC, 2006; Duncan, *et al.*, 2001). Some invasive weeds are also well established, notably *Hieracium* spp. in inter-tussock spaces at higher parts of the altitude range, and an invasive shrub *Rosa rubiginosa* in some lower parts. Tall pasture grasses are prominent at lower altitudes of this range, particularly *Anthoxanthum odoratum* (sweet vernal grass), *Agrostis capillaris* (browntop) and *Festuca rubra* (red fescue), with a scattered dispersion of *Trifolium* (clovers) and *Lotus* spp. Samples of the three pasture grasses were amalgamated. Tussock grassland and the proportion of native species in plant communities tends to increase with altitude, with patches of tall acuminate rosettes of *Aciphylla aurea* (golden spaniard). Native tussock grasses included *Chionochloa rigida* (narrow-leaved snow tussock), *Poa colensoi* (blue tussock) and *Festuca novae-zelandiae* (hard tussock). A native broom (*Carmichaelia petriei*), one of a small number of threatened native species of broom found across the station and in the high country (nzpcn.org.nz; DOC, 2006; Mark, 2012), was scattered mostly as individual plants across the sampling site. *Carmichaelia* spp. are commonly referred to as New Zealand brooms (Tan, 2014). Their role in soil development in chronosequences through a large build-up of soil nitrogen and facilitating forest species has been reported previously (Bellingham, *et al*., 2001).

Figure 6.1 Sampling locations at Mt Grand Station.

Altitude (m)	1093	900	1043	871
Plant Assemblages	*Carmichaelia petriei* growing with pasture grasses and *Poa colensoi*	*Carmichaelia petriei* growing with *Chionochloa rigida* and *Festuca novae-zelandiae*	*Lotus pedunculatus* growing with *Acyphylla aurea* and *Chinochloa rigida*	*Trifolium repens* growing with *Festuca novae-zealandiae*
				

Carmichaelia and the four groups of companion grasses (pasture grasses and three species of native tussock grasses) were sampled. Two exotic legumes, *Trifolium repens* (white clover) and *Lotus pedunculatus* (bird's-foot trefoil) and their companion native grass, *Festuca novae-zelandiae* (fescue tussock) and *Chionochloa rigida amara* (narrow-leaved snow tussock), were collected. *Lotus* was also sampled that was growing with or adjacent to *Aciphylla aurea*. Five replicates were sampled for each legume, each by excising five leaves across the canopy. Five replicates of each grass were sampled in the same way. All plant samples were collected at least 2 cm from the ground to avoid soil contamination of samples. All the plants were dried (48hrs, 65^{o}C), finely ground in a mill, then microwave digested followed by elemental analysis using ICP-MS (7500cx, Agilent Technologies) following standard protocols. Total nitrogen was analysed using an Elementar Rapid Max N Elemental Analyser.

6.4 Results

The native broom (*Carmichaelia petriei*) had significantly higher above-ground tissue concentrations of several elements when it was growing with companion species of grasses. Higher foliar concentrations of K, Ca, Mg and B were recorded when it was growing with pasture grasses and with *P. collensoi* at lower altitudes of the sampling range (Figure 6.3A) and higher Mn, Zn and Ni when growing with *C. rigida* and *F. novae-zelandiae* at higher altitudes of the sampling range (Figure 6.3B). Conversely, the two larger native tussock grasses tended to have lower foliar concentrations of nutrients when growing together with broom (Figure 6.4). This was much less evident in the pasture grasses and the small blue tussock, *P. colensoi*. Of all chemical elements, only K and Mn were elevated in grasses growing with broom.

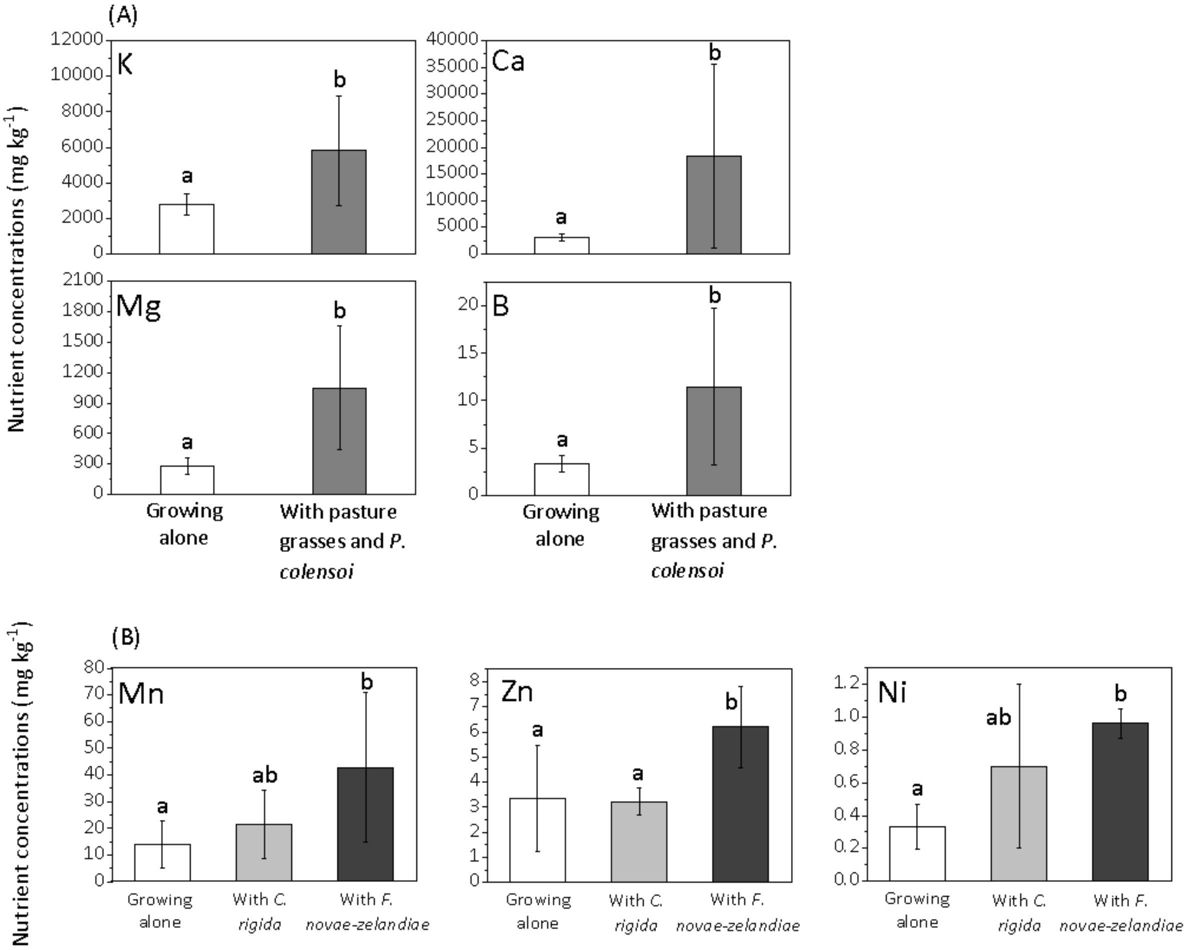

Figure 6.2 Nutrient concentrations in above-ground biomass of broom (Carmichaelia petriei) and the four groups of grasses (pasture grasses, Poa colensoi, Chionochloa rigida and Festuca novae-zelandiae). Figure illustrates only elements when significant differences were recorded. Histogram bars are means ± standard deviations. Different letters separately indicate significant differences ($p < 0.05$).

Foliar concentrations of eight nutrients in *Lotus* foliage were higher when it was growing with either *C. rigida* or *A. aurea*, or with both (Figure 6.5); Fe was the only element in lower concentrations. Snow tussock, *C. rigida*, foliage had significantly higher foliar concentrations of N, Zn, Cu and Mo when growing with *Lotus* (Figure 6.6). In contrast, higher P and Mn concentrations in the foliage of *Trifolium* were in when it was growing with *Festuca novae-zelandiae* were the only significant differences recorded in combinations of clover or grasses (Figure 6.7). No data are available for foliar concentrations of nutrients in *A. aurea*.

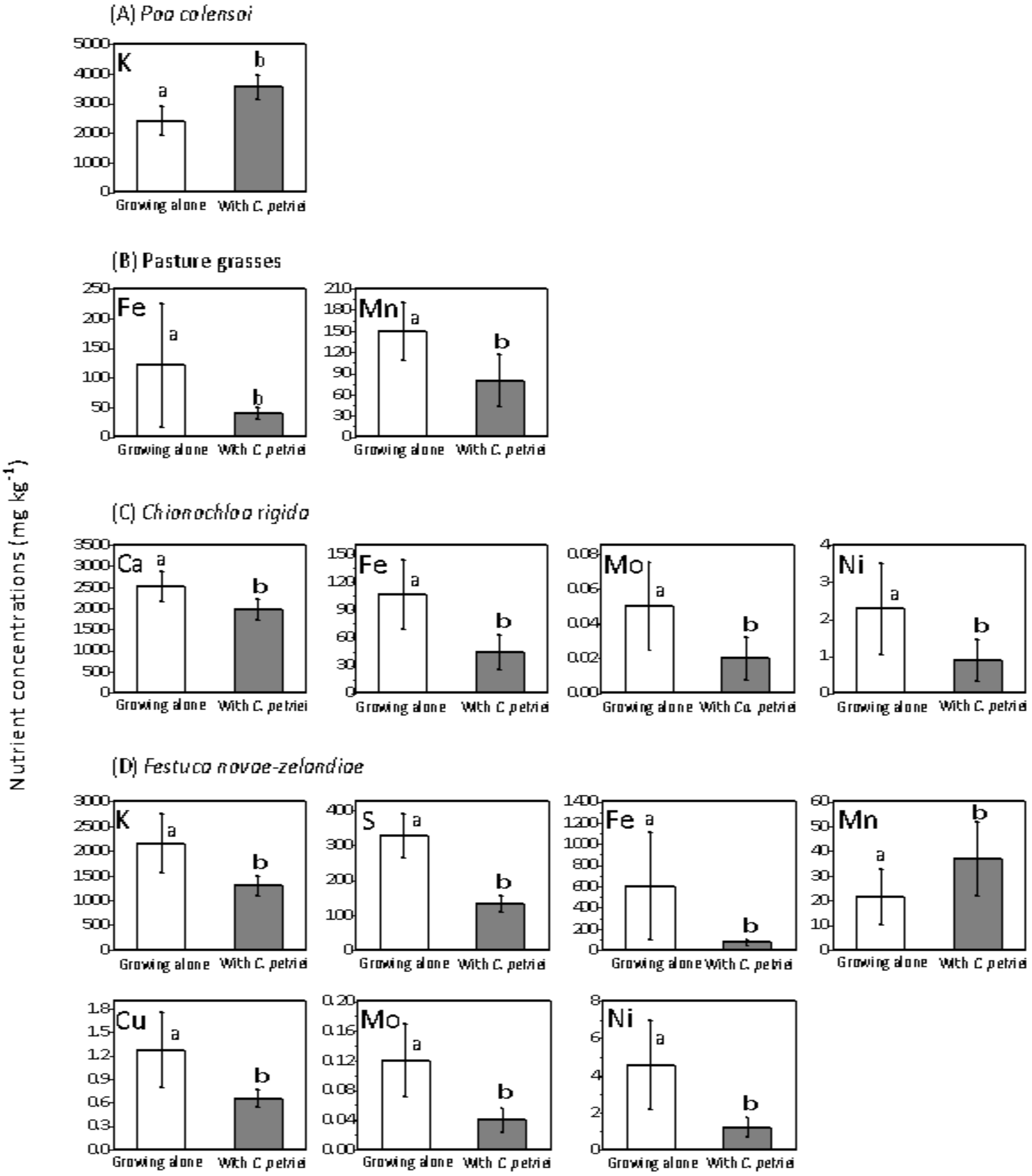

Figure 6.3 Nutrient concentrations in foliage of the four groups of grasses, (A) *Poa colensoi*, (B) pasture grasses, (C) *Chionochloa rigida*, and (D) *Festuca novae zelandiae* according to whether they were growing alone or with a native broom (*Carmichaelia petriei*). Figure illustrates only elements when significant differences were recorded. Histogram bars are means ± standard deviations. Different letters separately indicate significant differences ($p < 0.05$).

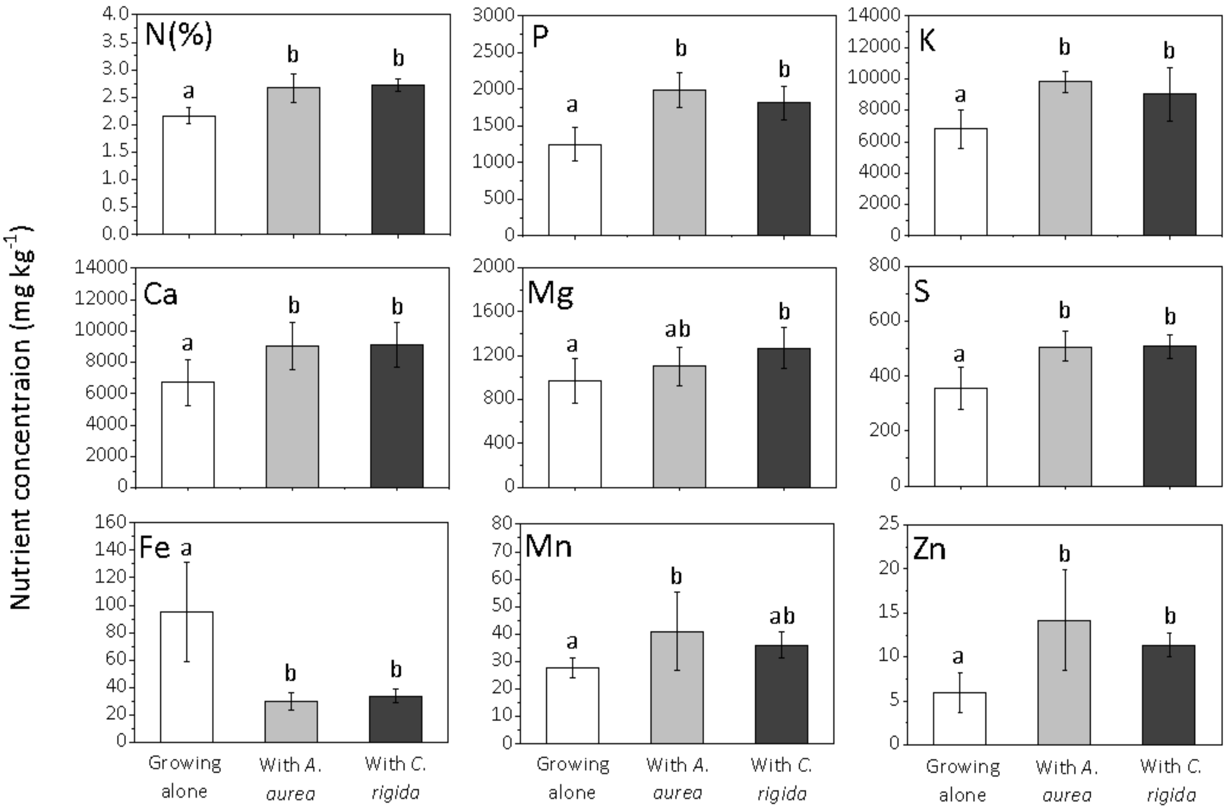

Figure 6.4 Nutrient concentrations in foliage of *Lotus pedunculatus* according to whether it was growing alone or together with *Acyphylla aurea* or *Chionochloa rigida*. Figure illustrates only elements when significant differences were recorded. Histogram bars are means ± standard deviations. Different letters separately indicate significant differences (p < 0.05).

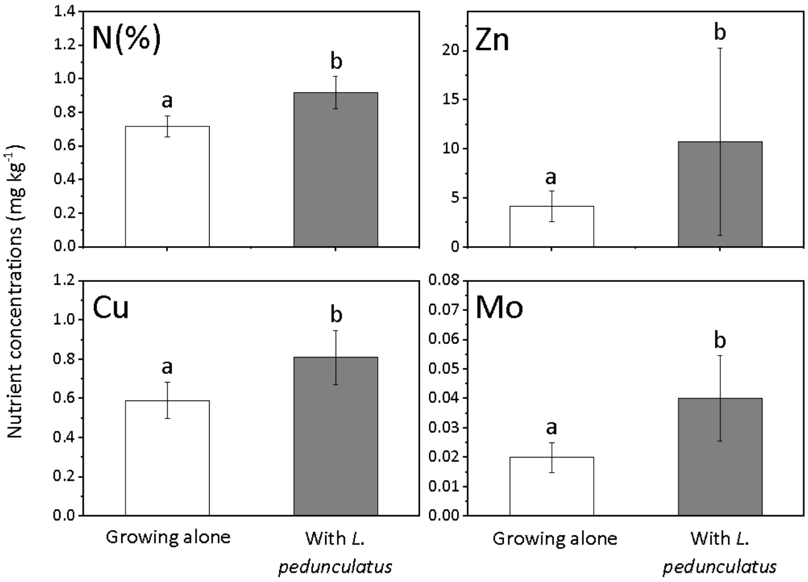

Figure 6.5 Nutrient concentrations in foliage of Chionochloa rigida according to whether it was growing alone or together with Lotus pedunculatus. Figure illustrates only elements when significant differences were recorded. Histogram bars are means ± standard deviations. Different letters separately indicate significant differences (p

< 0.05).

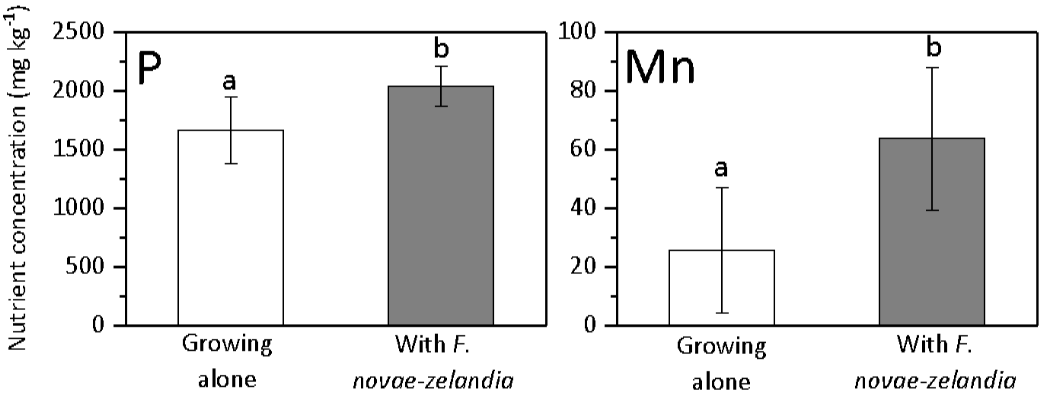

Figure 6.6 Nutrient concentrations in foliage of Trifolium repens (white clover) when it was growing alone or with Festuca novae-zelandiae. Figure illustrates only elements when significant differences were recorded. Histogram bars are means ± standard deviations. Different letters separately indicate significant differences ($p < 0.05$).

6.5 Discussion

There are natural differences in foliar trace element concentrations between different species of legumes. For example, in pot experiments in which the growth of twelve species of legumes (including nine species of *Trifolium* and *Lotus pedunculatus*) were grown in South Island high country soil, comparable to the site of the present study, Jordan (2011) found a wide range of shoot concentration of P (0.11 – 0.26%), Mo (0.23 – 2.3 µg g -1) and B (6.4 – 17.7 µg g -1). T repens and L. pedunculatus were near the upper part of the range for P, with concentration similar to those in the present study. Mo was much higher than the present study, but the soil had been limed in the other study which provided a supply of this element. Boron was at the lower part of the range in both these species. Foliar concentrations of nutrients in legumes are also likely to differ within the same species, largely dependent on the type of soil and its fertility (Nguyen *et al.*, 2020). Gounden *et al.* (2018) collected several species of Trifolium from different localities, and recorded large differences in Fe, Ca, and Mn. The process of symbiotic nitrogen fixation (Liu *et al.* 2018), requires the interplay of several variables involving rhizobial communities (Tan *et al.* 2015). These include the specificity and extent of rhizobial infection (Andrews and Andrews 2017), root nodule development (Schwember *et al.*, 2019) and other factors such as mycorrhizal associations (Sprent and James 2007), all interacting with multiple nutrient availability in soil and uptake by legumes and grasses (e.g. Becana *et al.*, 2018).

Undoubtedly, at the site of the present study there would be significant spatial variability in a range of soil nutrients associated with soil development, slope, aspect, erosion, vegetation cover and stock activity. However, this would be unlikely to explain differences in above-ground concentrations of nutrients recorded between legumes and grasses in the present study; every species sampled when growing singly or in combination were within 3 m of each other. In each case, all replicates for each species pair were collected within a maximum land area of approximately 100 m^2. The sampling procedure involved identifying locations containing each species combination, then immediately sampling adjacent patches where each species was growing alone, providing direct comparisons between plants growing alone and with companion species. This study provides field evidence of significant benefits of grasses to legume nutrition.

Native plants that fix nitrogen are largely lacking in New Zealand's grassland flora; brooms are one of only a few exceptions, together with a few woody shrubs (e.g., *Discaria toumatou* and *Sophora* spp.). The amount of nitrogen cycled was much less before vigorous N-fixing plants were introduced (Wardle 1991). It is well established that, in low fertility soils, legume-grass assemblages are more productive than grassland without nitrogen-fixing plants (e.g. (Berenji, *et al*., 2017)). Legumes have a higher demand than grasses for P, S and other trace elements essential for N-fixation (Caradus, 1980, Yuvaraj, 2020). However, many grass species have been shown to activate fixed phosphorus in the soil by releasing organic acid root exudates. This obviously provides scope for neighbouring nitrogen-fixing plants and grasses to exchange mobilised soil nutrients. Unraveling the likely explanations for changes in patterns of nutrient uptake were discussed in an earlier paper (Zhang, *et al*., 2022c).

The present study showed benefits to *Carmichaelia* in terms of acquisition of a range of nutrients, corresponding with declining concentration of several elements in companion grasses that support the hypothesis of this study. The genus *Carmichaelia* contains about 30 species, all but one from New Zealand, although only a handful of species extend into the high country (Mark, 2012). Rhizobial symbionts have been described for some species (Tan, 2014, Tan, *et al*., 2013) and there is evidence from chronosequences that native brooms provide nitrogen benefits to coexisting plant species and in soil and ecosystem development in (Bellingham, *et al*., 2001, Lagerstrom, Bellingham, *et al*., 2011). There is, however, far more research on an exotic *Cytisus scoparius* (scotch broom), which is highly invasive and widespread in New Zealand, including montane shrubland and tussock grasslands (Bellingham and Coomes, 2003), and of which more is known of its effect on soil nitrogen (Drake, 2011, Broadbent, *et al*., 2017). Little attention has been given to modification of soil biogeochemistry by native species. Legumes including brooms provide better nutrition than grasses by stock, but they are also preferentially grazed, suggesting this a possible example of how a threatened endemic species could provide a valuable component of pastoral grassland in the high country, even though grazing potentially threatens their resilience and conservation status.

Lotus pedunculatus also received nutritional benefits from companion grasses. A marked effect of facilitation in procuring eight nutrient elements including nitrogen by *Lotus* when growing with its two companion species was evident. This legume develops a dense superficial underground system of roots and rhizomes, although above-ground recovery from defoliation is slow and it thrives only under light grazing pressure (Espie, 1987). Old rhizomes breakdown in winter and spring but later propagate new discrete plants. This species thrives better than *Trifolium repens* on acid soils with low P (Charlton and Stewart, 2006), but is generally considered to have a lesser effect than clovers on the growth of companion grasses (Nordmeyer and Davis, 1977). The findings of the present study suggests that *Lotus* may provide a good example of a relationship between exotic legume and a native grass that is beneficial for grazing and stock production, and also to conservation of native species. Foliar concentrations of N and three key trace elements (Zn, Cu and Mo) were enhanced in snow tussocks at higher parts of the altitude range when growing together with *Lotus*.

The present study did not provide similar evidence to show that *Trifolium repens* benefited from associations with any of the grasses, apart from improved concentrations of P and Mn when growing with *Festuca novae-zelandiae*. Supply of P is critical to nitrogen fixation (Liu, *et al*., 2018). Manganese improves drought tolerance in legumes, being required for degradation of ureide, an acyl derivative of urea, which otherwise inhibits nitrogen fixation (Purcell, *et al*., 2000). *F. novae-zelandiae* was the most widespread native grass in lower altitudes of the sampling area and is typical of dry and windy locations in South Island. An early study found that *Poa colensoi* has VA mycorrhizal association, but the other two tussocks do not (Crush 1973). The relationship between different species of grasses requires further field investigation to support the finding of earlier pot experiments (Zhang, *et al*., 2022b, 2022c). In view of the long history of attempts to establish different species of annual and perennial clovers in the New Zealand hill county, this requires more research.

This study provides field validation that broadly supports the findings of earlier *ex situ* experimental work (Zhang, *et al*., 2022a, 2022c). Grasses provide benefits to legumes by facilitating the procurement and uptake of key nutrients. When growing with grasses, legume foliage frequently had higher concentrations of P, K, S and Mn. Improved uptake of six other elements (Ca, Mg, S, Zn, B, Ni) was recorded in more than a single study (Table 6.2). When growing with legumes, higher foliar concentrations of K, but lower Ca, Fe and Mn were recorded in grasses. In the two earlier *ex situ* studies, elevated concentrations of nutrients in legumes often corresponded with lower concentrations of the same nutrients in grasses but, in the present study, there appeared to be less evidence this was the case. Without vegetation yield data from the sampling locations, it is not possible to estimate the total mass of each nutrient extracted from the soil. However, mass balance calculations in the earlier studies showed that combinations of species enhanced overall exploitation of nutrients from defined volumes of soil, providing evidence of transgressive overyielding (Zhang, *et al.*, 2022a, 2022c). Undoubtedly, differences between studies can be attributed to differences between species in terms of requirements and rhizosphere biogeochemistry. Further study is required of the most significant species combinations that potentially could be managed to improve pasture productivity and to allow native species to be restored and sustained within this agricultural matrix.

Table 6.2 Comparison of the results with two earlier studies. The increase (+, dark shading), no significant change (light shading) or decrease (-, no shading) of foliar nutrient concentration in (A) legume spp. when growing with companion grasses, and (B) grass spp. when growing with companion legumes.

(A) Legumes

	Zhang, *et al.*, 2022c			Zhang, *et al.*, 2022a	Present findings		
Experiment type	Pot experiment		Field sample (Fertile paddock)	*Ex-situ* Soil core	Field sample		
Species Nutrients	*Trifolium repens*	*Lotus pedunculatus*	*Trifolium repens*	*Trifolium repens*	*Trifolium repens*	*Lotus pedunculatus*	*Carmichaelia petriei*
Phosphorus	+	+	-	+	+	+	
Potassium		+	+	+		+	+
Calcium		-	+			+	+
Magnesium		-				+	+
Sulphur		+		+		+	
Iron						-	
Manganese	-	-	+		+	+	+
Zinc						+	+
Copper		-	+				
Boron		-	+				+
Molybdenum		-	-				
Nickel		-		+			+

(B) Grasses

	Zhang et al, 2022 c	Zhang et al, 2022 a	Present findings
Experiment type	Pot	Soil core	Field sample

Species Nutrients	*Lolium perenne*	*Dactylis glomerata*	*Festuca novae-zelandiae*	Native grasses and Herbs	Pasture grasses	*Poa colensoi*	*Chionochloa rigida*	*Festuca novae-zelandiae*
Phosphorus	-		-					
Potassium	+	-	-	-		+		-
Calcium			-	-			-	
Magnesium	-			-				
Sulphur	+							-
Iron					-		-	-
Manganese				-	-			+
Zinc		+	-					
Copper	-			+				-
Boron	-		-	-				
Molybdenum	-						-	-
Nickel		+		+			-	-

6.6 Conclusion

The findings of the present work have shown that facilitation between species plays a role in nutrient procurement from soil in New Zealand's hill country grasslands. This points to a requirement for more detailed studies into the combined influence of mixed plant species on multiple nutrient availability in soil, and for better mechanistic explanations. There are synergies between legumes, grasses and other co-existing plant species to optimise acquisition of deficient chemical elements from soil. Clearly, there is variability between species and species combinations, but we have provided evidence of improved uptake of P, K, S and Mn, also extending to six other elements. Informing ecological knowledge of the role of nutrient acquisition in the origin and maintenance of biodiversity in grassland is an additional outcome of this work.

Novel native plant community assemblages in this agroecological mosaic represent a potential opportunity to refine pasture management by exploiting combinations of plant species that facilitate optimal exploitation of nutrients, with less reliance on fertilisers. Incorporating more native species into this mid-altitude pastoral landscape would provide undoubted benefits to protection of biodiversity through land sharing. Generally, however, native plants have little resilience to or protection against grazing, whether or not they are preferentially grazed by stock. Prior to relatively recent human arrival in New Zealand, the endemic flora evolved and existed largely without fertile soils and in the absence of mammals. Native brooms provide one of only a small number of legumes that provide an obvious nutrition contribution to grazers through fixing N. Otherwise, native species persist in contemporary pastoral grassland occasionally through their physical defences, as in golden spaniard, or though being a secondary choice for grazing, as in snow tussocks. Nonetheless, grasses and other species that are less desirable from an agricultural perspective play a facilitation role in nutrient procurement by species that are more desirable for agriculture or conservation. Combinations of plants enhance the acquisition of key soil nutrients. These findings justify more attention to enhancement of biodiversity in the New Zealand hill country.

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Chapter 7

Supplementary Information - Additional Findings

This chapter contains details of experimental work and results not yet published or described in earlier chapters.

7.1 Invasive weed disrupts facilitation of nutrient uptake in grass-clover assemblage

7.1.1 Abstract

To investigate the interplay of competition and facilitation between plants in low-fertility pasture grasslands of New Zealand, we compared nutrient uptake and acquisition of key nutrients of three species from different functional groups. Combinations of Pilosella officinarum (mouse-eared hawkweed, an invasive weed), Trifolium repens (white clover, a nitrogen fixer) and Dactylis glomerata (cocksfoot, a pasture grass) were planted into a soil with low-to-deficient concentrations of key nutrients. Highest yields were achieved by the grass growing alone but, when the clover and grass had grown together, there were complementary benefits in terms of procurement of a wide range of nutrients from soil despite lower root biomass. The invasive weed negated these benefits, and soil nutrients were exploited less efficiently when Pilosella had grown alone or in a mixture with the other species. Competition from the weed removed the benefits of grass-legume coexistence. These findings are interpreted to suggest that requirements for legumes to be the main source of nitrogen in pasture grasslands may be compromised unless competitive weeds are controlled to avoid disrupted procurement of key nutrients. It is likely these constraints to nutrient procurement would similarly impact

7.1.2 Introduction

Pastoral grasslands in New Zealand were developed for sheep farming only within the last two centuries, with exotic species of grasses and legumes introduced to improve productivity (Laidlaw, 2014; Bork et al., 2017). Excluding the intensively fertilised and irrigated lowlands, nearly a third of the land area is at higher altitudes that support most of the extensively managed grasslands where exotic and native species co-exist to varying extents (Wardle, 1991; StatsNZ, 2021; Yao et al., 2022). This presents a somewhat unique challenge of maintaining and improving productive pasture (Thom 2016; Caradus et al. 2021), particularly by increasing the legume component of the pasture (Maxwell et al. 2016), whilst also protecting indigenous grasslands for conservation (Rissman et al., 2021; Tozer et al., 2021). Most of these soils naturally have low pH and low fertility, particularly in terms of phosphorus, sulphur, molybdenum and boron (Hendrie et al., 2021). An additional confounding factor is that these grasslands are frequently impacted by exotic pasture grasses and other weeds that are less desirable for forage. Invasive weeds also threaten native plant diversity (Duncan et al., 2001, Sage et al., 2009). Sustainability or improvement of plant assemblages in these grasslands undoubtedly requires a better understanding of the factors that influence the coexistence of species and susceptibility of this habitat to invasion by weeds (Sage et al., 2009). Elsewhere, for example in nutrient-poor meadows of Central Europe, the declining occurrence of plant species has been explained by a combination of elevated soil nutrients and invasive competitive species (Hockendorff et al., 2021). In the present paper, with a focus on New Zealand grasslands, we report the findings of a study of the ability of plants to procure limiting plant nutrients from soil constrained by deficient nutrients. Using a pot experiment, we investigate the apparently contrasting roles of competition and facilitation.

Competition between plants for resources is known to be an important driver of species diversity and processes of vegetation change (Burrows, 1990, Yao et al., 2022), shaping both the structure and dynamics of plant communities (Klinerova & Dostal 2020). Exotic plants become invasive through adaptations that are better suited to local or modified environmental conditions, in New Zealand's grasslands as elsewhere (Buddenhagen et al., 2022). However, in broader terms, competition also allows coexistence when each species can dominate in at least one combination of abiotic and biotic variables at any particular location (Rebele, 2000). In low fertility grasslands, species abundance is regulated by competition for soil nutrients (Mamolos et al., 1995); it appears to be the case that compatibility of competitive abilities between species allows diversity to be maintained (Schippers et al., 1999). An example is seen in root system structures that vary according to both dispersion patterns of soil nutrients and the presence of competitive plants (Craine, 2006) which suggests some degree of complementarity between species.

The role of complementarity or facilitation between species in grassland plant communities is best known in the context of legume-grass mixtures (Annicchiarico et al., 2015). Atmospheric nitrogen (N) fixed by rhizobial symbionts in the root nodules of legumes spills over in soil to facilitate improved growth of neighbouring grasses (Lüscher et al., 2014). This beneficial effect may also result in transgressive overyielding in which the combined herbage yield exceeds that of the most productive of these species growing in monocultures (Sturludóttir et al., 2014). In intercropping systems, nutritional benefits within the rhizospheres of combinations of plant species extend beyond N to provide better access to phosphorus and improved uptake of a range of trace elements from the soil leading to increased productivity (Li et al., 2014). Our own earlier studies on low-fertility grassland pasture found that grasses provide reciprocal nutritional benefits to legumes by supplying key nutrients that are required for N fixation (Zhang et al., 2022a). Thus, when grasses and clovers grow together in unfertilized grassland, more nitrogen is procured by both species, and other limiting plant nutrients in the soil are better utilized (Zhang et al., 2022b). This type of complementarity between species effectively shares the differing abilities of each species to procure limiting nutrients from the soil (Zhang et al., 2022c).

The aim of the present study was to quantify nutrient acquisition by plants from soil, using three species with differing functional traits that are commonly found growing together in low fertility grasslands. We compared combinations of a pasture grass, an invasive weed and a clover grown in different combinations in a soil with defined elemental deficiencies. Our hypothesis was that appropriation of nutrients by the more competitive invasive weed might curtail the development of a beneficial relationship between the grass and clover.

7.1.3 Materials and methods

The three species of plants used in this experimental work were all exotic species that have become naturalised in New Zealand. Species of Pilosella (hawkweeds) are prevalent invasive weeds, widely naturalised over more than 500,000 ha of the South Island, particularly in hill country tussock grasslands (Scott & McIntosh, 2001). Once established, Pilosella spreads rapidly and outcompetes other species (Rinella & Shelley, 2002; Williams & Holland, 2007). It is quite easy to see the repellent effect that Pilosella has on other species, sometimes with a visual halo of bare ground surrounding its dense mats. Dactylis glomerata (cocksfoot) is a common and widespread perennial species of pasture grass that was naturalised in New Zealand in the mid-19th century. In our earlier experimental studies, D. glomerata consistently provided evidence of the facilitation of nutrient procurement as a companion species to other plants. Trifolium repens (white clover) is the most common legume planted or oversown into grassland in New Zealand and worldwide (Annicchiarico et al., 2015). It has stolon and petiole traits that enhance its capacity to spread and forage (Annicchiarico et al., 2019), although a decline in the abundance and diversity of clovers has been associated with increasing N supplies (Tognetti et al., 2021).

Pilosella officinarum (syn. Hieracium pilosella, mouse-ear hawkweed) was collected from a non-agricultural site at McLeans Island near Christchurch then transplanted into pots (3.5 L plastic pots, diameter 15 cm, height 20 cm). This species is a small perennial rosette-forming herbaceous plant that often uses stolons and rhizomes to form interconnecting plants in dense clumps, growing particularly in grasslands, including tussock grasslands, with low to moderate fertility (nzpcn.org.nz). Both D. glomerata and T. repens were collected from paddocks on the Lincoln University campus.

Individual plants of P. officinarum and D. glomerata and, and tillers of T. repens (7-8 cm) were planted in low nutritional potting mix soil either alone or in combination with individuals of one or both of the other species. The nutrient content of the soil was routinely analysed at a commercial laboratory. Each of the seven treatments had five replicates. Pots were randomly arranged in a single-block design on a bench in unheated glasshouse and were watered sparingly as required. The experiment was set up in December 2021 and plants were harvested after two months. Aboveground biomass was harvested, sorted into separate species, oven-dried (65o C, 48 hrs) then weighed. Roots were carefully separated from the soil, washed, and dried in the same way. All plant samples were then finely ground and microwave-digested in 5M HNO3 before nutrient analysis. Nitrogen was analysed using an Elementar Rapid Max N Elemental Analyser and other nutrients were analysed by ICP-OES following the standard method. Data not normally distributed were log-transformed before analysis. Differences between means were determined using one-way ANOVA, with a post-hoc Fisher LSD test. All analyses were conducted using Minitab 19.

7.1.4 Results

7.1.4.1 Soil fertility

The growth medium was low to deficient in several key elements (Table 7.1) including available concentrations of N, P and key trace elements.

Table 7.1 Significant determinants of fertility in the soil used in the pot experiment, with typical ranges in agricultural soils in New Zealand

Analysis	Units	Concentration	Typical Range*
pH [1]	pH Units	6	5.2-6.5
Electrical Conductivity (EC) [2]	mS cm $^{-1}$	0.3	0.5-1.8
Nitrate-N [3]	mg L $^{-1}$	5	20-80
Ammonium-N [4]	mg L $^{-1}$	2	1-20
Phosphorus [5]	mg L $^{-1}$	6	5-20
Sulphur [5]	mg L $^{-1}$	10	10-20
Potassium [5]	mg L $^{-1}$	35	20-80
Calcium [5]	mg L $^{-1}$	13	30-70
Magnesium [5]	mg L $^{-1}$	12	7-25
Sodium [5]	mg L $^{-1}$	14	5-40
Iron [6]	mg L $^{-1}$	27.6	20-50
Manganese [6]	mg L $^{-1}$	15.6	1-15
Zinc [6]	mg L $^{-1}$	1.88	0.3-10.0
Copper [6]	mg L $^{-1}$	0.48	0.4-10.0
Boron [6]	mg L $^{-1}$	0.11	0.1-0.65

*Typical range for agricultural soils in New Zealand. Method: [1] 1:1.5 (v/v) Water extraction followed by potentiometric pH determination. In-house. [2] 1:1.5 (v/v) Water extraction followed by potentiometric conductivity determination (25°C). In-house. [3] 1:1.5 (v/v) Water extraction followed by Salicylate colorimetry. In house. [4] 1:1.5 (v/v) Water extraction followed by Berthelot colorimetry. In house. [5] 1:1.5 (v/v) Water extraction followed by ICP-OES. In-house. [6] 2 mM DTPA extraction (Australian Standard 3743-1989) followed by ICP-OES. In-house

4.2 Plant yield

Species growing alone produced more biomass, as would be expected (Figure 7.1), which was a particularly large difference for the grass. The presence of *Pilosella* was detrimental to growth of *Trifolium* in both 2- and 3-species combinations. Total biomass produced in pots (Figure 7.2) was higher when *Pilosella* had grown in mixtures with other species rather than alone. There were no significant differences in above-ground pot yields in the presence of *Dactylis*, whether growing alone or in any combination with other species, although root biomass was lower when this species grew with *Trifolium*.

Figure 7.1 Individual plant species yields when each species was growing alone or in a mixture with other species (n=5). Symbols are means ± s.d. Letters indicate significant differences ($p<0.05$) within each block

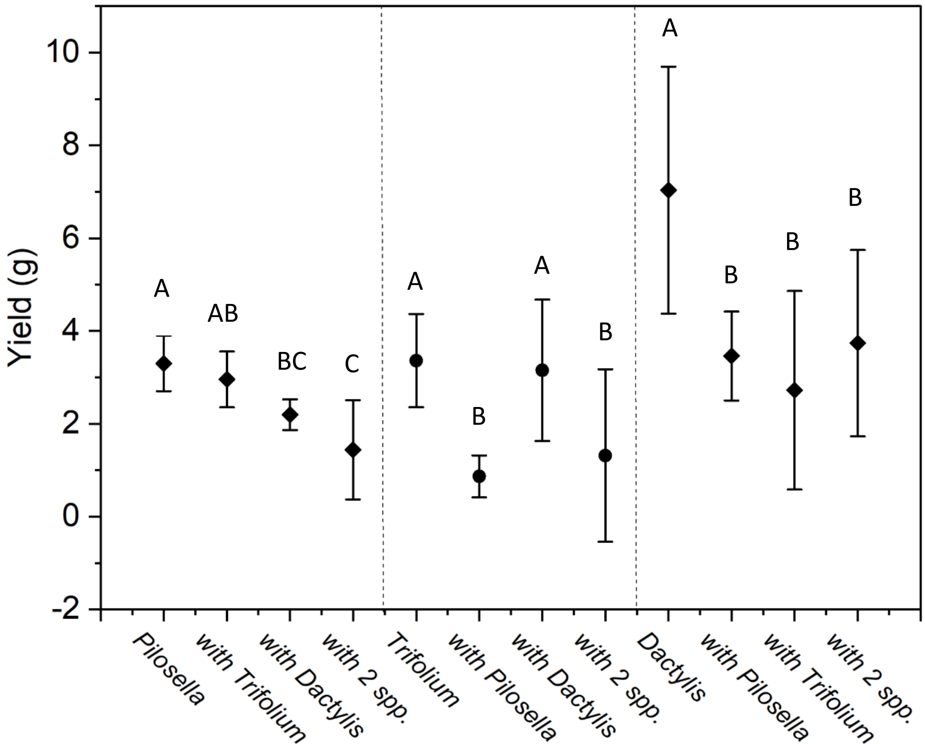
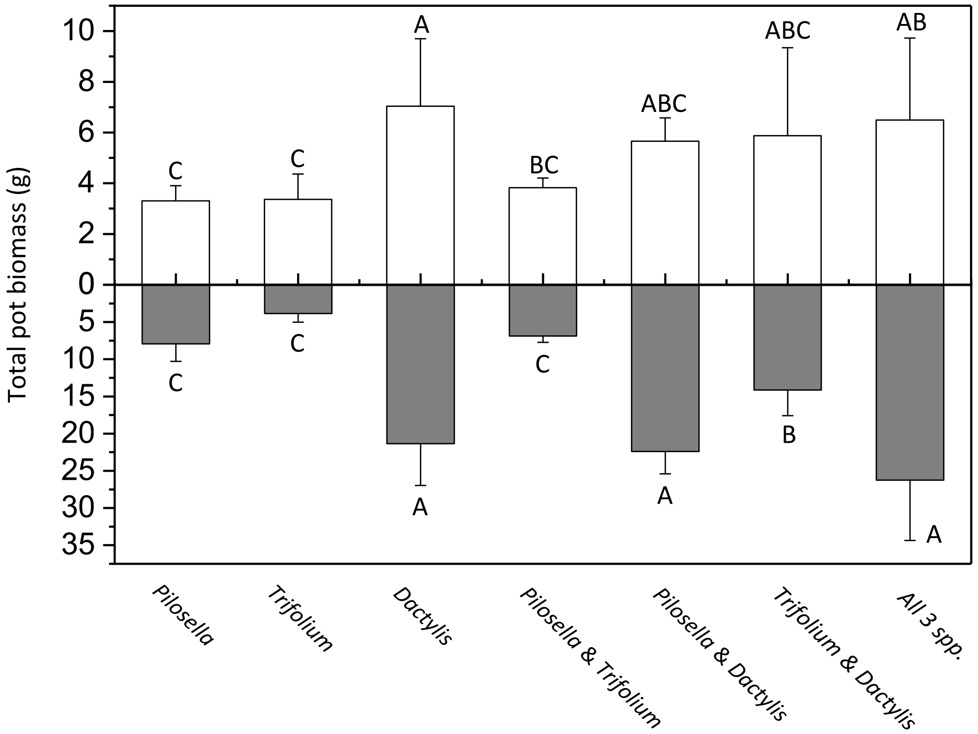


Figure 7.2 Total pot biomass (aboveground and underground, n=5). Histogram bars are means + standard deviations. Values are means ± s.d. Different letters indicate significant differences (p<0.05) within each block



7.1.4.3 Foliar uptake of nutrients

Trifolium had higher N and Zn concentrations when growing with *Dactylis* than when growing alone. Additionally, *Trifolium* had higher K and Ca when growing with *Pilosella*. *Dactylis* had more N, S and Cu when growing with *Trifolium*, but lower concentrations on P and K in mixtures with the two other species. *Pilosella* had higher concentrations of Ca and Mg when it had grown with *Trifolium* but its foliar concentrations of N, P and B were reduced when it had grown with other species (Table 7.2).

Table 7.2 Foliar nutrient concentrations (mg kg $^{-1}$) when (A) *Pilosella*, (B) *Trifolium* and (C) *Dactylis* had grown as single species or together with the other species (n=5). Different letters and bold font alphanumeric indicate significant differences (p<0.05)

(A)

Species/Combinations	N (%)	P	K	Ca	Mg	S	Fe	Mn	Zn	Cu	B	Mo	Ni
Pilosella	2.06ab	**4040a**	32800ab	7300b	2840b	3560ab	307a	80.8a	46.7a	5.29a	**71.1a**	0.90ab	0.95a
With *Trifolium*	**2.40a**	**4150a**	37200a	**8650a**	**3340a**	4170a	290a	102a	52.8a	4.62a	**70.5a**	1.22a	0.78a
With *Dactylis*	1.60c	3150b	29700b	7230b	2790b	3020b	349a	74.1a	51.5a	5.47a	54.6ab	0.64b	0.81a

With 2 Spp.	1.84bc	2950b	34900ab	7070b	2800b	3450ab	304a	90.2a	48.3a	4.92a	48.5b	0.76b	0.79a

(B)

Species/Combinations	N (%)	P	K	Ca	Mg	S	Fe	Mn	Zn	Cu	B	Mo	Ni
Trifolium	3.38ab	2770a	286002b	12800a	3720a	3490b	415a	60.3a	25.5b	4.84ab	27.7a	2.66a	**0.85a**
With *Dactylis*	**3.88a**	4280a	37700b	13100a	3670a	4650a	150a	43.6a	**32.90a**	4.96a	28.2a	3.58a	**0.43b**
With *Pilosella*	3.05ab	3910a	**48300a**	**9450b**	3660a	5020a	142a	43.8a	30.1ab	3.61b	28.1a	4.84a	0.49ab
With 2 Spp.	2.59b	4000a	38400ab	12100a	3350a	5170a	191a	62.3a	29.3ab	4.58ab	28.7a	2.98a	0.66ab

(C)

Species/Combinations	N (%)	P	K	Ca	Mg	S	Fe	Mn	Zn	Cu	B	Mo	Ni
Dactylis	0.88b	1660ab	25700ab	2380a	1530ab	3260ab	95.3a	78.50a	22.9ab	3.13b	4.17a	0.89b	0.81a
With *Trifolium*	**1.3a**	2010a	31300a	2350a	1850ab	**4760a**	99.9a	90.1a	30.9a	**4.68a**	4.41a	2.40a	0.87a
With *Pilosella*	1.01ab	1910ab	28800ab	2630a	1910a	4430ab	83.9a	77.5a	27.5ab	4.04ab	4.96a	1.77ab	1.14a
With 2 Spp.	0.97ab	**1390b**	**21100b**	2050a	1360b	3010b	77.8a	65.5a	19.7b	2.83b	10.0a	1.20b	1.17a

Dactylis and *Trifolium* mixtures procured a larger amount of 10 soil nutrients than when either species was growing alone of in any of the other combinations of species (Table 7.3). *Pilosella* negated this advantage.

Table 7.3 Total pot nutrient uptake (µg) into foliage of *Trifolium*, *Dactylis* and *Pilosella* when they were growing either alone or in mixtures with other species (n=5). Different letters and bold font alphanumeric indicate significant differences (p<0.05)

Species/Combinations	N (G)	P	K	Ca	Mg	S	Fe	Mn	Zn	Cu	B	Mo	Ni
Pilosella	0.07b	13200ab	108000abc	24200bc	9380a	11800bc	1000a	266bc	152ab	17.3a	242a	3.02b	3.15bc
Trifolium	0.11ab	8410b	90000c	43700ab	12800a	11100c	1790a	203c	83.2b	16.1a	90.1bc	9.33ab	3.19bc
Dactylis	0.06b	11700ab	180000ab	16100c	10700a	23300abc	601a	529a	156ab	22.1a	28.7c	6.53b	5.55ab
Pilosella & *Trifolium*	0.06b	10100ab	106000bc	24200bc	9330a	11000c	892a	195c	137ab	15.0a	144b	5.49b	2.20c
Pilosella & *Dactylis*	0.07b	13600ab	165000abc	25100bc	12800a	22000abc	1080a	443ab	207a	25.9a	136b	7.49ab	5.92a
Trifolium & *Dactylis*	**0.15a**	**16300a**	**195000a**	**49100a**	**16400a**	**25900a**	684a	371abc	**182a**	**27.3a**	102b	**15.6a**	3.2bc
All 3 Spp.	0.1ab	15000ab	181000ab	36300abc	14400a	24000ab	1010a	473ab	196a	25.9a	138b	9.31ab	5.85a

7.1.5 Discussion

Previous field-based studies in nutrient-poor grasslands have shown that biomass production is enhanced in species-rich swards (Lüscher et al., 2014). Coexistence of grasses and legumes has generally been explained in terms of differing functional traits that provided for better exploitation of light, nutrients and other resources than can be achieved by monocultures (Gaëtan et al., 2018). This was not the case in the present study in which interspecific competition was evident; all three species produced higher above ground biomass when growing alone within the confines of the pots. The highest biomass yield was achieved by the grass and in combinations of species that included the grass. However, the results demonstrate the complementary benefits of growing clover and grass together. In terms of mass balance, a larger amount of N and nine other elements were transferred from soil to foliage, and this was achieved through a smaller root biomass than when clover or grass grew alone. Mutual facilitation of nutrient procurement between the same species has been similarly demonstrated in our earlier work (Zhang et al., 2022a), where it was found that increased uptake of trace elements facilitated by the grass allows increased nitrogen fixation by the clover. Nyfeler et al. (2011) found stimulatory effects from an accompanying grass on the symbiotic N fixation activity of clover, with acquisition of symbiotic N by the mixture similarly stimulated by the grasses. This is valuable to legumes which have higher nutritional demands than grasses for P and S and other trace elements that are essential for N fixation, which already creates considerable metabolic demand (Zhang et al., 2022b). Phosphorus and K play critical role in productivity and forage quality (Ineichen et al., 2020). Foliar concentrations of various elements were elevated in each of the species when they were growing in combination, with higher N, K, Ca and Zn recorded in *Trifolium*, higher S and Cu in Dactylis and higher Ca and Mg in *Pilosella*. Variable herbage concentrations of Ca, Mg and Na associated with graminoid and forb ground cover and different species composition has been recorded in other recent studies reported in this journal (Kaizrová et al., 2022; Kang et al., 2022). Likely mechanistic explanations in grass and clover are discussed in an earlier paper (Zhang et al., 2022a). Increased Ca and Mg in *Pilosella* foliage when it had grown with clover may be due to small changes of pH in the rhizosphere altering mobility of these elements, although there is no evidence to support this.

The most remarkable findings concern the increased total foliar uptake of 10 nutrients when *Trifolium* and Dactylis grew together, that was disrupted in the presence of *Pilosella*. This is likely to be a substantial practical significance. Since the 1960s in New Zealand, the role of legumes in agricultural pastures has been displaced by fertilizers, and there has been a continuing decline in the abundance and diversity of forage legumes (Laidlaw, 2014), with an eight-fold increase in N use on dairy farms from 1988 to 1994 (Harris et al, 1996) and a further 3.65-fold increase in N fertiliser sold from 1994 to 2019 (StatsNZ, 2021). In a likely reversal of this trend, it is now widely recognised that a return to increased incorporation of clovers and other legumes into grassland species mixtures will be a critical component of future sustainable agricultural pasture management, with less reliance on urea and ammonia fertilizer (Rubiales et al., 2021). However, the process of nitrogen fixation requires a supply of other key elements, as described above, and mobilising these elements in the rhizosphere appears be the role of companion grasses through facilitation. The effects of plants on abiotic (and also biotic) soil components that in turn influence plant performance, is referred to as plant-soil feedback (PSF). Klinerova and Dostal (2020) found PSF reduced differences in competitive ability amongst 46 plant species. Dostál (2021) showed that nutrient addition attenuated negative PSF. If invasive weeds disrupt the acquisition of nutrients by legumes, as in the present study, there is little possibility of increasing pastoral productivity without the use of fertilisers.

In a review of 39 studies of pairs of coexisting plant species, Adler et al. (2018) found that 96% of the studies featured both intraspecific competition and interspecific facilitation. They concluded these are processes that promote stable coexistence. This is likely to be applicable to low-fertility grasslands that are managed for conservation in New Zealand, particularly in the high country. However, the impact of interspecific competition from a recently introduced invasive weed, such as *Pilosella* in the present study, requires further consideration, particularly if its successful establishment is a consequence of environmental change. Grazing mammals were absent from New Zealand until two centuries ago, and their introduction and management has undoubtedly altered soil fertility. It is known that nutrient accumulation in soil due to grazing can reduce the interspecific competitive ability of native plants and favour the invasion of exotic species (Ba & Facelli, 2022). This has consequences for protection of native species; increasing soil nutrients in combination with invasive competitive species has explained the declining occurrence of plants species in grasslands (Hockendorff et al., 2021).

7.1.6 Conclusions

An improve understanding of the impact of coexisting plant species on procurement and management of soil nutrients is clearly required. This represents an opportunity to refine pasture management and also to protect biodiversity in conservation grasslands. Exploiting combinations of plant species that facilitate nitrogen fixation and optimal exploitation of nutrients could reduce fertiliser requirements and enhance and protect biodiversity in pastoral grasslands. In the present study, increased foliar uptake of 10 nutrients when *Trifolium* and *Dactylis* grew together was disrupted in the presence of *Pilosella*. Invasive competitive weeds may disrupt this procurement of key nutrients achieved by facilitation through complementary plant species assemblages. The impact of other biotic and abiotic environmental variables in these processes and of increased soil fertility in nutrient-poor grasslands remain poorly understood.

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7.2 Supplementary results for Objective 3 – Clover and tussock grass in a high-country hill pasture with a history of sulphur fertilisation

7.2.1 Introduction

Sulphur is a critical element for the nodulation of legumes (Ohkama-Ohtsu, 2021). New Zealand hill country soils have limited ability to retain plant-available inorganic-sulphate sulphur on their exchange sites (Sakadevan, 1993). My earlier studies (Zhang, *et al*., 2022a) found that clover contained more S when it was grown with grasses. We question whether the value of a mixed crop of legume with grass can offset the need for S fertilizer applications.

7.2.2 Methods

Field sampling was carried out at a New Zealand hill (43°11'30.3"S 171°41'45.6"E). This site had a sulphur application history two years ago, as part of an Honours project supervised by Richard Lucas from Lincoln University. Plant leaves were collected from where clover was growing alone, clover growing with extra S applied, and clover growing with tussock grass. Leaves were then dried (65°C, 48 h), ground, digested and analysed by ICP-OES following the standard method.

7.2.3 Results

The results indicated that clover growing with additional S and clover growing with tussock grass had higher concentrations of N, P and S compared to clover growing alone. More N and Fe were found in clover leaves; and more Fe was found when tussock grass was growing alone.

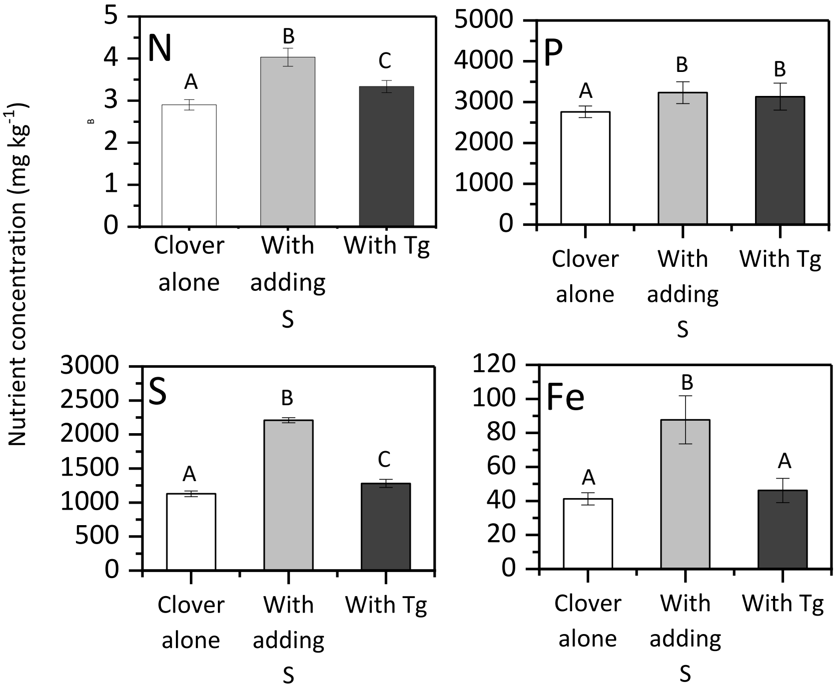

Figure 7.3 Nutrient concentration in clover when it was growing with added sulphur and tussock grass.

Figure illustrates only elements when significant differences were recorded. Histogram bars are means ± standard deviations. The different letters indicate significant differences ($p < 0.05$).

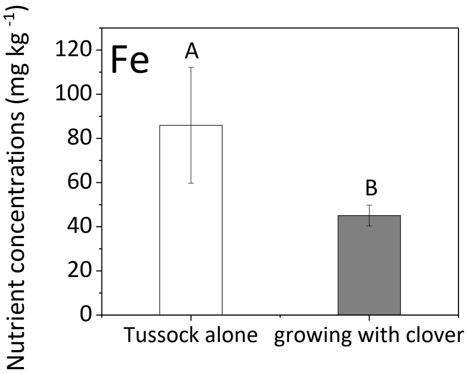

Figure 7.4 Nutrient concentration in tussock grass foliage when it was growing with clover. Figure illustrates only elements when significant differences were recorded. Histogram bars are means ± standard deviations. The different letters indicate significant differences ($p < 0.05$)

7.2.4 Conclusions

Clover had more S in its tissues after a history of added S fertilizer to the site. The naturally-mobilized S by grass was negligible compared to the input of S fertilizer. The clover plants where S was applied were visibly larger than when it was grown elsewhere. The level of sulphur concentration also determined the proportion of nitrogen in the plants. However, the coexistence of legumes with grass can reduce the input of chemical fertilizers.

7.3 Supplementary results for Objective 4 - Lupin and Grevillea 'Bronze Ramble' vs. Cocksfoot

7.3.1 Introduction

This experiment is an extension study of the cluster roots species vs. grass species experiment (Chapter 4), to see whether *Dactylis glomerata* (cocksfoot) has the same effect when grown at the same time as other species. From the results shown in Chapter 4, *Dactylis glomerata* (cocksfoot) was found to be the most effective companion species to cluster roots species of Proteaceae. The hypotheses is that both cluster roots species and grass can procure key nutrients under low-nutrient soils, and the uptake of nutrients are relatively high compared to species growing alone. The nutrient concentration changes are unknown for these combinations.

7.3.2 Method

An additional experiment was prepared using only cocksfoot grown with *proteas*. Two cluster roots species were chosen: white lupin (*Lupinus albus*) and *Grevillea* 'Bronze Ramble' (a cross between *Grevillea rivularis* and *Grevillea* 'Poorinda Peter'). Lupin is a species that has both cluster roots and rhizobia. *Grevillea* 'Bronze Ramble' belongs to the Proeaceae family and also has cluster roots. The two cluster roots species and one grass were grown either singly or in pairs. Each treatment had seven replicates (there were 10 seeds and transplants prepared, but while many of the lupin seeds did geminate well many transplants of *Grevillea* died after being transplanted). All the pots were arranged in a randomized single block design on a glasshouse bench in natural daylight at the Lincoln University Nursery. Water was supplied every day. This experiment lasted approximately two months, from 26th October to 2nd December. The harvested plant tissues were dried (65°C, 48 h), finely ground, digested and chemically analysed using ICP-OES following standard methods.

7.3.3 Results

The results showed that the lupin leaves had higher S concentrations and *Grevillea* had higher Mo concentrations when growing with cocksfoot. Cocksfoot also had high concentrations of nine other elements (P, K, Mg, Fe, Zn, Cu, B, Mo, and Ni) when growing with lupin. Calcium and Mg occurred at higher concentrations when growing with *Grevillea*. The data were also analysed to show total uptake of each element into lupin and cocksfoot, calculated as biomass x element concentration.

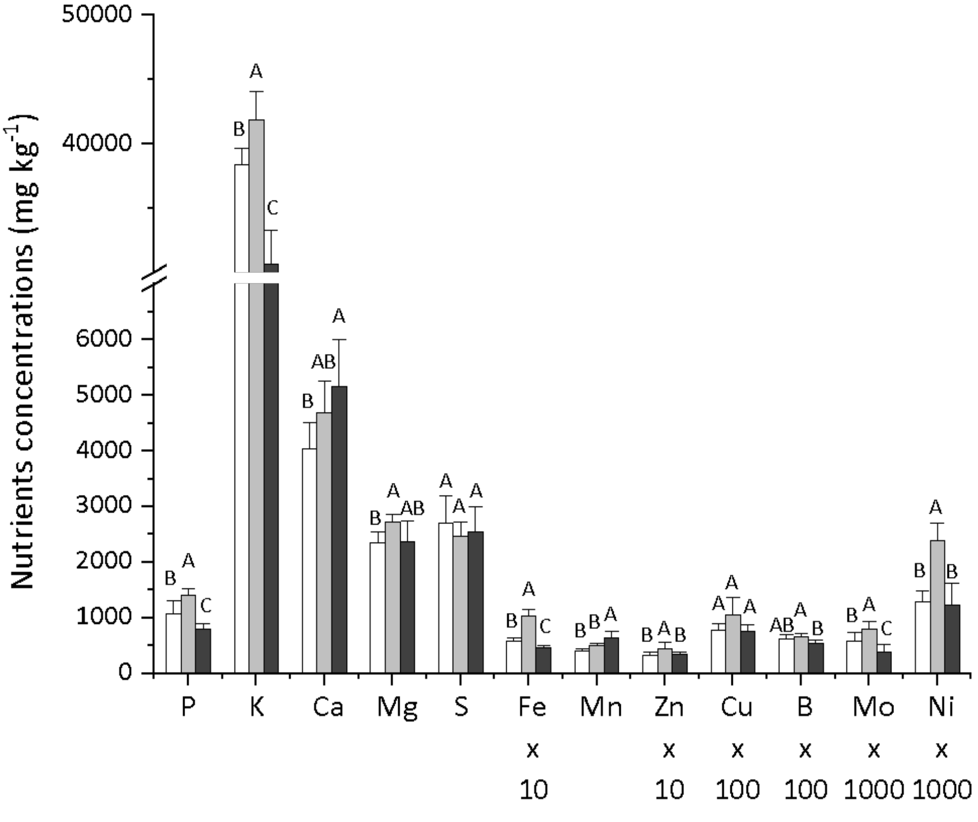

Figure 7.5 Nutrient concentration in cocksfoot foliage when it was growing with two cluster roots species (*Lupin*, grey bars; *Grevillea*, black bars). Histogram bars are means + standard deviations. Different letters

separately indicate significant differences (p < 0.05).

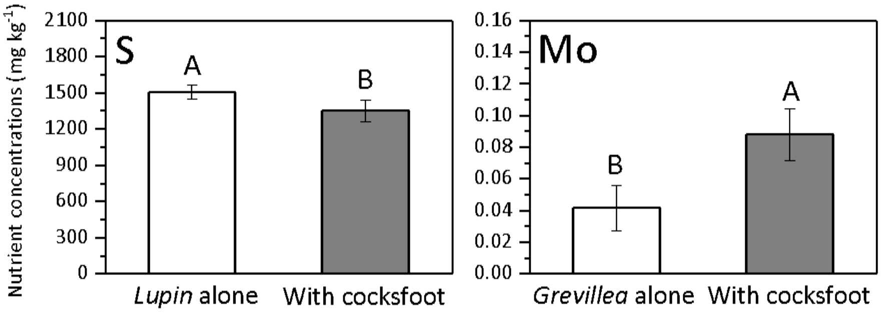

Figure 7.6 Nutrient concentration in cluster root forming species when growing with cocksfoot. Figure illustrates only elements when significant differences were recorded. Histogram bars are means ± standard deviations. Different letters separately indicate significant differences (p < 0.05).

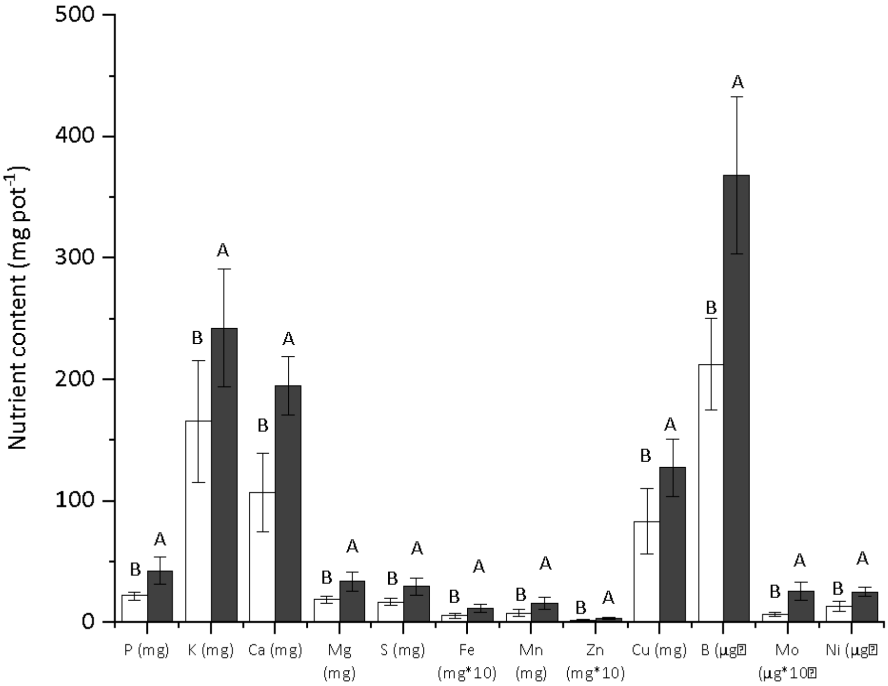

Figure 7.7 Nutrient uptakes in *Grevillea* when growing alone or/with cocksfoot. Histogram bars are means ± standard deviations. Different letters separately indicate significant differences (p < 0.05).

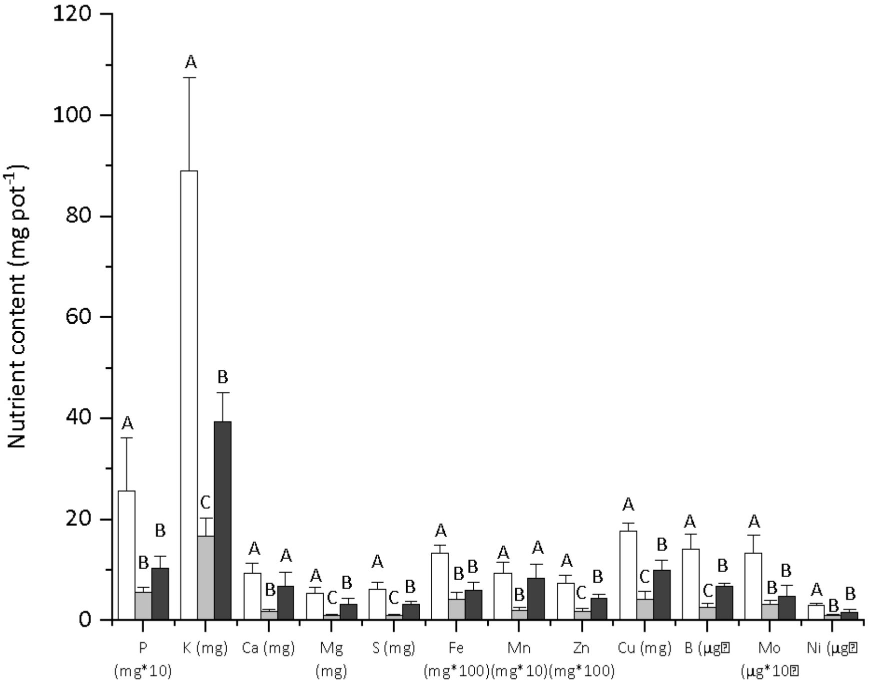

Figure 7.8 Nutrient uptakes in cocksfoot when it was growing with *Lupin* (grey bars) and *Grevillea* (black bars). Histogram bars are means ± standard deviations. Different letters separately indicate significant differences ($p < 0.05$).

7.3.4 Conclusions

These results support the hypothesis that plant diversity can enhance root explorations of the nutrient pool in a degraded soil. The exploitation of specific elements in soil by different plants occurs to provide for the needs of adjacent plants. We made a small extension on the basis of the original experimental species in Chapter 4. Both *Grevillea* spp. and lupin (legume) had a positive effect when growing with cocksfoot. When plants co-exist, the role of grass cannot be ignored.

7.4 Rhizon box observation experiment

7.4.1 Introduction

Interactions between plants start at the roots (Li and Watkinson, 1998). The morphology of roots are not only affected by soil pressure but also by the distribution of water and nutrients. When plants are under environmental and nutrient stress, some will release secretions to improve the utilization of nutrients (Wu *et al,* 2014) by dissolving fixed elements in the soil, which indirectly provides an environment for other adjacent plants to obtain nutrients. How the roots present underground can reflect the interactions interplants. The hypothesis of this study is that the roots of the two species cross each other or one moves towards the other. Meanwhile, we will investigate when the cluster roots form under P-deficient conditions.

7.4.2 Method

To observe the roots' activity underground, rhizonboxes were set up at the Lincoln University nursery. Three different plant combinations (lupin vs. cocksfoot, *Hieracium* vs. clover and *Hieracium* vs. cocksfoot) were chosen. Soil (1-20 cm deep) was collected from a site near Lyndon Lake in Canterbury (611 m). This was planted mainly with hardy shrubs and grasses, with no known fertilization history, and with low calcium, sulphate-sulphur, soluble phosphorus, copper, magnesium, and manganese levels. Two types of rhizonbox were used in this experiment, one was wide but shallow, and the other was narrow but deep. The distance (cm) between the panes of glass was 5 cm. Black plastic covers covered the glass preventing sun damage for the roots. Individual plants were set up 25 cm (left) and 50 cm (right) apart. Photos were recorded every two days from the time when the cluster roots appeared until they disappeared. Water was supplied daily.

7.4.3 Results

The first cluster roots appeared 59 days after sowing. All observable cluster roots grew towards the grass in the wide rhizonbox, and most cluster roots appeared to grow underneath the grass or at the intersections in the narrow rhizonbox (Figure 7.6).

Figure 7.9 Rhizon box observation experiment. Bottom left shows the long-distance growth, and bottom right shows the short-distance growth. Marks showed where the cluster roots were.

7.4.4 Discussion

Visual evidence showed the interaction between the roots. To a great extent lupin roots extended toward the roots of the grass. The development of cluster roots is affected highly by several nutrients, especially phosphorus (Cheng et al, 2011). The over exploitation of P underneath the grass prompted the formation of cluster roots. However, when the cluster roots died, P will be released into the soil and able to be utilized by grass (Muler, *et al*., 2014). This is a process in which lupin passively received stimuli and the grass actively benefitted. The formation of cluster roots enlarge/increased acquiring other elements as well, e.g., Fe and Mo.

Chapter 8

General Discussion and Conclusions

8.1 Discussion and conclusions

Substantial interaction between plants occurs belowground, between roots systems of adjacent species. Intercropping provides the best example of species complementing each other, e.g. in legume-grass mixtures. Clearly, grasses or other companion plants of legumes can utilize the nitrogen fixed by the legume. However, phosphorus and other trace elements still limited plant growth where no fertilizer had been applied. As grasses, or species that have cluster roots, can mobilize the fixed phosphorus underground, my intention was to investigate the interactions between plants in terms of changes in nutrient uptake of plants when growing together. The findings throughout this research showed that a mutualistic relationship occurred between selected plant combinations, where one species derived nutritional benefits from the other.

In New Zealand, lowland farming systems have impacted indigenous grasslands drastically, but it is common to find some native plant assemblages and species that persevere despite some level of intensification. The present study has provided a better understanding of how plant species diversity in grasslands may benefit stock production and land management. This exploration of soil and plant nutrients in plant communities that contain combinations of exotic species alongside native grasses has provided a better understanding of plant diversity in New Zealand nutrient-poor soils. The progression of the study is described below:

8.1.1 Investigation of whether there are benefits to nitrogen-fixing plants when grown in combination with grasses, rather than vice versa (Objective 1, Chapter 3)

This investigation found that the nutrition of legumes nutrition was improved by neighbouring grasses. Meanwhile, the grasses also benefitted from the presence of legumes. The extraction of nutrients in the soil (especially in poor soil) by mixed cultivation was obviously better than that of a monoculture. These findings have clear implications for understanding the value of plant diversity in pastoral agriculture, but they also advance understanding of the coexistence of native and exotic plants that are highly relevant to the conservation and management of New Zealand's native biodiversity. Exotic legumes established on native grassland not only enhanced plant diversity but also reduced the need for fertilizers. The main findings were:

- Legume and grass species growing together enhanced productivity and the higher uptake of the key nutrients.
- Clovers were recorded to have more P, K and S when growing with grass under poor nutrient soil conditions.
- Grasses contained greater concentrations of K, S and Zn when they were growing with legumes.
- Legume and grass combinations can exploit more soil nutrients (P, Zn, Mn and Mo) compared to species growing alone.

8.1.2 Investigation of transgressive overyielding and phosphorus biogeochemistry, using plants with cluster roots in combination with grasses (Objective 2, Chapter 4)

Proteas are known for their P-mobilisation (Lambers, 2015). This objective determined the nature of the relationships between P mobilizers and grasses. The growth of both *Proteas* and grass nutrients were found to significantly improve when they were growing together (apart from *P. nerifolia*). This paper draws attention to the lack of fundamental and applied research knowledge on the functional biodiversity and complementarity of plant species in the context of soil biogeochemistry, especially in low-fertility and degraded soils, and suggests enhancing species diversity may be a better way to manage low-fertility or degraded soils. The main findings were:

- *Grevillea* spp. had elevated concentrations of P and Mn when growing with grasses.
- *Grevillea barklyana* had higher foliar concentrations of most chemical elements out of 12 tested nutrients when growing together with grasses, particularly cocksfoot.
- Other three elements (B, Mo, Ni) in *G.* Robin Hood foliage had significantly different concentrations when growing with grasses.
- Higher concentrations of P, K and Ca were found in ryegrass and cocksfoot when they were growing with *proteas.*
- When the *proteas* and grasses were growing in combination with each other, compared to growing as monocultures, they extracted substantially increased amounts of nutrients from the soil.

8.1.3 Investigation of whether bidirectional complementarity also exists in terms of exploitation of the soil nutrient pool (Objective 3, Chapter 5)

This is a further study for Objective 1. The findings showed that grasses provided an advantage by enhancing N fixation by clovers, while also increasing the mobility and uptake of other major nutrients and trace elements in mid-altitude grasslands. We concluded that more attention needed to be paid to enhancing diversity rather than by attempting to simplify species assemblages in grassland pastures. Clovers and grasses have different complementary functional roles. Advancing this knowledge through further research could also help to optimize the use of fertilizer amendments in rangeland farming systems. The main findings were:

- Nutrient concentrations in pore water under legume-grass mixture tended to be higher in lower pH soil cores for six elements (P, S, Fe, Ni, B, Zn).
- P, Mo and Ni had significantly higher foliar concentrations in grasses than in clovers, and K, Ca, Mg, Zn, B were in much higher concentrations in clover foliage.
- Clover had higher foliar concentrations of the major nutrients, N, P, K, S and Ni, when growing in combination with grasses.
- Legume-grass assemblages extracted significantly larger quantities of four elements (P, S, and Fe) from soil than when either grasses or clovers were growing alone.

8.1.4 Investigation of whether coexistence of species provides an opportunity to facilitate enhanced uptake and improved procurement of key soil nutrients by assemblages of plants in these mid-altitude grasslands (Objective 4, Chapter 6)

Plant complimentary in mid-altitude grassland is poorly understood. We found that when native broom (*Carmichaelia petriei*) is growing with native grass, its uptake of nutrients is enhanced. An increased uptake of eight nutrients was recorded when the exotic legume, *Lotus pedunculatus,* was grown with native grasses or with *Aciphylla aurea* (Golden Spaniard). When white clover (*Trifolium repens*) was grown in combination with grass, only the foliar concentrations of P and Mn increased. Overall, the mutual promotion of nutrient uptake by co-growing species combinations is evident. This is also a study of the diversity of alpine plants, so it has great value to understanding how native plants perform when facing exotic species for nutrient uptake. The main findings were:

- Native brooms had higher foliar concentrations of K, Ca, Mg and B recorded when growing with pasture grasses and with *P. collensoi* at lower altitudes of the sampling range and higher Mn, Zn and Ni when growing with *C. rigida* and *F. novae-zelandiae* at higher altitudes of the sampling range.
- K and Mn were elevated in grasses when they were growing with native broom.
- Foliar concentrations of eight nutrients in *Lotus* foliage were higher when it was growing with either *C. rigida* or *A. aurea*, or both.
- Snow tussock foliage had significantly higher foliar concentrations of N, Zn, Cu and Mo when

growing with *Lotus*.

- Higher P and Mn concentrations in the foliage of *Trifolium* occurred when it was growing with *Festuca novae-zelandiae*.

8.1.5 Additional experimental work

These experiments supplement and expand on these four objectives. Not all the experimental results were consistent, but they did provide evidence that plants can cooperate with each other in a nutrient-deficient soil. At the same time, it further elucidated that nutrient exploitation by plant interactions cannot completely replace f112ertiliser inputs, but may reduce them. The *Hieracium* experiments indicated that not all plants maximize the use of soil resources when they are mixed, as plants also compete with, and repel, other species. It was found that only certain combinations provided sharing of nutrients between species. This study showed that coexistence and facilitation occur along competition and exclusion in terms of nutrient acquisition by plants.

8.2 Critical evaluation

The findings in this thesis is a first report to show that the spillover and transfer of N from clovers to grasses is likely to be an adaptational strategy to procure *quid pro quo* other limiting nutrients from neighbouring grasses. This reveals the importance of grass species in legume-grass and *Protea*-grass mixtures. Plant-plant relationship was also found to provide nutritional benefits in terms of procurement of P and trace elements. Limitations of the study included a restricted number of plant species. Experimental work using more plant species combinations, especially native species, would have increased the value of the research. The amount of replication in the experimental had to be limited for practical reasons and, in many cases, there was considerable variability of the data around the means. Furthermore, there was limited scope to repeat some of the experimental that would have provided increased confidence in the findings. Glasshouse and growth-chamber studies never accurately replicate the field environment and the present study would undoubtedly have been improved by more studies in the field. Limited scope to travel the > 400 km from the university to Mt Grand, lack of more time and difficult accessibility to the farm at certain times of year (for example due to weather extremes, lambing and Covid restrictions) provided unavoidable constraints to this study.

8.3 Further Research

Further research on the topic of this thesis would be targeted towards:

- Elucidation the mechanism of the interactions between plant rhizospheres.

- Study of the mobility of key elements within the rhizophere of different species.
- Testing of more species combinations.
- Further investigation of key limiting nutrients (e.g. S, Mo, B).
- Increased focus on the coexistence of native and exotic species
- Further evaluation of the opportunities to incorporate more native species into hill country grasslands, with more emphasis on ‘land sharing’ for both agriculture and conservation.

8.4 Contribution of the study to the science space

Soil biogeochemistry and the nutrition of plants was a key factor that determined whether, and how, species can be sustained, especially indigenous species. Nowadays, overgrazing, soil erosion and plant invasion seriously threatens native grasslands. New Zealand’s native grassland ecosystems are now made up largely of assemblages of native plants together with naturalised exotic species and soils that are naturally low in plant nutrients. Economic pressure to increase farm productivity and nutrient spill overs from farmland continues to have a major impact on indigenous grassland biodiversity and sustainability. The outcome of this research identifies species combinations of native and exotic pasture plants that better exploit critical soil macronutrients and micronutrients. This will reduce demands for topdressing on grazed grassland by contributing towards both agronomic and conservation goals of indigenous grassland management, by providing new opportunities for sustainability of native flora through a better understanding of the interactions and compatibility between productive pasture species, native species and weeds. The findings from this work will contribute to an understanding of how indigenous grasslands can be protected and sustained and avoid further degradation by focusing on critical aspects of soil and plant nutrition. It is hoped this will also provide knowledge to sustain the indigenous component of New Zealand’s grazed grasslands through management by enhancing the protection of native species.

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