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Agronomic potential of *Lupinus polyphyllus* L. for dryland Merino properties in the Mackenzie Basin

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A thesis

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of the requirements for the Degree of

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Travis P. Ryan-Salter

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

Agronomic potential of *Lupinus polyphyllus* L. for dryland Merino properties in the Mackenzie Basin

By

Travis P. Ryan-Salter

This research examined the potential of *Lupinus polyphyllus* L. (also known in New Zealand as perennial lupin and Russell lupin) as a productive pasture component for high-country pastures. To do this, a combination of on-farm studies, controlled field experiments and glasshouse experiments were carried out. Throughout the thesis, lucerne (*Medicago sativa* L.) was used as a control.

The use of perennial lupin as a suitable feed for a commercial Merino ewe flock was evaluated in an on-farm study at Sawdon Station, Lake Tekapo. The performance of ewes and lambs grazing a lupin-based pasture was compared with a control flock that was predominantly grazing lucerne. The lupin pasture grew rapidly during the spring period and average pasture cover increased by about 4500 kg of dry matter (DM)/ha under a stocking rate of 15-17 stock units/ha. At tailing in December 2012 and 2013, lambing averaged 111% and ewes averaged 58 kg for the lupin pasture, and 105% and 62 kg for the control flock, while lambs averaged 19 kg for both mobs. At weaning in February 2013, ewes and lambs on the lupin pasture averaged 58 kg and 28 kg compared with 63 kg and 31 kg for the control flock, respectively. During autumn, ewes on the lupin pasture gained 3.8 kg compared with 5.5 kg for the control flock before mating in May. In September, the ewes were shorn and wool averaged 4.62 kg/ewe for the lupin mob and 4.92 kg/ewe for the control flock, with a mean fibre diameter of 18.5 μm. A selection of *L. polyphyllus* samples were subjected to wet chemistry analyses, which was later used to calibrate a near infrared spectroscopy analyser and predict the nutritional composition of lupin material. Lupin lamina was consistently high in metabolisable energy (11.3-12.6 MJME/kg DM) and crude protein (24-33%) throughout the growing season

Under controlled experimental conditions at Lincoln University, sheep liveweight gain from a dryland mixed cocksfoot (*Dactylis glomerata* L.)-lupin pasture was 810 kg/ha compared with 1227 kg/ha from dryland lucerne over a 15-month period. This difference was primarily driven by the amount of palatable feed consumed by the sheep, which was 7330 kg DM/ha for cocksfoot-lupin

compared with 10,922 kg DM/ha for lucerne. The abundance of lupin in the mixed pasture declined from 40% of the total herbage offered, during the first autumn after sowing (March to May, 2014), to 22% of total herbage offered in the first full growth season (August 2014 to May 2015). This meant that at least 60% of the diet of these sheep was comprised of cocksfoot. In late spring/summer, the growth rate of cocksfoot-lupin pastures slowed to 20 kg DM/ha/day compared with 43 kg DM/ha/day for lucerne. Despite these differences in feed consumption and pasture composition, the feed conversion efficiency of sheep liveweight gain was the same for both pasture types and averaged 131 g of liveweight gain/kg of DM consumed from August 2014 to May 2015.

The greater sheep liveweight gain and total feed consumed on lucerne compared with cocksfoot-lupin was associated with a greater DM yield through improved water-use efficiency for lucerne. During the 2014/2015 growth season, the cocksfoot-lupin pasture produced 6612 kg DM/ha compared with 8122 kg DM/ha for lucerne before a soil moisture deficit started to limit pasture growth in summer 2014/15. To produce this yield, lucerne used 404 mm of water whereas cocksfoot-lupin used 360 mm of water. This equated to a water use efficiency of 20.9 kg DM/ha/mm for lucerne and 18.6 kg DM/ha/mm for cocksfoot-lupin. The 'Monteith framework' was successfully applied to both pasture types and provided an accurate description of water extraction patterns to a depth of 165 cm. Lucerne extracted an additional 53 mm of moisture than cocksfoot-lupin from the soil profile between 55 and 155 cm. These results helped to explain the superior sheep liveweight gain on lucerne.

Lupinus polyphyllus plants were heavily nodulated at 10 field sites sampled across the South Island. Twenty-two bacterial isolates formed functional nodules on *L. polyphyllus,* which indicated that rhizobia, capacble of nodulating *L. polyphyllus,* were present across a wide range of sites in the South Island. Gene sequences identified all 22 isolates and the Group G commercial inoculant as *Bradyrhizobium* sp. Eleven isolates and the Group G inoculant were tested for their effectiveness on growth of *L. polyphyllus* under glasshouse and field conditions. Plants were grown in a high-country soil under glasshouse conditions and all plants nodulated regardless of inoculum treatment. However, plant growth was variable and further quantification was required. Field studies showed that inoculation was beneficial for the growth of *L. polyphyllus* seedlings under high-country conditions. Therefore, despite the presence of effective rhizobia throughout the South Island, inoculation is recommended for newly established stands.

Both *L. polyphyllus* and lucerne responded to increasing levels of soil nitrate by reducing N fixation. Application of potassium nitrate at rates up to 600 kg N/ha had little effect on the growth or herbage N concentration of *L. polyphyllus* or lucerne. However, the proportion of N derived from soil (%Ndfs) increased consistently with fertiliser applications between 50 and 600 kg N/ha. For both species, %Ndfs was highest at a fertiliser rate of 600 kg N/ha, where %Ndfs was 62% for *L. polyphyllus* and 74% for lucerne.

This thesis confirmed that *L. polyphyllus* is a suitable forage option for dryland, high-country, farmers. Rather than replacing lucerne, *L. polyphyllus* should be viewed as a complementary species that can considerably improve the productivity of areas of lower soil fertility. In this context, *L. polyphyllus* is best suited to areas that are often uneconomical to develop for traditional legumes. Farmers will need to carefully manage *L. polyphyllus* during establishment, with a focus on seedbed preparation and careful grazing management during the first season. However, once established, *L. polyphyllus* will likely remain as a persistent dryland species that will produce significant amounts of spring forage on high-country farms.

List of publications from this thesis

Ryan-Salter, T. P., Black, A. D., Andrews, M., Moot, D. J. (2014). Identification and effectiveness of rhizobial strains that nodulate *Lupinus polyphyllus*. *Proceedings of the New Zealand Grassland Association* **76**, 61-66.

Black, A. D., Loxton, G., Ryan-Salter, T. P., and Moot, D. J. (2014). Sheep performance on perennial lupins over three years at Sawdon Station, Lake Tekapo. *Proceedings of the New Zealand Grassland Association* **76**, 35-40.

Black, A. D., Ryan-Salter, T. P. (2016). Evaluation of perennial lupin/cocksfoot pasture relative to lucerne pasture under summer dry conditions. *Journal of New Zealand Grasslands* **78**, 123-132.

Jiang, S., Keaney, D., Ryan-Salter, T.P., Black, A.D. (2014). Developing NIRS calibrations to predict the nutritional value of Russell lupin (*Lupinus polyphyllus*). 16th Australian Near Infrared Spectroscopy Group Conference, Coffs Harbour, NSW.

Black, A.D., Ryan-Salter, T.P., Loxton, G., Moot, D.J. (2015). Liveweight gain of young sheep grazing perennial lupin-cocksfoot pasture compared with pure lucerne pasture. Proceedings of the XIV International Lupin Conference, 21-26 June 2015, Milan, Italy. Pg. 32-35.

Black, A.D., Ryan-Salter, T.P., Liu, W.Y., Moot, D.J., Hill, G.D., Andrews, M. (2015). Bradyrhizobia with a distinct nodA gene nodulate *Lupinus polyphyllus* in New Zealand soils. Proceedings of the XIV International Lupin Conference, 21-26 June 2015, Milan, Italy. Pg. 45-48.

Black, A.D., Loxton, G., Ryan-Salter, T.P., Moot, D.J. (2015). Merino lamb and wool production from a commercial stand of perennial lupin (*Lupinus polyphyllus*) on a high-country farm in New Zealand. Proceedings of the XIV International Lupin Conference, 21-26 June 2015, Milan, Italy. Pg. 36.

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1   General introduction

The high-country of the South Island of New Zealand (NZ) is characterised by extensive grazing areas and harsh landscapes that cover 3.4 million hectares (Scott *et al.*, 1995a). The annual productivity of these areas is predominantly limited by low winter/early-spring temperatures and severe summer soil moisture deficits (Chapman and Macfarlane, 1985). Within these constraints, properties also experience considerable variability in pasture production due to changes in topography, aspect, elevation, rainfall, climate and soil fertility (Chapman and Macfarlane, 1985). This requires site-specific adaptations in subdivision, grazing management, soil fertility and plant species to gain productive efficiency and meet the seasonal requirements of livestock (Allan and Keoghan, 1994; Floate *et al.*, 1987; Pedofsky and Douglas, 1987; Scott and Williams, 1996).

The South Island high-country is traditionally associated with the production of fine Merino wool of 12-24 μm and market weight lambs of 30-35 kg within 4-5 months after lambing. Merino sheep are well suited to the high altitudes and mountainous landscapes of NZ's South Island high-country. Environments such as these are much too harsh for other domesticated sheep breeds which often require a constant supply of high quality feed to be productive. Because diet quality and wool fineness are often inversely related, the desire to produce fine wool has often come at the expense of other production traits such as reproductive efficiency and liveweight gain potential. Thus, it has been traditional to consider Merino sheep as having a low reproductive efficiency and 'poor' mothering ability (Alexander *et al.*, 1989). However, more recently, improvements in nutrition and refinement of breeding programs has allowed this breed to become more productive (Anderson *et al.*, 2014). It is for this reason that high-country farms now require an improved feed regime that can support a higher rate of reproductive efficiency than was previously possible.

This challenge can be met, at least in part, by the introduction of forage species to the pasture mix with the potential to increase liveweight gain, carrying capacity and wool production. Therefore, introduced plant species must be able to efficiently produce high-quality feed during periods where soil moisture and temperature are non-limiting. In high-country pastures, this is often achieved by introducing legumes, which hold a competitive advantage over grasses due to their ability to fix atmospheric N_2 (Scott and Maunsell, 1981). This ability is often associated with improved water use efficiency (Brown *et al.*, 2005; Moot *et al.*, 2008) and feed quality (Brown and Moot, 2004; Cosgrove *et al.*, 1999; Dorland *et al.*, 2007).

Experiments at high-country sites have demonstrated that many traditional legumes such as white clover (*Trifolium repens*) are usually unable to thrive without inputs of irrigation and fertiliser (Scott, 1998, 2000c; Scott *et al.*, 1989). This is due to the severity of summer drought but also the challenging soil conditions that have evolved through intensive leaching, poor nutrient cycling and only minor inputs of fertiliser and lime. In many cases, soils are acidic (pH <5.5), which is often associated with an increase in soluble Al (Edmeades *et al.*, 1983; Moir, 2013). The elevated levels of soluble Al are commonly associated with poor root growth and nodulation failure in legumes (Berenji *et al.*, 2015; Macleod and Jackson, 1965), which can have considerable impacts on their persistence. This effect can be reversed with inputs of fertiliser and lime and/or the introduction of legume species that are tolerant of these conditions.

Under suitable soil conditions, lucerne (*Medicago sativa*) has become a widely adopted legume that is valued for its forage quality and productivity in the high-country (Anderson *et al.*, 2014; Avery *et al.*, 2008; Stevens *et al.*, 2012). It is mostly used in lower areas of flat to rolling land, that often accommodate 80% of the stock but represent only 20% of the land that has been developed (Scott *et al.*, 1995a; Scott and Williams, 1996). As a tap-rooted perennial, it is able to extract water from greater depths than other species which extends its period of growth under dryland conditions (Brown and Moot, 2004). However, its sensitivity to soluble Al (>3 meq/kg) in acidic soils (pH <5.5) has limited its application in marginal high-country zones (Moir and Moot, 2010a).

In contrast, perennial lupin (*Lupinus polyphyllus*), otherwise known in NZ as Russell lupin, is a legume species that is widely recognised for its tolerance of acidity and growth under moderate inputs of fertiliser (Scott, 1989; Scott *et al.*, 1995a; Scott *et al.*, 1989; White *et al.*, 1995). In particular, it has been recognised as a 'niche' species with moderate acceptability to livestock that will become the dominant pasture legume under modest fertiliser regimes. It has thrived in soils throughout the high-country of the South Island, even where Al is toxic for most clovers (*Trifolium* spp.) and lucerne (Berenji *et al.*, 2018; Hendrie *et al.*, 2018). However, *L. polyphyllus* is noted as having only moderate acceptability to livestock (Scott, 1994) and is vulnerable to competition during its establishment year (Scott, 1989; Scott *et al.*, 1995a). Furthermore, attempts to quantify seasonal production, nutritional value and suitability for year-round grazing are limited (Kitessa *et al.*, 1993; Moot and Pollock, 2014; Scott *et al.*, 1994). Thus, it has remained as a minor species in the high-country with limited uptake among farmers.

The productive areas of inland mid-altitude properties (500-1000 metres above sea level) has been mostly confined to areas of moderate fertility and rainfall. The use of crops such as lucerne has offered considerable improvements in the productivity of these areas. However, there are still large areas of mid-altitude land where soil fertility strongly restricts the choice of legume. The use of *L. polyphyllus* in these areas has been shown to offer significant improvements in pasture productivity with only minimal inputs of fertilizer. However, its uptake has been predominantly limited by a lack of information surrounding its productivity, nutritive value and tolerance of grazing. Quantifying these aspects will offer an improved understanding of its agronomic value and potential to improve the productivity of marginal areas.

1.1   Research objectives and thesis structure

The primary aim of this thesis is to evaluate the potential of *L. polyphyllus* as a forage option to improve the productivity of high-country Merino farms in South Island NZ. To assess this, *L. polyphyllus* was grazed in mixed-pastures and animal productivity was considered the primary indicator of pasture performance. Pasture performance was quantified with a range of agronomic measurements that define pasture quality and quantity and used as explanatory variables for live weight measurements.

Underpinning this aim is the assumption that *L. polyphyllus* is a persistent legume that can initiate N cycling in high-country pastures. Therefore, the ability of *L. polyphyllus* to form a successful symbiotic relationship with suitable rhizobia is essential. Thus, a secondary aim was to understand the requirements for successful nodulation and N fixation in *L. polyphyllus*.

To meet the primary and secondary aims, five specific objectives were developed:

Objective 1: To quantify the productive performance of sheep grazing a perennial lupin pasture.

Objective 2: To assess the agronomic traits that drive sheep performance on pastures containing perennial lupin.

Objective 3: To quantify the temporal and spatial patterns of water extraction of perennial lupin pastures as an indicator of its ability to survive in a dryland environment.

Objective 4: To identify the requirements for successful nodulation and N fixation in *L. polyphyllus*.

Objective 5: To determine whether N fixation of *L. polyphyllus* is affected by increasing levels of inorganic soil N.

To investigate these objectives, this thesis is structured in seven chapters. A literature review is provided in Chapter 2, which describes the highc-ountry environment in South Island NZ and outlines the primary constraints to pasture productivity in this environment. Following this, data from previous experiments that included *L. polyphyllus* as a pasture legume, are presented and evaluated. Four experimental chapters meet the five objectives.

In Chapter 3, the suitability of a perennial lupin-based pasture for a commercial Merino ewe flock is evaluated on a high-country station near Tekapo (Objectives 1 and 2). This benchmarks the performance of Merino ewes grazing a perennial lupin pasture with those grazing on conventional

pastures. Animal performance was supported with monthly pasture measurements and collection of herbage samples for nutritive analysis. Herbage samples were used to develop a calibration for future nutritive value measurements using near-infrared spectroscopy (NIRS).

Chapter 4 also reports sheep performance but from a controlled experiment. To do this, a mixed perennial lupin-cocksfoot (*Dactylis glomerata*) pasture was compared with a lucerne monoculture at Lincoln University (Objectives 1 and 2). Weekly agronomic and animal measurements are used to quantify pasture performance on a seasonal and annual basis.

Chapter 5 explains the environmental variables that contributed to the results of Chapter 4 (Objective 3). This chapter primarily focuses on the seasonal water use and extraction patterns of the mixed perennial lupin-cocksfoot pasture, to evaluate its suitability in a dryland farming system.

To meet the secondary objective related to N cycling (Objectives 4 and 5), Chapter 6 focuses on characterising the rhizobial symbiont partners to *L. polyphyllus* in the South Island of NZ. This was followed by an assessment of whether inoculation was required for improved growth of *L. polyphyllus*. To meet Objective 5, glasshouse experiments were used to quantify the effect of increased levels of inorganic soil N on N_2 fixation in *L. polyphyllus* and comparative pasture legumes.

In Chapter 7, a general discussion of the results from all experimental chapters is used to evaluate their implications for both dryland and high-country farmers from production and environmental perspectives.

2 Literature review

2.1 Introduction

In this literature review, the main factors that affect the productivity of high-country grasslands within the South Island of New Zealand are first considered. This will provide a basis for the agronomic traits that are required for a legume to be successful in the New Zealand high-country. Based on this, a number of traditional and potential options will be listed and described. Finally, the review will focus on the potential of *L. polyphyllus* as a legume option for low-input high-country pastures. Literature containing agronomic data about Russell lupin is limited. Therefore, the majority of information presented for Russell lupin is from the extensive pasture trials at Mt John, in Tekapo.

2.2 High-country pasture development

In N deficient soils, such as most soils in the New Zealand high-country, legumes have a competitive advantage over grasses due to their ability to fix atmospheric N_2 (Scott *et al.*, 1995a). During a short growing season of 220 – 270 days (with irrigation), Scott and Maunsell (1981) estimated that grasses required N inputs of 300 – 400 kg N/ha/year to either match or exceed the growth of legumes. The dominance of each forage species in high-country grasslands is therefore strongly dependent on the N status of the soil, which can be modified with legumes or fertiliser application.

The relative dominance of grasses and legumes under varying soil conditions was demonstrated by Scott (2012), who quantified the relative abundance of different forage species, including Russell lupin, within developed and undeveloped grassland at Mt John, Tekapo. When sown into previously developed land areas, legumes were low in abundance and had a mean contribution of 12.5% compared with non-leguminous species over an 18-year period (Table 2.1). This was most likely due to the dominance of non-leguminous species in response to the relatively high soil fertility of previously developed land at the site. During the first 6 years, the contribution of Russell lupin to yield was marginal; however, it slowly increased in abundance to 11.2% in years 13-18, which was far higher than the 1.3% of any other legume. In contrast, when sown into undeveloped pastures, Russell lupin and clovers contributed up to 52% of the herbage yield for the first 6 years of the experiment. The success of the clovers was short-lived and lupin became the dominant legume, which eventually comprised 41% of the herbage grown. It is likely that the continued drought and considerable selection pressure from grazing Merino sheep caused a rapid decline in the abundance of conventional clover species in this long term study.

Table 2.1 **Mean pasture composition (%) of seven species groups in three periods following over-drilling in a prior developed block compared with an undeveloped block. Average within-year standard error for group proportions was 1.02%. Taken from Scott (2012).**

Period (yrs)	Developed			Undeveloped		
	2-6	7-12	13-18	2-6	7-12	13-18
Clovers	12.1	4.3	1.3	37.6	7.9	0.7
Lupin	0.6	1.2	11.2	14.4	28	41.2
Cocksfoot	7.1	4.4	0.7	4.4	5.8	1.1
Tall oat	6.5	11.4	10.8	0.5	5.5	9.1
Small grasses	20	27.3	26.6	6.9	18.1	18.1
Tussock	15.2	24.8	25.2	8.4	21.5	19.2
Hieracium	6.3	10.6	17.6	22.8	9	8.6

In the broader experiment of Scott (2012) at Mt John, introduced species tended to respond differently to the range of grazing and fertiliser combinations imposed. Initially, species had a rapid response to different fertiliser levels with slower changes in later years. Legumes tended to dominate yields in the first 4 years due to their ability to grow in the N-depleted soil at the site. However, there was a general transition from legume to grass dominance that occurred as soil N fertility improved over the years (Scott, 2001, 2008). In assuming that legume-based pastures are desired, the following section will describe the various facets of achieving legume-dominant pastures.

2.3 Legume-based pastures

Nitrogen fixation in legumes is generally considered to be consistent between species and environment. In general, legumes are expected to fix approximately 25 kg N for every tonne of herbage dry matter (DM) they produce (Lucas *et al.*, 2010). This agrees with data presented in Peoples *et al.* (2009), which showed a linear relationship between nitrogen fixation and shoot growth across several different species and climates (Figure 2.1). Thus, the amount of N_2 fixed is primarily dependent on the amount of legume herbage grown. In high-country pastures, this will be limited by the suitability of a particular legume species to combinations of climate, soil fertility and grazing management.
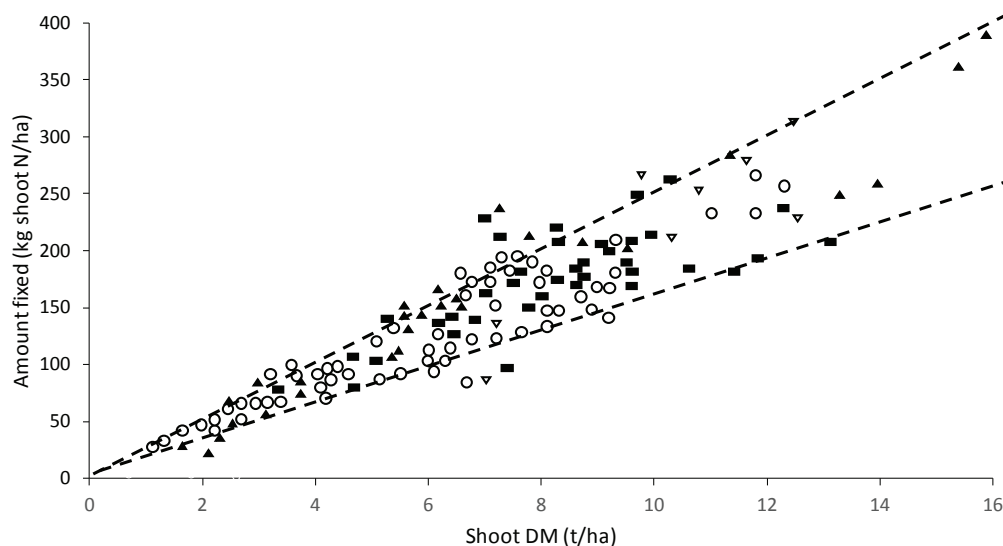
Figure 2.1 Examples of the relationship between amounts of shoot N fixed and shoot dry matter (DM) production for legume crops. The upper broken line indicates a 25 kg N fixed/t DM relationship, while the lower broken line represents a 15 kg N/t DM relationship. The figure uses data from dryland warm-season crops (▲), dryland cool-season crops (○), irrigated warm-season crops (▽) and irrigated cool-season crops (■). Redrawn from Peoples *et al.* (2009).

2.3.1 Limitations under high-country conditions

The introduction of legumes and fertiliser initiates a cycle of organic matter (OM) and N accumulation that will tend toward a new and improved equilibrium depending on site, climate and management (Nordmeyer and Davis, 1977). Where this equilibrium lies is dependent on the cost:benefit ratio of development. It is therefore the role of the farmer to assess where these 'niches' lie on their property and where each species can be applied for maximum efficiency.

One of the main limitations to pasture productivity is the acidity, and often associated increase in exchangeable Al, of high-country soils (Moir, 2013; Moir and Moot, 2010b; Scott, 1999a; Scott, 2003). This is known to have an impact on conventional legumes such as white clover (Caradus *et al.*, 1996; Davis, 1981b) and lucerne (Moir and Moot, 2010a). In high-country soils, the levels of exchangeable Al seem to increase to toxic levels (>3 mg/kg) as soil pH decreases (Figure 2.2). This increase is known to depress root growth (White, 1967), reduce persistence (Schmehl *et al.*, 1950) and has also been shown to have detrimental effects on rhizobia (Asanuma and Ayanaba, 1990; Graham *et al.*, 1994; Munns, 1978). Thus, there are two methods for overcoming this limitation; with fertiliser and lime amendments and/or the use of acid-tolerant species.
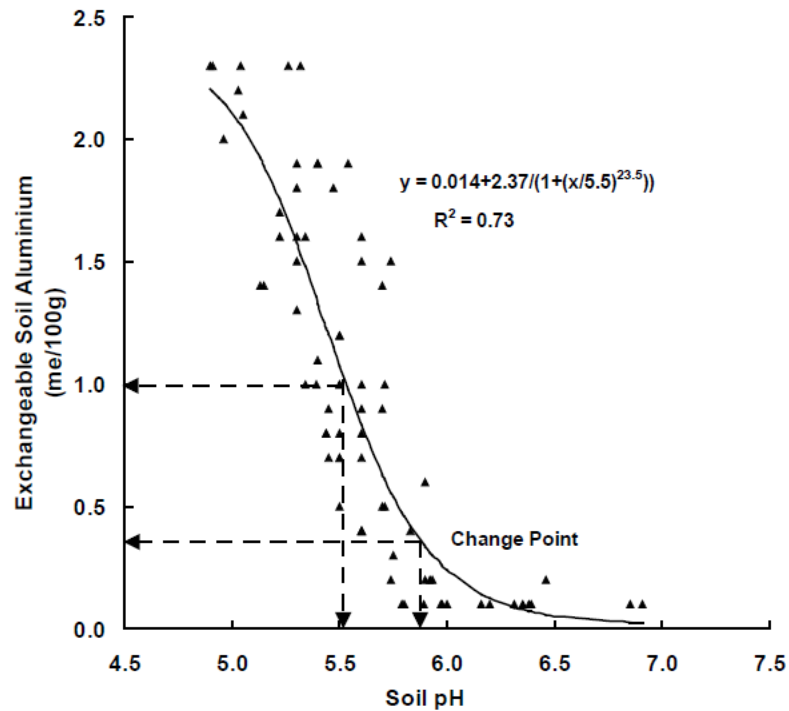
Figure 2.2 The relationship between exchangeable soil Al and soil pH at Lees Valley, North Canterbury. Taken from Moir and Moot (2010b).

Lime ($CaCO_3$) is commonly used to reverse soil acidity. Under acidic soil conditions, its application is closely associated with improved legume growth. Berenji *et al.* (2015) found that a lime application of 2 t/ha on an acidic soil (pH = 5.2) increased yield of lucerne from 3 t DM/ha/year to 4 t DM/ha/year at Burnham, Canterbury. In addition, the use of a suitable rhizobial inoculant did not have an effect on the growth of lucerne in the establishment year, but caused an increase in lucerne growth from 2.4 to 7.8 t DM/ha/year in Year 2. Growth in the establishment year may have been achieved through uptake of soil mineral N. However, lower availability of soil mineral N in the second season would have caused plants to revert back to N fixation, which uninoculated plants appeared to be incapable of. This difference could also be due to the reduction in Al toxicity which is shown to dramatically limit root growth in lucerne (Berenji *et al.*, 2015; Macleod and Jackson, 1965; Mullen *et al.*, 2006; Schmehl *et al.*, 1950). Similarly, Davis (1981b) found that the addition of lime led to considerable improvement in the productivity of lucerne whilst noting that its sensitivity to Al toxicity was considerably higher than white clover and lotus (*Lotus uliginosus* syn. *pedunculatus* L.).

Inputs of lime have been noted to have a considerable effect on livestock production from forages grown in acid soils (Li *et al.*, 2010; Li *et al.*, 2003; Li *et al.*, 2006b; Lowther *et al.*, 1987). For example, Li *et al.* (2006a) found that inputs of lime increased the carrying capacity of pastures by 29%, which led to an increase in lamb liveweight productivity of 27%. Similarly, lime had a profound effect on

the productivity of acid soils in New South Wales, Australia (Chen *et al.*, 2009). In that study, perennial pastures that received treatments of lime were able to support a greater number of lambs that grew at a similar rate as those on unlimed control pastures (Table 2.2). This led to the production of 506 kg/ha of liveweight over four grazing cycles compared with 423 kg/ha for control pastures. In both studies, additional liveweight production was attributed to an increase in pasture palatability through increased abundance of legumes.

Table 2.2   Average stocking rate (lambs/ha), daily liveweight gain (g/head) and liveweight gain of lambs (kg/ha) from perennial pastures with (+) and without (-) lime on an acid soil in New South Wales, Australia. Adapted from Chen *et al.* (2009).

Treatments	Cycle 1†	Cycle 2	Cycle 3	Cycle 4
Stocking rate (lambs/ha)				
- lime	24.7	29.6	32.1	32.1
+ lime	39.5	44.4	37	34.6
Daily liveweight gain (g/head)				
- lime	249	224	155	27
+ lime	213	215	145	-24
Live weight gain (kg/ha)				
- lime	140	152	104	27
+ lime	201	206	113	-14

†Cycle: grazing cycles, 1-week grazing, 2-weeks resting

2.3.2   Fertiliser inputs

Lime is considered 'cheap' to purchase ($/t). However, it is argued that once its considerable bulk and high application costs are considered, it may not be the most economically feasible amendment (Scott and Williams, 1996). Therefore, if farms have a certain amount of expenditure dedicated to amendments, it is important to understand where these can be most efficiently spent. This is particularly important during the introduction of new species which are often adapted to higher fertility environments than the resident vegetation. This was highlighted in Scott (2008) where the low success of introduced species was observed in treatments that did not receive fertiliser. Pastures that did not receive fertiliser had a carrying capacity of 0.8 stock units (SU)/ha which was considerably lower than 3.1 SU/ha that was achieved with the additions of 50 kg superphosphate (9% P, 12% S) per year. This experiment also showed that there was approximately a 15% advantage to the mob stocked treatments across all rates of fertiliser application. The increased growth of pastures receiving inputs of P and S was also reflected in the nutrient pools of the soil under these experiments where the total amount of C increased from 120 to 134 t/ha and N from 8.9 to 10.4 t/ha as fertiliser input rose from 0 to 50 kg/ha/year, respectively. In a similar

experiment, Scott (1989) noted that some species were more efficient at using fertiliser inputs than others. Perennial lupin became the dominant species in all but the highest level of P in the sixth year of the experiment. He concluded that the most financially effective treatment of fertiliser was an annual application of 50 kg/ha of sulphur superphosphate 30 (7% P, 30% S), which would provide 4 kg P/ha and 16 kg S/ha. This was compared with the recommendation of 20 kg P/ha (250 kg/ha superphosphate) at establishment and 10 kg P/ha (125 kg superphosphate) in ongoing annual applications (Floate *et al.*, 1985). Sulphur makes up a larger component of superphosphate than P. Therefore, it is suggested that S may be more important in maintaining pasture production than P (Jarvis *et al.*, 1997; Scott, 1998; Scott and Covacevich, 1987) and was found to be universally limiting during the development of the 3500 ha Tara hills property in the Mackenzie Basin (Pedofsky and Douglas, 1987).

There is a strong positive relationship between fertiliser input and stocking rate in high-country pastures (Luscombe, 1980; Pedofsky and Douglas, 1987; Scott, 1998; Scott, 2000a; Scott, 2000c). This was quantified in Scott *et al.* (1989) where an additive regression model was used to estimate the effects of P and S inputs on the carrying capacity of pasture (Equation 2.1). Whilst this shows that it is possible to estimate grazing capacity and, therefore, economic viability of fertiliser inputs, calculations are still largely dependent on commodity prices that undergo constant fluctuation. Therefore, maintaining consistent inputs should be the focus, to prevent "humping and hollowing" of production throughout different growing seasons.

Equation 2.1
$$SU/ha = 1.7 + 0.28\sqrt{P} + 0.17\sqrt{S}$$

2.3.3 Animal productivity

Forage legumes are known for their high-palatability which, when animals are given the choice, is reflected in their preferential grazing. For example, Cosgrove *et al.* (1999) found that when given the choice, sheep consumed a diet comprising 70% legume and 30% grass. There are several possible explanations for this preference. Often, grasses will tend to increase in the amount of neutral detergent fibre (NDF) present in their leaves as the plant matures (Buxton and Marten, 1989). This increase in NDF can have a negative effect on animal productivity due to the strong link between NDF and intake potential (Buxton, 1996).

The higher crude protein (CP) content of legumes may also influence feed consumption in ruminants. Kyriazakis and Oldham (1993) showed that lambs tended to select a diet based on their

daily CP requirements, with a greater preference for components that were high in palatability. However, studies by Cosgrove *et al.* (2002) showed that the relationship between diet preference and CP content may not be as critical as initially thought (Table 2.3). When clover was offered with either high-N grass or low-N grass, sheep selected 82% and 78% clover, respectively. However, this experiment did illustrate that, when given a choice between high-N grass or low-N grass, sheep spent 78% of their grazing time in high-N plots. Therefore, the high palatability (Ates *et al.*, 2008; Black *et al.*, 2007; Dorland *et al.*, 2007) and lower seasonal fluctuations in nutritive value of leaf material (Black *et al.*, 2014b; Brown and Moot, 2004) will likely contribute to the higher intakes generally found for legume herbage (Buxton, 1996).

Table 2.3 **Total time spent grazing (minutes/day) and preference (ratio of foods eaten) by sheep offered choices from two food types comprised of ryegrass having N at a high or a low concentration (HNG_LNG), and each type of ryegrass with white clover (HNG_C, LNG_C), and time spent grazing by sheep offered ryegrass alone, having a high or a low concentration of N (HNG, LNG). Adapted from Cosgrove *et al.* (2002).**

Treatment	Time spent grazing (minutes/day)			Preference
	Total	Food A[1]	Food B	Food A: Food B
HNG alone	360			
LNG alone	350			
Signif.	NS			
HNG_LNG	410	320	90	78:22
HNG_C	290	220	70	76:24
LNG_C	280	230	50	82:18
Signif.	$P<0.01$	$P<0.05$	NS	

[1] For each treatment read Food A and Food B as follows: HNG_LNG: Food A = high N grass, Food B = low N grass; HNG_C: Food A = clover, Food B = High N grass; LNG_C: Food A = clover, Food B = Low N grass.

There is an important balance to strike between individual animal performance and productivity per area of land. Whilst it is tempting to focus on individual animal performance, a focus on overall productivity is generally more profitable (Dove, 2010). This comes from a balance between stocking rate and livestock performance. In the stocking rate experiment of Ates *et al.* (2008), a stocking rate of 8.3 ewes/ha (with twin lambs) led to a mean lamb liveweight gain of 356 g/head/day which was significantly higher than 269 g/head/day for those at a higher stocking rate of 13.9 ewes/ha. However, the higher stocking rate led to a mean overall productivity of 7.3 kg/ha/day compared with 5.4 kg/ha/day at the lower stocking rate. This was attributed to the greater consumption of DM whilst there was an apparent increase in the utilisation of annual clover. However, as pasture availability decreased in early summer, ewes at the high stocking rate were losing about 120 g/head/day, which would need to be reclaimed to ensure reproductive potential in the following

season. Adjustments may also need to be made based on early-season premiums or meeting specific carcass weights of young stock.

The concept of individual animal production and total productivity was quantified in the NZ high-country by Scott (1994). The growth rate of 1-year old Merino wethers was 58 g/head/day on perennial lupin compared with 110 g/head/day on red clover (*Trifolium pratense*). Red clover also produced double the biomass of lupin in its first year of grazing which was reflected in a stocking rate that was also double the lupin (Table 2.4). This undoubtedly improved the early productive potential of red clover. However, as the abundance of lupin began to increase, the lower stock acceptability and associated lower liveweight gains were offset by an increase in the carrying capacity of the lupin pastures. One of the probable reasons for the low growth and carrying capacity of the lupin pastures during the early years was likely due to a low plant population at establishment. The sowing rate was not reported by the author but was probably lower than the suggested optimum of 8 kg/ha (Moot and Pollock, 2014).

Table 2.4   Mean stocking rate of Merino wethers and feed on offer over five measurement years for perennial lupin, alsike clover and red clover at Mt John, near Tekapo, New Zealand. Taken from Scott (1994).

Attribute/Year	Species		
Stocking rate (sheep/ha)	Lupin	Alsike clover	Red clover
1	6	10	12
2	12	12	12
3	14	12	12
4	16	8	12
5	22	8	11
Feed on offer (t DM/ha)			
1	0.5	0.0	1.0
2	1.3	0.9	1.1
3	2.0	1.1	1.4
4	3.3	1.7	1.7
5	3.5	1.9	2.0

Legume abundance and productive performance will often be positively correlated. However, the seasonality of production for each legume species must be considered. Black and Moir (2015) found that tall fescue (*Festuca arundinacea* L.) pastures containing subterranean clover (*Trifolium subterannean* L.) were 30-50% more productive than those containing white clover. This difference was primarily attributed to white clover's inability to thrive under dry summer conditions (<215 mm rainfall; Dec-Apr), which limited its abundance and recovery in subsequent seasons. The

productive difference was primarily due to the considerable spring growth of subterranean clover, which improved the metabolizable energy (ME) and CP content of the pastures. Similarly, Brown *et al.* (2006) focused on productivity of five different legume x grass combinations and a lucerne monoculture. Lucerne appeared to be superior in its annual biomass production (Figure 2.3). However, subterranean clover pastures produced the most sheep liveweight in spring (Figure 2.4). These studies also showed that cocksfoot gave more production in summer than ryegrass without any sacrifice in spring yield. This agrees with Fasi *et al.* (2008) who found that cocksfoot produced more herbage from mid-December to February than perennial ryegrass (*Lolium perenne*) under high-country conditions. Similarly, Mills *et al.* (2015a) found that grass-based pastures produced 64-70% of their total annual liveweight during spring compared with 57% for lucerne. Cocksfoot/subterranean clover pastures yielded 8.7-13 t DM/ha/year compared with 10-18.5 t DM/ha/year for lucerne monocultures (Mills *et al.*, 2015b).
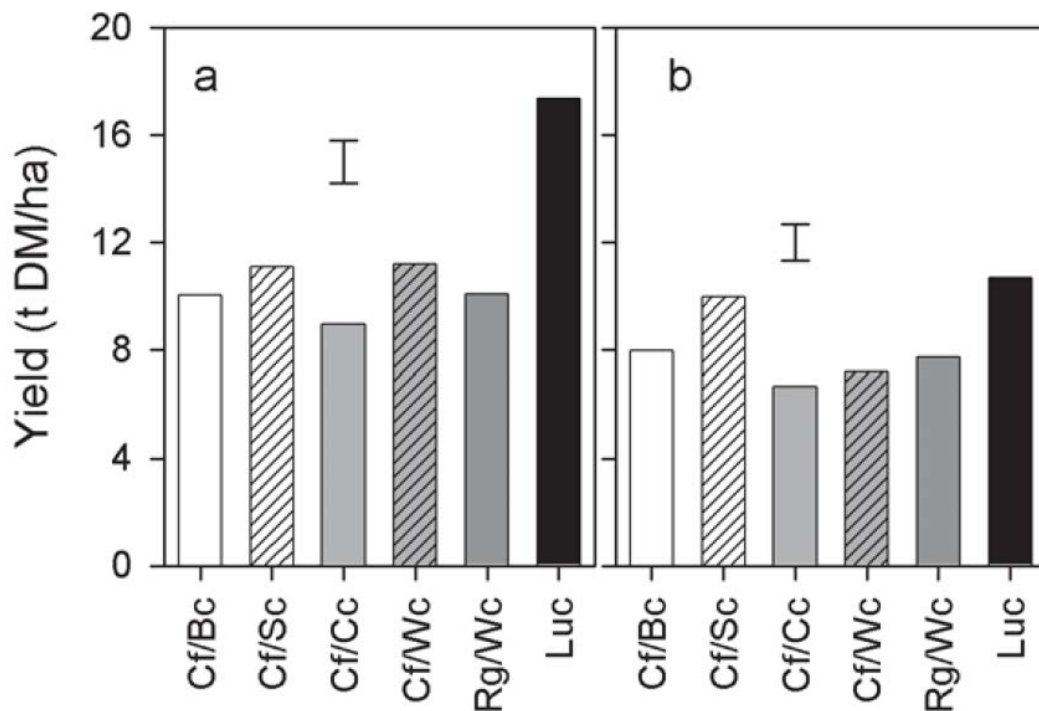
Figure 2.3 Annual dry matter (DM) yields in, a) 2004/05 and b) 2005/06 of six dryland pastures grown at Lincoln University. Cf = cocksfoot, Bc = balansa clover, Sc = subterranean clover, Cc = caucasian clover, Wc = white clover, Rg = perennial ryegrass, Luc = lucerne. Error bars represent LSD. Taken from Brown *et al.* (2006).
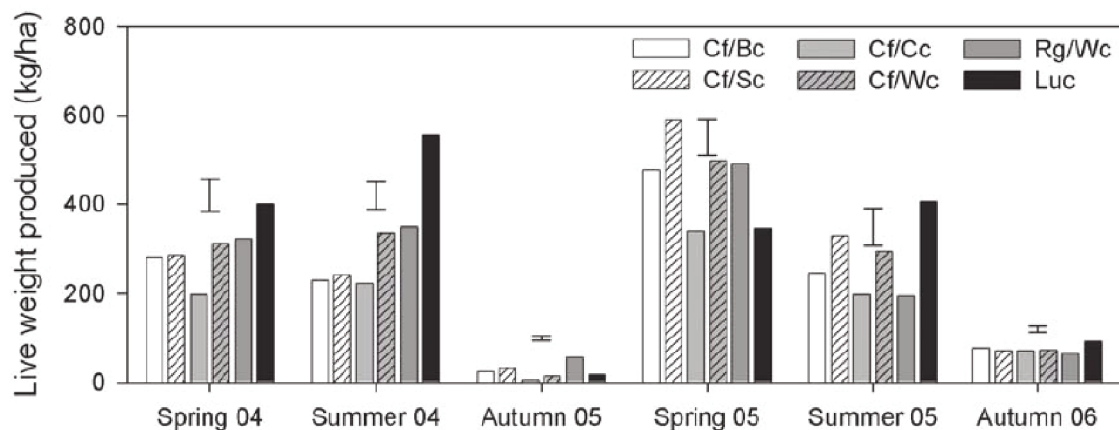
Figure 2.4 Liveweight produced per hectare from six dryland pastures at Lincoln University. Cf = cocksfoot, Bc = balansa clover, Sc = subterranean clover, Cc = caucasian clover, Wc = white clover, Rg = ryegrass, Luc = lucerne. Error bars represent LSD for each period. Taken from Brown *et al.* (2006).

2.3.4 Water extraction and use efficiency

Late spring and summer herbage growth is often limited by considerable soil moisture deficits in high-country areas. The implication is that pastures need to convert moisture to herbage more efficiently or be capable of extracting water from greater soil depths. Improved water use efficiency (WUE) is often achieved through an increase in the concentration of leaf N, induced by either N fixation or N fertiliser application (McKenzie *et al.*, 2006). Moot *et al.* (2008) found that low N nutrition cocksfoot had an annual WUE of 6.7 kg DM/ha/mm compared with 40 kg DM/ha/mm in lucerne. However, once N was added to cocksfoot monocultures, the spring WUE increased from 17 to 38 kg DM/ha/mm. This was due to a leaf N concentration, in plots receiving no inputs of N fertiliser, that was below the 4.5% required for optimum photosynthetic efficiency in cocksfoot (Mills *et al.*, 2009; Peri *et al.*, 2005).

The N concentration of pastures, and related photosynthetic efficiency, can affect the WUE of pastures but does not affect the total amount of evapotranspiration. For example, Black and Murdoch (2013) found that the yield of perennial ryegrass/white clover pastures under different N regimes did not affect the total amount of water used. In this example, pastures receiving 25 kg N/ha after each grazing had an annual WUE of 27 kg DM/ha/mm compared with 19 kg DM/ha/mm for pastures that did not receive any inputs of N fertiliser.

Sim *et al.* (2012) found that the yield of lucerne was highly correlated ($R^2=0.98$) to water use, regardless of the soil type. This pattern remained true for multiple species where lucerne, red clover and chicory (*Cichorium intybus* L.) showed near-identical relationships between yield and water use (Brown *et al.*, 2003). Thus, the amount of water that can be extracted from the soil profile

will be the ultimate driver of productivity in dryland situations. This is affected by extraction depth and soil texture, with the latter being of greater importance for deep-rooted crops such as lucerne. For example, Moot *et al.* (2008) found that, despite no difference in rooting depth, soils with a plant available water content (PAWC) of 130 mm produced 50% of the yield that was produced by lucerne on soils with a PAWC of 330 mm. Shallow stony soils did not affect the rooting depth of lucerne (2.3 m) but the lower PAWC led to a 50% reduction in yield.

2.3.5 Effects on soil structure

In Scott (2000b), the decay rate and relative OM content of soils under the imposed fertiliser and management conditions was measured. The legume species experienced a higher decay rate than the grasses and lost one fifth of their litter weight in the first two weeks. The lower C:N ratio of *L. polyphyllus* seemed to give the highest decay rate of all species, with a half-life of 0.2 years compared with 38 years for the considerably less palatable *Festuca novaezelandie* litter. This experiment concluded that the application of fertiliser was the primary driver of soil OM creation due to its effects on improved growth that was primarily related to the growth of legumes. Similarly, Drinkwater *et al.* (1998) associated improvements in soil quality with the low C:N ratio of legume litter. In this case, the quality of material being returned to the soil had a large influence on its incorporation as soil OM. However, other studies have also suggested that it is the amount of litter content that is important for improving the OM content of soil (Havlin *et al.*, 1990; Larson *et al.*, 1972), which is probably true of soils that have a high background N status but may not be as applicable in the severely N depleted soil of the high-country.

2.3.6 Species selection – perennial

The highly weathered soils of the NZ high-country are often low in nutrient abundance due to intensive leaching and low nutrient turnover. The challenging conditions and moderate affordability of inputs means that pasture growth is often dominated by resident species that offer little in terms of forage productivity and often have low palatability (e.g. browntop; *Agrostis capillaris*). For example Kearney *et al.* (2010) found that resident pastures, dominated by browntop, were growing <1.5 t DM/ha/year. However, after development, this was improved to 3.1-5.3 t DM ha/year for ryegrass/white clover pastures and 4.2-8.4 t DM/ha/year for lucerne. Whilst this was remediated with lime and fertiliser, the considerable inputs may not be economically sustainable under all situations where other limiting factors may be present.

When considering pasture development in the high-country, there are several considerations to be made, which include:

- Climate and soil fertility and their compatibility with species
- Competitiveness and abundance of resident or introduced vegetation
- Livestock acceptability and productive potential
- Long-term persistence

In general, it is a combination of these factors that will determine the choice of legume that will be sown.

2.3.6.1 White clover

White clover is the most common legume in NZ and is primarily used as the secondary pasture component, behind perennial ryegrass, in mixed-pastures (Caradus *et al.*, 1996; Cosgrove *et al.*, 2002; Frame *et al.*, 1998). It is valued for its productivity, survival under a range of conditions and its herbaceous spread through above-ground stolons. It has been traditionally oversown with fertiliser in the high-country. However, its application is limited in the lower fertility areas of the high-country, particularly those that are subjected to severe moisture stress (Scott *et al.*, 1995a). This was evident in Scott and Covacevich (1987) where its productivity, compared to other legumes, was only significant under irrigation in the high fertility treatment that received 500 kg of superphosphate per year. This was also reported by Nordmeyer and Davis (1977) where its P requirements for maximum growth were twice that of *Lotus pedunculatus*. Furthermore, its inability to thrive when subjected to drought would definitely limit its application in areas that receive minimum rainfall between December and April (Black and Moir, 2015; Knowles *et al.*, 2003). Thus, white clover is not a realistic option for low-input pastures that receive < 800 mm/year rainfall.

2.3.6.2 Lucerne

Lucerne is well-recognised for its feeding value and summer productivity in dryland environments of NZ (Brown *et al.*, 2006; Brown *et al.*, 2003; Mills *et al.*, 2015b; Moot *et al.*, 2008). Its deep tap-root allows it to exploit large volumes of soil for water extraction (Brown *et al.*, 2003; Sim *et al.*, 2017) whilst its N fixation ability allows it to maintain a high leaf N concentration that is important for the efficient use of water (Moot *et al.*, 2008) and for palatability (Brown and Moot, 2004). Its use in high-country pastures has also showed it to be a valuable tool for increased productivity (Anderson *et al.*, 2014; Avery *et al.*, 2008; Stevens *et al.*, 2012). In a high rainfall dryland

environment (approximately 1,400 mm/year), the extended summer productivity of lucerne (Figure 2.5) offered a considerable improvement in farm Gross margins over traditional ryegrass/white clover pastures (McGowan *et al.*, 2003). The reduced winter carrying capacity of the area under lucerne meant that stocking rate had to be reduced by about 10%. However, this increased the availability of feed for livestock during spring and summer which ultimately improved productive efficiency (Table 2.5).
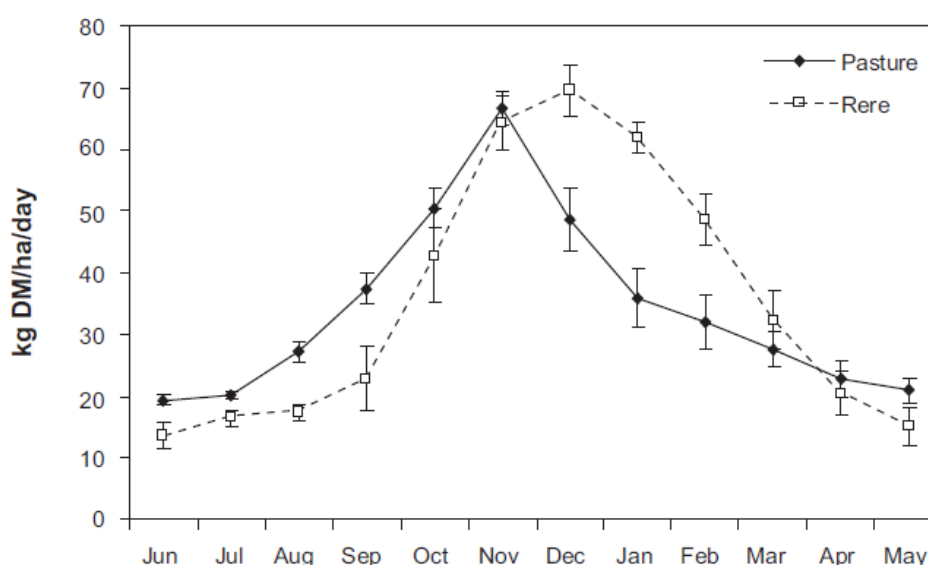
Figure 2.5 Mean monthly growth rates of pasture and 'Rere' lucerne grown on moist (~1,400 mm rainfall/yr) hill country (20° slope) over five years at Whatawhata, Waikato. Error bars indicate standard error of the mean. Taken from McGowan *et al.* (2003).

Table 2.5 Modelled farm performance after lucerne incorporation on hill country property in Whatawhata, Waikato. Taken from McGowan *et al.* (2003).

	Pasture	12.5% lucerne 87.5% pasture
Lambing (%)	116	136
Stocking rate (su/ha)	10.6	9.8
Lamb sale weight (kg)	15.5	18
Lucerne establishment and maintenance ($/ha)	NA	41.5
Gross margin ($/ha)	611	629.5

NB: All performance figures are based on per ha over the whole farm

The use of lucerne in the high-country is limited by its sensitivity to acidity (Berenji *et al.*, 2015; Caddel *et al.*, 2004; Davis, 1981b) and related aluminium toxicity (Moir, 2013). These conditions can severely restrict its root growth (Macleod and Jackson, 1965; Munns, 1965), which ultimately reduces its competitive advantage as a deep-rooting legume and makes it prone to competition from other species. The acidity has also been shown to reduce survivability and effectiveness of the

rhizobia that are required for successful N fixation (Stout *et al.*, 1997). This suggests that lucerne is primarily adapted to higher fertility sites in the lower altitudes of properties.

2.3.6.3   Caucasian clover

Caucasian clover (*T. ambiguum*) has also been recognised as a potential species for development in dryland areas (Black *et al.*, 2014a; Black and Lucas, 2000; Black *et al.*, 2007; Black *et al.*, 2002; Brown *et al.*, 2006; Widdup *et al.*, 2001). It is productive and persistent under dryland conditions where it has produced 10% more annual liveweight gain than mixtures containing white clover, which was directly related to its greater abundance on offer (Black *et al.*, 2007). Despite encouraging experimental results, its adoption among farmers has remained limited due to its slow establishment that can limit its persistence in pastures. The unnaturalised rhizobia are specific and are generally not present in NZ soils which has been attributed to establishment failures in the high-country (Black *et al.*, 2014a). It is also very susceptible to competition during establishment (Hurst *et al.*, 2000) due to the considerable investment in below-ground structures that it makes early in its life (Woodman *et al.*, 1996). However, its use as a legume is still valued in high-country pastures (Pollock and Moot, 2016) and it is probably most useful as a secondary legume component that can provide appreciable yields once established.

2.3.7   *Lupinus polyphyllus*

2.3.8   History

In NZ, there are three main forms of *Lupinus* sp. that include *L. polyphyllus*, *L. arboreus* and *L. perennis*. *Lupinus polyphyllus* and *L. arboreus* are the most widespread of the three species and have colonised several riverbeds and roadside areas of the South Island of NZ (Liu, 2014; Weir, 2006). Originating from North America (Gladstones, 1970), *L. polyphyllus* was introduced to Eastern Europe in the late 1700s/early 1800s and was eventually recognised for its potential as an ornamental plant. In its original form, *L. polyphyllus* was a primarily blue-flowered plant that was used as the parent plant for a new multi-coloured hybrid termed the "Russell" lupin. It is suggested that the majority of Russell lupins' parental material is that of *L. polyphyllus* with small contributions from *L. arboreus* and *L. nootkatnensis* (Gladstones, 1970).

The Russell lupin was introduced into NZ in the 1950s as a garden horticultural species. Its spread in the Mackenzie District, and several other regions, has occurred from garden escapes and deliberate spreading on roadsides and other areas (David Scott, Pers. Comm.). It was the success of *L. polyphyllus* on the bare, well-drained roadsides, which were often acidic and low in nutrients,

that indicated its possible use as a pioneering plant or potential forage legume species for challenging high-country environments (Lambrechtsen, 1986). For this reason, Russell lupin, or perennial lupin as it became known in NZ, was included in the extensive pasture trials at Mt John, Tekapo (Scott, 2008; Scott and Covacevich, 1987; Scott *et al.*, 1989), which will be discussed later in this review.

2.3.9 Physiology

2.3.9.1 Seed characteristics

The large-seeded nature of *L. poylphyllus* suggests that it may be less sensitive to adverse seedbed conditions than other legumes. Wangdi *et al.* (1990) found lupin emergence was highest at a depth of 20 mm where establishment was 55%. Establishment varied between 35 and 40% at depths between 0 and 3 cm. In comparison, traditional small-seeded legumes had optimum emergence at a depth of 13 mm with establishment becoming extremely poor below this (Woodman *et al.*, 1997). Greater tolerance of sowing conditions may be attributed to the greater energy reserves often associated with a large seed. However, providing establishment depth is consistent, several alternative high-country legumes, such as *Lotus pedunculatus*, have shown that they can match the seedling weight of lupin with small inputs of fertiliser (Davis, 1981a). Whilst tolerant of a wide range of sowing conditions, lupin plants still require a significant period between sowing and first grazing.

2.3.9.2 Growth characteristics

Perennial lupin is a tap-rooted (Plate 2.1), perennial legume that grows from a central crown (Scott, 1989) (Figure 2.6). A mature plant with reproductive stems can grow to a height of 1.5 m, but stems usually remain inconspicuous during its vegetative growth period. Large petioles (10-40 cm) extend the leaves from the crown. Each leaf contains 9-16 pointed palmate leaflets of 5-15 cm long. The plant produces woolly seed pods which are 2.5-5.0 cm long with each containing about 9 seeds. The seeds are brown to black in colour with a glossy sheen (Horn and Hill, 1982; Horn *et al.*, 1987).
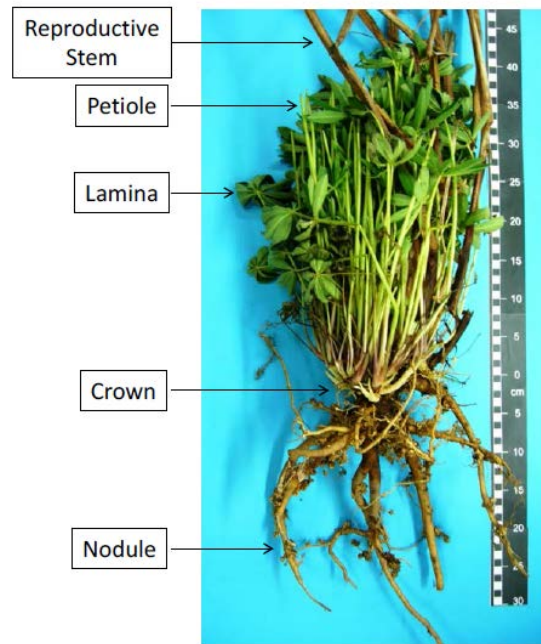
Figure 2.6 A whole *L. polyphyllus* plant taken from the 9-year-old crop at Sawdon Station, 21 May 2012.

L. polyphyllus is strongly apical dominant with plant response determined by the apical growing point at the tip of each stem. Continued vegetative growth or repeat flowering in the growing season is strongly dependent upon the absence of any previous flowering stem, even if partly dead (David Scott, Pers. Comm.). Therefore, autumn vegetative growth will be encouraged by removal of dead stems, which in some cases can lead to the occurrence of a second flowering (Kitessa, 1992; Scott and Tesfaye, 2000).

Plate 2.1   The deep tap-root of an established plant of *L. polyhphyllus* at Sawdon Station, 2013.

The flowering of *L. polyphyllus* is indeterminate and not considered to be influenced by day length and vernalisation, but this has not been quantified. In general, flowers appear in November and December, but flowering can occur in any season as perennial lupin will try to flower under any conditions suitable for growth.

2.3.9.3 Nitrogen fixation

As with most legumes, *L. polyphyllus* is capable of fixing atmospheric N via symbiotic bacteria in the root nodules. This ability gives lupin an advantage in soils that are low in N provided that other factors are favourable for growth (Andrews *et al.*, 2011; Vitousek and Howarth, 1991). To maintain this productive advantage, particularly on sites with no prior history, many legumes require the use of rhizobial inoculants at establishment (Black *et al.*, 2014a; Brockwell *et al.*, 1975; Lowther and Kerr, 2011; Wigley *et al.*, 2015; Wigley *et al.*, 2012). There are several reports in the literature of lupins nodulating at sites that have no known exposure to inoculants (Liu, 2014; Weir, 2006). However, despite indications that there may be a suitable symbiont partner in NZ soils, Scott (1989) suggested that inoculation of lupin would improve seedling growth.

Lupins are nodulated by the slow-growing, acid-tolerant strains of rhizobia belonging to the *Bradyrhizobium* genus (Barrera *et al.*, 1997; Jarabo-Lorenzo *et al.*, 2003; Stepkowski *et al.*, 2007). Studies in Australia suggested that *Bradyrhizobia* isolated from Lupin *spp.* have European origins (Stepkowski *et al.*, 2005; Stepkowski *et al.*, 2011), though the exact source has not been defined. NZ studies have found *Bradyrhizobia* to be the main symbiont partner to the widespread invasive weeds such as gorse (*Ulex europaeus*) and common broom (*Cytisus scoparius*) (Liu, 2014; Weir *et al.*, 2004). Therefore, it is hypothesised that these plants are in fact 'promiscuous' in their selection of symbiont partners and can freely nodulate with a range of different *Bradyrhizobium* species. In an agricultural context, this could be an important consideration for farmers sowing perennial lupins, where inoculation could have considerable influence on the persistence of the crop. Additionally, selection of 'elite' rhizobia strains has been successful for other crops (Asanuma and Ayanaba, 1990; Brockwell, 1975; Thies *et al.*, 1991) and may offer some growth advantages for perennial lupin.

2.3.9.4 Acidity tolerance

The ability of perennial lupin to tolerate soil acidity, and associated high levels of soluble Al (Davis, 1981b; Scott, 1989; Scott *et al.*, 1995b), makes it a noteworthy plant in areas where only low

fertiliser inputs are used. Several authors have suggested lupin-specific adaptations to tolerate acidic soils that include:

1. Tolerance of low soil P levels and capability of utilising soil P which is unavailable to most other plants (Borie, 1990; Davis, 1981b). Borie (1990) listed four probable root adaptations of lupins growing on P-deficient soils: (i) root excretion of acid substances, (ii) deep roots and other geometry of rootlets, (iii) exudation of root phosphatases, and (iv) formation of mycorrhizal associations. The review of Lambers *et al.* (2013a) acknowledged the ability of lupins to mobilise unavailable P, but found no explanation for this. This was also supported by Gardner *et al.* (1983) and Scott (2000b) who suggested that the citric acid release from lupin roots was the probable mechanism for the release of primary minerals in soil. However, this is disputed for *L. polyphyllus* in Lambers *et al.* (2013b) where the plant was not recognised as having any specific physiological adaptations that would lead to improved P uptake. The fact that *L. polyphyllus* can survive under lower nutrient conditions than most does suggest that it has some type of adaptation for coping with low levels of available P. However, it has not been as strongly studied as other lupin varieties and therefore this ability has had only minimal exploration. Also, the extensive cross-breeding of perennial lupin may have caused some inheritance of P acquisition strategies from other species of lupin.

2. The potential to mobilise unavailable P in excess of their own requirement (Borie, 1990). This may increase P availability to companion species and further increase the productivity of low input pastures.

3. Large root system improves soil structure and aids erosion control on loose-textured soils by increasing the soil OM content, which forms and stabilises aggregates (Rowland *et al.*, 1986) and encourages N cycling (Scott *et al.*, 1995b).

2.3.9.5   *Alkaloids*
Most lupin species contain some form of alkaloid (e.g. quinolizidine) that is present in foliar and seed tissue (Gladstones, 1970; Wink, 1987; Wink *et al.*, 1995). A commonly accepted explanation for the presence of alkaloids is their use as a defence mechanism that assists plant survival (Williams and Harrison, 1983; Wink, 1983b). However, there are also other possibilities for their presence that include being a form of protein storage in seeds (Wink and Witte, 1985) or an allelopathic compound (Wink, 1983a). In the context of grazing, the presence of alkaloids may limit

animal intake and, therefore, livestock productive potential despite *L. polyphyllus* containing about half the alkaloid content than other lupin species (Gibbs, 1988).

Scott (1989) suggested that reduced acceptability of *L. polyhphyllus* over the summer was probably due to high levels of alkaloid in the leaves. This was similar for Kitessa (1992) who found that relative grazing pressure needed to be increased to encourage consumption when *L. polyphyllus* was grown at high plant densities (approximately 90% of diet). Both authors did acknowledge that sheep did eventually adapt to the taste of lupins, whilst Scott and Covacevich (1987) noted that the preference for the plant tended to return in autumn. Other studies (Gibbs, 1988) have suggested that *L. polyphyllus* has an alkaloid content of approximately 2.4% (varying between 1.5% and 3.1%) compared with the higher alkaloid content of 5.2-7.9% found in *L. arboreus*. There is no evidence to suggest that the presence of alkaloids in Russell lupin has detrimental effects on animal health in NZ.

The evaluation of several parental lines of *L. polyphyllus* indicated that there was some potential for selecting low-alkaloid varieties that would have improved stock acceptability (Scott and Tesfaye, 2000). This has also been recognised overseas (Kurlovich *et al.*, 2008), where the dominance of lupins in sub-optimal environments has triggered interest in breeding them for improved grazing value. However, breeding lupins for low-alkaloid content is somewhat of a paradox. In one case, improved livestock acceptability would improve the potential for increased animal performance. However, it would undoubtedly increase the grazing pressure on the lupin plant which can have negative effects on its persistence – as is often the case when small amounts of palatable legumes are present in a pasture.

2.3.10 Place in high-country pastures
The majority of information surrounding the use of *L. polyphyllus* in the high-country has come from the 25-year species mixture experiment at Mt John, Tekapo (Scott, 1999a, 2012). In the following section, a considerable amount of this literature will be reviewed, and other supporting information will be added when available.

2.3.10.1 Forage growth
Many high-country trials, that investigated *L. polyphyllus*, used animal productivity to evaluate its potential. After reviewing the literature, there was one report from Mt John of lupin-based pastures producing 5-7 t DM/ha/year with low-to-moderate fertiliser inputs (0-100 kg of

superphosphate per year) (Scott, 2000b). However, there appears to be only one study that has quantified the DM yield and yield components of *L. polyphyllus* in any detail. That study was a Master's project completed in 1992 by Soressa M. Kitessa that was carried out at Lincoln University (Kitessa, 1992). Plots were established two years prior to the commencement of measurements, and regular herbage cuts and grazing were used to evaluate the seasonal productivity of the lupin monocultures. Lupins reached a maximum biomass of 160 g DM/plant at the dry pod stage (Figure 2.7), which was at the end of summer. However, 75% of this had been produced by full-bloom in mid-November. Yield increased considerably in mid-spring when plant size rose from 42 to 100 g DM/plant. The second largest increase was from 28 December to 18 January when plant weight increased from 120 to 160 g DM/plant, which was mostly attributed to unpalatable components (dry stem/pods). This illustrated the productive potential of lupins during spring/early summer. However, its application was limited due to the high-fertility site that the experiment was undertaken on.
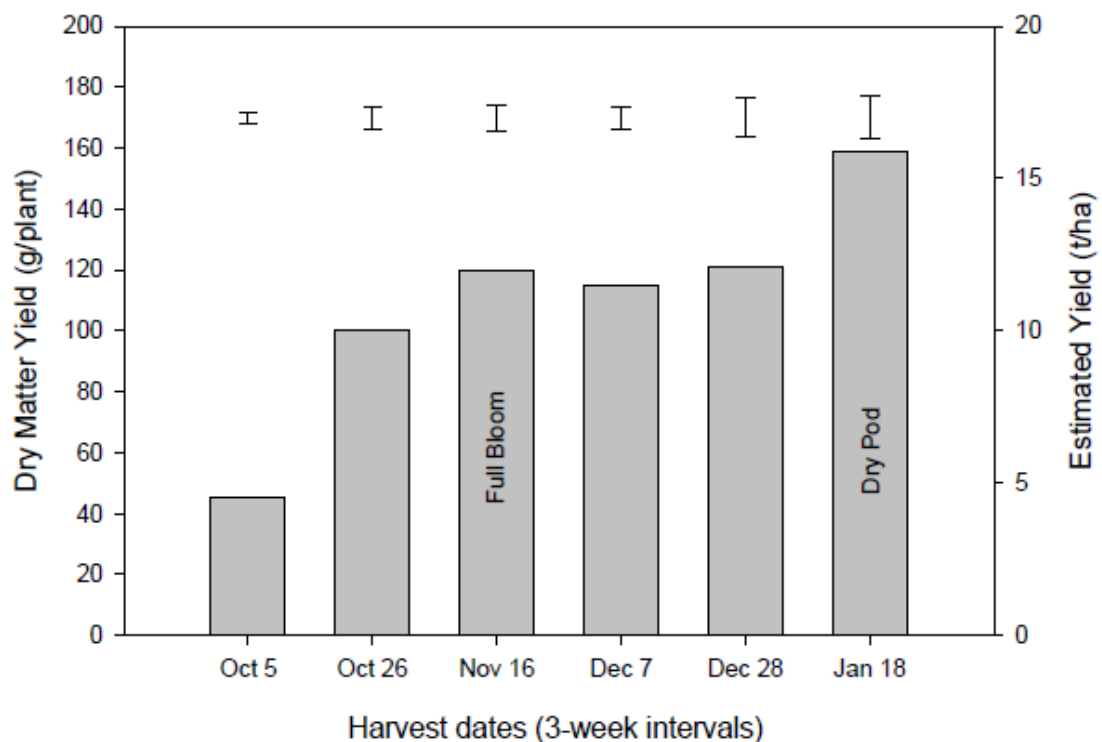
Figure 2.7 Dry matter (DM) yield of individual perennial lupin plants at different growth stages in 1988/89 at Lincoln University, Canterbury. Estimated yield was based on a plant population of 10 plants/m^2. Redrawn from Kitessa (1992)
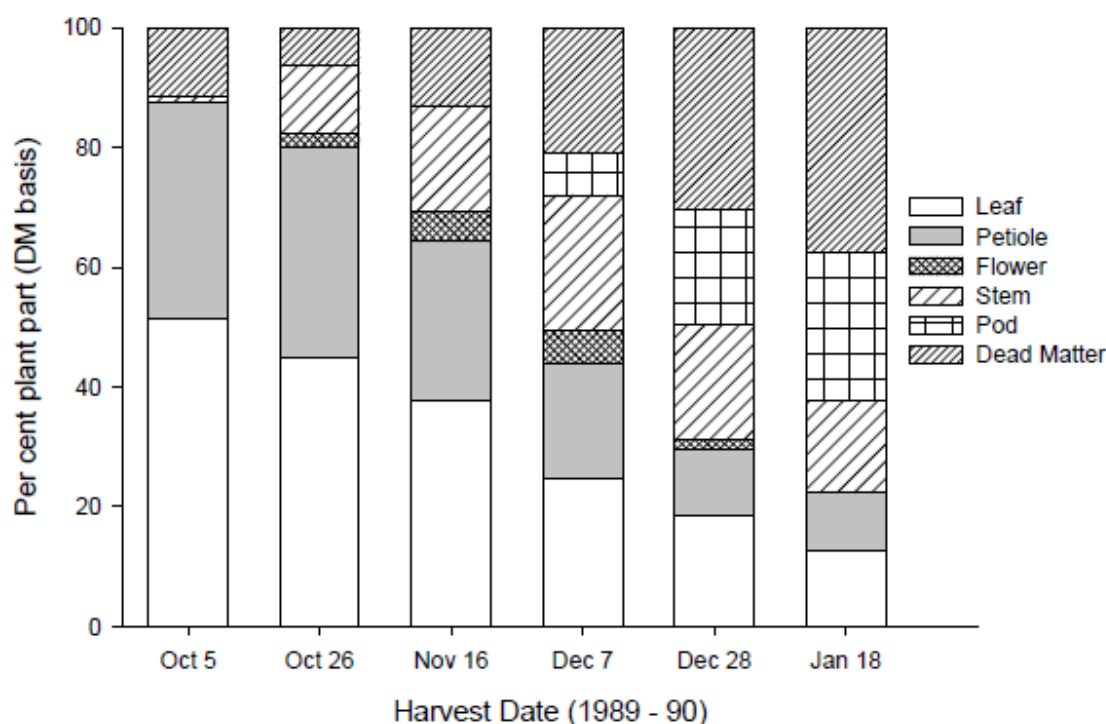
Figure 2.8 The distribution of plant parts in the total dry matter (DM) yield per plant of perennial lupin at 3-week intervals from 5 October 1989 to 18 January 1990 at Lincoln University, Canterbury. Redrawn from Kitessa (1992).

The findings of Scott (2014) suggest that lupins can take time to become dominant in newly developed pastures. The low sowing rate of 2 kg/ha in these experiments meant that initial populations of lupin were low. However, the withholding of stock from these experiments for the first two growth seasons allowed the lupin plants to reseed and the swards to naturally 'thicken'. This meant that they eventually provided 1560 grazing days per year under a mob-stocking regime. In comparison, Moot and Pollock (2014) found that perennial lupin could achieve dominance (approximately 80%) as a pasture legume in the establishment year with stands that were producing >10 t DM/ha/year. This experiment also showed that sowing rates of >8 kg/ha did not offer notable improvements in lupin yield throughout the two-year experiment. Therefore, as seed becomes commercially available, it is suggested that moderate sowing rates of 8 kg/ha should be used to achieve maximum productive potential.

2.3.10.2 Nutritive value

Information surrounding the nutritional value of *L. polyphyllus* as forage is limited and there is a need for further investigation in this area. The main source of lupin nutritional data currently available comes from the Masters research of Kitessa (1992).

Kitessa (1992) reported that the leaves and flowers of perennial lupin retained a high metabolisable energy (ME) concentration (12.2-12.8 MJ/kg DM) throughout all harvest periods, whereas the ME of stem reduced from 13.2 to 3.8 MJ/kg DM and of petiole from 10.1 to 5.8 MJ/kg DM (Table 2.6). The results indicated that leaf material remained the most acceptable of all yield components regardless of growth stage.

Table 2.6 **Within-harvest comparison of the mean metabolisable energy (MJ/kg DM) of different plant parts of perennial lupin at various growth stages from 5 October 1989 to 18 January 1990 at Lincoln University, Canterbury. Taken from Kitessa (1992).**

| Harvest Date | Plant Part | | | | | | [1] L.S.D. | [2] CV |
	Stem	Petiole	Leaf	Flower	Pod	Dead matter		
Oct-05	13.2	10.1	12.2	-	-	2.6	0.05	0.04
Oct-26	11.5	9.1	12.8	11.4	-	4.2	0.07	0.05
Nov-16	7.1	7.1	12.4	12.2	-	7.5	0.04	0.11
Dec-07	5.0	6.9	12.5	12.2	11.8	5.2	0.07	0.05
Dec-28	4.4	6.9	12.6	11.9	8.1	4.0	0.06	0.05
Jan-18	3.8	5.8	12.2	-	6.5	4.5	0.17	0.15

[1] L.S.D. = Least significant difference at $P<0.05$. [2] CV = Coefficient of variation (%).

Sheep showed a strong preference for lamina material when introduced to lupins in late spring (Kitessa, 1992). Over the four-day grazing period, they removed 99% of the lamina material before preference was shown for other yield components (

Figure 2.9). There was also evidence that sheep performance might decline once the flowers, leaves and immature green pods of the lupin have been removed (Table 2.6). Dr David Scott (Pers. Comm.) also indicated that young flower buds are often the first part to be eaten from the plant. During spring, he observed that all parts of the plant are eaten, but then leaves are progressively grazed less (Scott and Covacevich, 1987). Thus, despite evidence that ME and probably CP remain high in the leaf during this period, the preference may be reduced due to the elevated presence of secondary compounds such as alkaloids (Wink, 1987; Wink and Hartmann, 1981; Wink *et al.*, 1995).
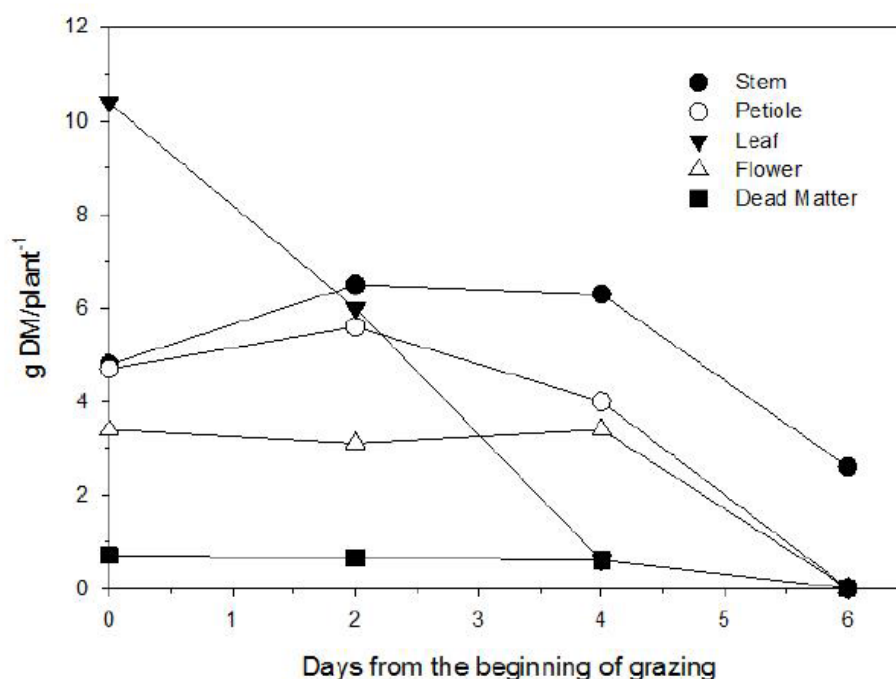
Figure 2.9 The pattern of disappearance of individual yield components of perennial lupin over successive days of grazing from 27 November 1990 to 3 December 1990 at Lincoln University, Canterbury. Redrawn from (Kitessa, 1992).

When sheep grazed the lupin at full bloom, residual herbage was 270 kg DM/ha, which was considerably lower than the 960 and 1920 kg DM/ha left after the green and dry pod grazings, respectively (Table 2.7). The increasing proportions of stem, pod and dead matter over time would have decreased whole-plant quality and reduced grazing preference towards the lupins. Regrowth after grazing in late November at full bloom was 4900 kg DM/ha, which was three times greater than the regrowth of 1600 kg DM/ha for plants grazed at the dry pod stage. Vegetative regrowth was limited after grazing at the dry pod stage in late summer, with plants having little time to recover before autumn grazing. Grazing at the full bloom stage provided a balance between herbage quality and quantity and added flexibility in autumn.

Table 2.7   Effect of timing of spring/summer grazing on autumn regrowth of perennial lupin at Lincoln University, Canterbury. Taken from (Kitessa, 1992).

Growth stage at spring/summer grazing	Autumn regrowth yield	
	Residual (kg/ha)	Regrowth (kg/ha)
Full Bloom (27/11/90)	270	6690
Green Pod (17/12/90)	960	2818
Dry Pod (21/01/91)	1920	362

2.3.10.3 Responses to fertilizer inputs

A 25-year pasture trial at Mt John evaluated the success of different species, including *L. polyphyllus*, under a range of induced soil and grazing conditions (Scott, 2008, 2012). The goal was to define the specific 'niche' of a range of species. This was based on the assumption that each species will become dominant under a specific soil fertility, soil moisture and grazing regime. The treatments included five rates of superphosphate (0, 50, 100, 250 and 500 kg/ha/year), two stocking methods (sustained grazing or short-term mob stocking with Merino wethers), and three grazing pressures (two (lax), three (= best guess), and four (hard) sheep per plot), with two spatial replications and 500 mm/ha/year of irrigation. In a separate fertiliser trial (Scott, 1998), which investigated the interactions between P and S supply, there were 31 combinations of P and/or S rates of 0, 5, 10, 20, 50 and 100 kg applied to the same multi-species swards. At the commencement of the experiment, the Mt John site was dominated by depleted fescue tussock and hawkweed (*Hieracium pilosella*) (Scott, 1999b). Soil test values were taken to a depth of 5 cm at the start of the experiment (1982) and were pH 5.2, exchangeable Al of 2.8 mg/kg, Olsen P of 40 mg/kg, and sulphate S of 5 mg/kg. The Olsen P was relatively high in the upper 5 cm but decreased rapidly to a value of 5 mg/kg at a depth of 10 – 20 cm. The following section will review the performance of perennial lupin in response to the various regimes.

Plant species responded most strongly to differences in fertilizer rate, which was followed by the effects of the different grazing regimes (Figure 2.10). With respect to perennial lupin, it became dominant, and remained dominant at the low to moderate fertiliser levels, but not without fertiliser. The dominance of perennial lupin at the low rate of fertiliser (50 kg superphosphate/ha/year) led to a four-fold increase in sheep carrying capacity compared to the zero fertiliser treatments (Scott, 2000a). Additional fertiliser did not improve the productivity of either lupin or any other species significantly and, therefore, did not offer any improvements in carrying capacity above the low fertiliser treatment. In this example, the significant improvement in carrying capacity with superphosphate suggested that P and S were highly limiting for pasture production. However, given that there was no increase at higher fertiliser rates, the results suggest that productive potential was met. Given that carrying capacity was doubled when plots received irrigation, this experiment showed that high fertiliser inputs are unlikely to result in greater pasture growth when uncontrolled factors such as soil moisture are severely limiting for seasonal and annual productivity.

There was no discernible effect of grazing intensity on the abundance of lupin during the first decade, but it subsequently decreased in abundance under the moderate, and more particularly,

the high stocking rate treatments (Table 2.8). The heavier grazing treatments of this study probably reduced persistence due to consistent grazing pressure that did not allow lupins to become apically dominant and also the possibility of damage to the growing point. It should also be clear that these stands were allowed to establish for two years before grazing treatments commenced. This would have allowed lupins sufficient time to establish. Thus, the effects of moderate and heavy grazing treatments may have become evident much earlier in the experiment if grazing had commenced during the establishment year, when lupins are particularly sensitive (Scott, 1989).
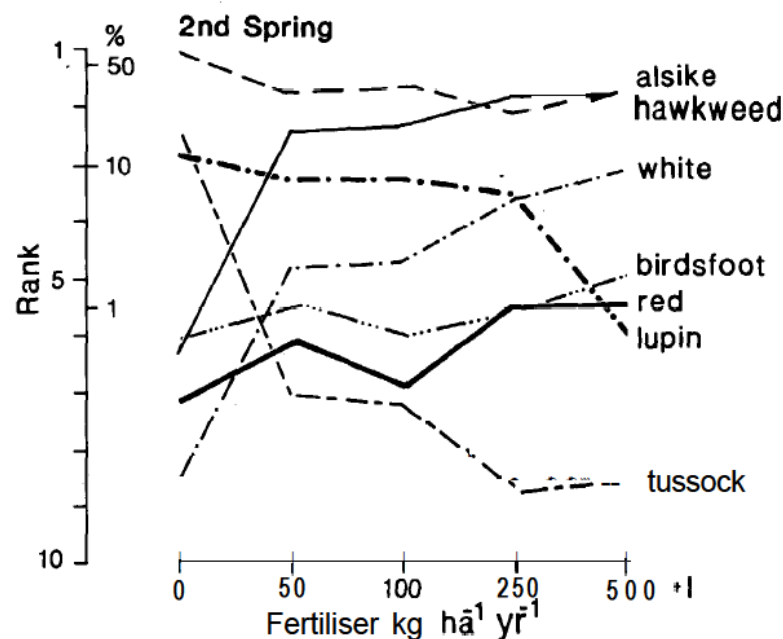
Figure 2.10 Effect of five rates of superphosphate fertiliser with irrigation on the relative abundance of the most dominant species in multi-species swards in the second spring after sowing at Mt John, Tekapo. Names: alsike = alsike clover (*Trifolium hybridum*), hawkweed = *Hieracium pilosella*, white = white clover (*Trifolium repens*), birdsfoot = birdsfoot trefoil (*Lotus corniculatus*), red = red clover (*Trifolium pratense*), lupin = *Lupinus polyphyllus,* tussock = fescue tussock (*Festuca novaezelandiae*). Taken from Scott and Covacevich (1987).

Table 2.8 **Change in species dominance over six periods across 25 years in response to five rates of superphosphate (1 = 0, 2 = 50, 3 = 100, 4 = 250 and 5 = 500 kg/ha/year) and grazing management (H = high stocking rate, M = moderate, L = low, and s = set stocking and m = mob stocking) with irrigation (500 mm/ha/year). A = alsike clover, C = Chewings fescue (*Festuca rubra*), D = cocksfoot, H = hawkweed, K = Caucasian clover, L = perennial lupin, O = tall oat grass (*Arenatherum elatius*), W = white clover, and Z = fescue tussock. Adapted from Scott (2008).**

Grazing	Year 2 - 4					Year 5 - 8					Year 9 - 12					Year 13 - 16					Year 17 - 20					Year 21 - 24				
	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5
Hs	L	A	A	L	A	H	A	A	H	W	H	L	K	K	C	H	K	K	K	C	H	K	K	K	C	H	K	K	K	C
Hm	L	L	L	L	W	L	L	L	L	D	H	L	L	L	K	L	K	K	L	K	L	K	K	K	K	L	K	K	K	K
Ms	L	L	A	L	A	H	L	L	A	W	H	L	L	K	C	H	L	L	K	C	H	L	L	K	C	H	L	L	K	C
Mm	L	L	L	L	A	H	L	L	L	D	H	L	L	L	D	H	L	L	L	C	H	K	K	K	K	H	O	O	K	C
Ls	L	L	L	L	A	H	L	L	L	D	H	L	L	L	C	H	L	L	L	C	Z	K	K	K	K	Z	O	O	K	C
Lm	L	L	A	L	A	H	L	L	L	L	H	L	L	L	L	Z	L	L	L	C	Z	O	O	O	K	Z	O	O	O	C

Other studies have also reported that perennial lupin can survive and be productive under low fertiliser inputs in the high-country. In soils low in P, perennial lupin produced more biomass than clovers (*Trifolium* spp.) (Davis, 1981b) and has demonstrated an improved tolerance of acidity and exchangeable Al than most other legumes (Davis, 1981a; Moot and Pollock, 2014; Scott, 1989; Scott *et al.*, 1995b). However, similar to the Mt John experiment, lupin productivity will likely be severely limited by deficiencies in S that are common throughout inland areas (White *et al.*, 1995). At Mesopotamia Station, White (1995) found that growth of perennial lupin was very poor in the absence of applied S at a low fertility site (Olsen P - 6 mg/kg, SO_4 - 2 mg/kg, pH - 5.3) receiving 700 mm rainfall (Figure 2.11). The perennial lupins showed little or no response to increasing rates of applied P, as either triple superphosphate (21% P, 1% S) or rock phosphate (14% P, 0% S) under nil S treatments. However, they showed a strong response to P in the presence of S (40 kg/ha elemental S applied at sowing in September 1990 and a further 20 kg/ha applied in October 1991); particularly those receiving soluble triple superphosphate. In the same experiment, Jarvis *et al.* (1997) highlighted the importance of post-establishment S application (Figure 2.12). Lupin showed an eight-fold increase in biomass production (0.5 vs. 4.1 t DM/ha) with a 60 kg/ha topdressing of S, 45 months after sowing, in plots that received 25 kg P/ha at sowing. Furthermore, the lupins that did not receive P at establishment had a three-fold increase with S at establishment and after 45 months. The authors concluded that, in the absence of fertiliser P, the addition of S was necessary to increase yield and that perennial lupin was able to extract P from soils with low natural P content. After 6 years, there were no productivity differences for lupin that related to the varying P levels. Therefore, inputs of fertiliser remain important for maintaining a productive lupin stand, however,

it appears to be a more efficient user of P compared to perennial legumes such as white clover that require regular applications of both S and P (White, 1995).
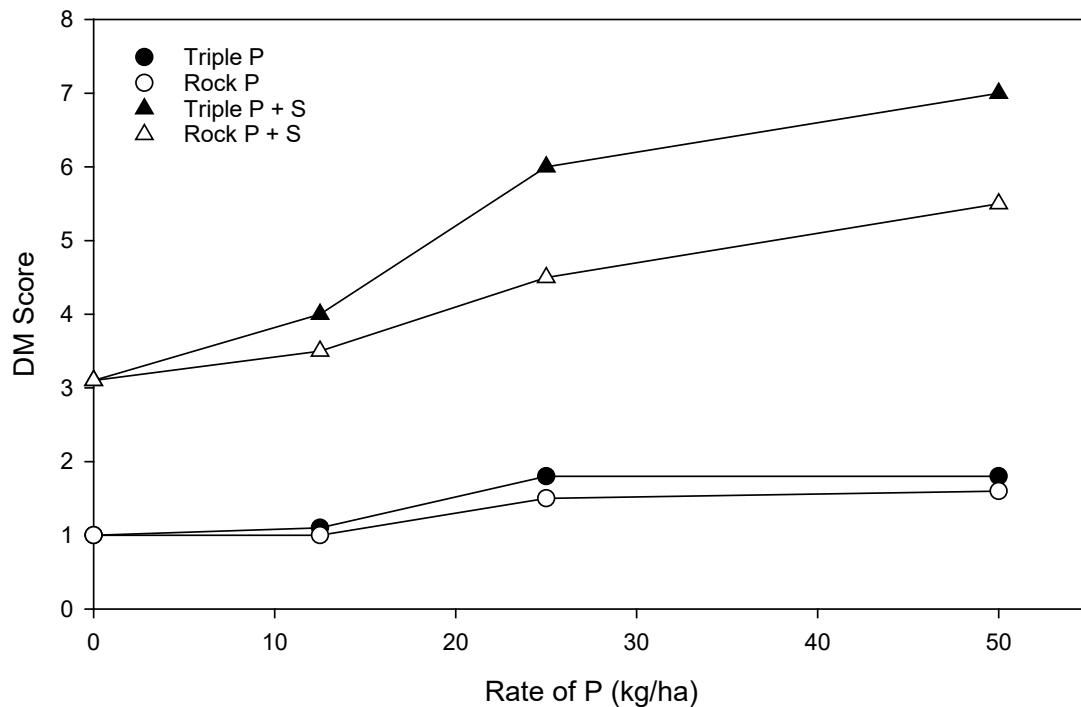
Figure 2.11 Effect of phosphorus (P; 0, 12.5, 25 and 50 kg/ha) and sulphur (S; 0 and 40 kg/ha) on the visual dry matter (DM) score of perennial lupin in December of the second growing season at Mesopotamia Station, South Canterbury. Phosphorous was applied as triple superphosphate (21% P; Triple P), rock phosphate (13% P; Rock P) and sulphur was applied as elemental sulphur (90% S). Plots were visually scored on a scale of 1-10. Redrawn from White (1995).
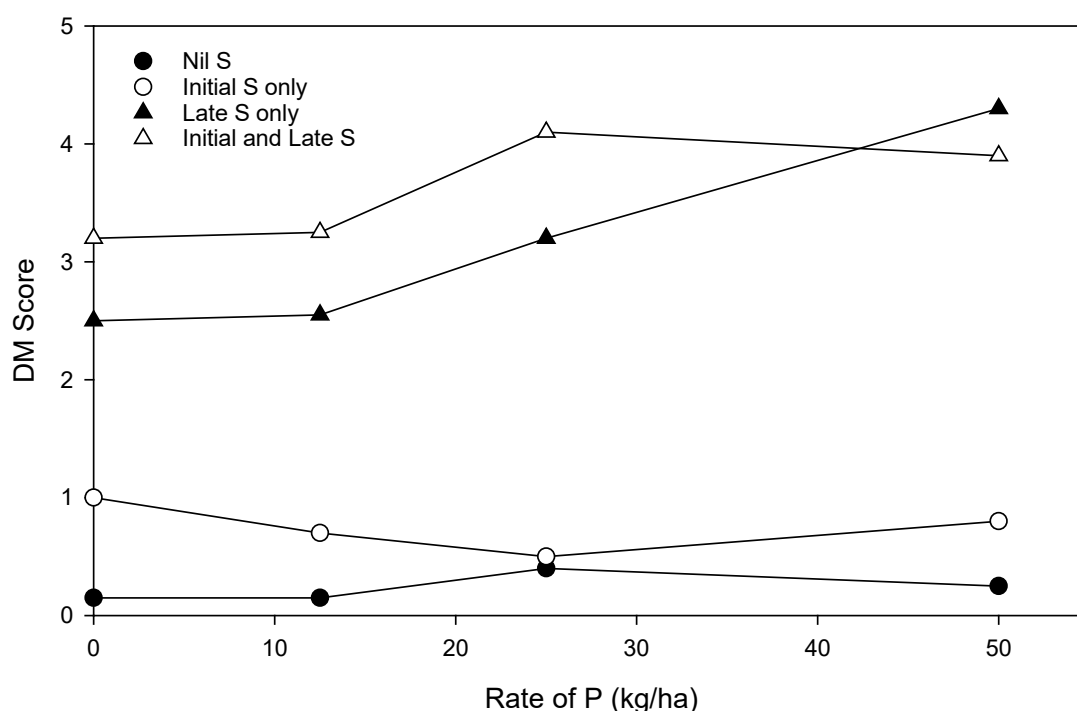
Figure 2.12 **Effect of phosphorus (P; 0, 12.5, 25, and 50 kg/ha) and sulphur (S; 0, early S (40 kg/ha at sowing in September 1990 and 20 kg/ha in October 1991), late S only (50 kg/ha in May 1994), and a combination of early and late S, on the yield (kg DM/ha) of perennial lupin at Mesopotamia Station, South Canterbury. Phosphorous was applied as triple superphosphate (21% P; Triple P) and sulphur was applied as elemental sulphur (90% S). Redrawn from Jarvis *et al.* (1997).**

2.3.10.4 Effects on animal health

There is no indication of animal health problems for sheep grazing perennial lupin in NZ. It is probable that the alkaloid-induced bitterness limits feed intake. However, this does not appear to have caused any notable issues during any of the high-country grazing experiments (Scott, 1989, 1994, 1998).

Lupinosis, a mycotoxicosis caused by the ingestion of toxins produced by the fungus *Phomopsis leptostromiformis* (Edgar *et al.*, 1986; van Warmelo *et al.*, 1970), which commonly colonises dead lupin tissue (Gardiner and Petterson, 1972), does not appear to be an issue for perennial lupin in NZ. The disease poses a considerably greater problem to the use of lupins for stock feeding than alkaloids, particularly in Australia where out-of-season rains can result in serious outbreaks (Gladstones, 1970; Kitessa, 1992). However, this information is mostly based on annual lupin and outbreaks are not known to occur in Russell lupin.

2.3.10.5   Responses to soil moisture

The drought tolerance and response of perennial lupin to soil moisture has not been quantified. Perennial lupin is noted for its deep tap-root (Plate 2.1) and reasonable resistance to dry conditions (Scott, 1989). For example, one 9 year trial at Tara Hills Research Station, Omarama, examined the persistence of lucerne, birdsfoot trefoil (*Lotus corniculatus*) and perennial lupin over-drilled with and without companion grasses at a site representative of the dry, acidic outwash soils common in the southern Mackenzie Basin (Woodman *et al.*, 1996). Plant population, seedling regeneration and stand recruitment were examined over three consecutive years under grazing by sheep and cattle. The results indicated that perennial lupin was the best adapted legume species and, therefore, one of the best species available for the future development of sustainable pastoral systems in drought-prone outwash landscapes in the Mackenzie Basin (Woodman *et al.*, 1996). However, studies have indicated that lupins prefer to grow on loose textured soils in areas with moisture seepage or moderate to high levels of rainfall (> 500 mm/year) (Scott, 1989; Scott *et al.*, 1995b). This agrees with Wills *et al.* (2003) who found that the primary reason for lupin failure in high-country experiments was due to rainfall conditions of < 550 mm/year. The drought conditions of that trial were primarily related to altitude and the experiment found that lupins were well represented in the mid (500-800 m) and high (>800 m) altitude bands.

2.4 Conclusions

- Considerable variations in topography, soil moisture, soil fertility and temperature gradients will determine economic suitability of inputs and, therefore, choice of species in the high-country environment.

- Soil fertility is a strong dictator of pasture productivity in high-country pastures and ultimately determines their productive potential and carrying capacity. Amendments of S and P are crucial for legume growth, which are required to initiate N cycling.

- Legumes have a strong ability to colonise depleted high-country soils due to their capacity to fix N_2. Therefore, rapid nodulation is crucial for ensuring persistence and maximal legume growth.

- *L. polyphyllus* is a suitable legume for low-moderate input pastures on soils that are often considered too acidic (pH < 5.5) for other legume species. Information around growth seasonality is limited for the high-country and its use as a pasture for breeding ewe flocks has not been investigated.

- Addition of S is of considerable importance for *L. polyphyllus* production in the high-country.

3 Merino performance and nutritive value of a perennial lupin pasture at Sawdon Station, Lake Tekapo

3.1 Introduction

In Chapter 2, the literature review identified perennial lupin as a useful legume under moderate inputs of fertiliser in the South Island high-country. However, prior to this study, information surrounding the nutritional value of perennial lupin and livestock production from perennial lupin pastures was limited to two studies: Kitessa (1992) and Scott (1994). As a result, perennial lupin has remained a minor species with low adoption among high-country farmers.

Sawdon Station, near Lake Tekapo in the Mackenzie Basin, is one of a few farms in the South Island high-country that has been using perennial lupin as a low-cost approach to pasture development. The idea of using perennial lupin on the farm was based on the species' ability to tolerate frosts, to start growth early in spring, and to survive and fix N for associated grasses with only 600-650 mm of annual rainfall and modest inputs of fertiliser and lime (Section 2.3.10.3). The initial grazing of perennial lupin pastures at Sawdon Station appeared to be successful and the possibility of using lupins as the sole feed source for a commercial ewe flock was recognised. To understand the application of perennial lupin in grazed pastures, further work would need to a) quantify livestock performance under continued seasonal grazing, b) understand the seasonal patterns of growth and quality of perennial lupin, and c) benchmark perennial lupin-based pastures with conventional options. Feed analyses were also required to determine the nutrient composition of perennial lupin. However, traditional analytical methods that rely on wet chemistry are time-consuming and costly. By developing NIRS calibrations for perennial lupin, we would be able to estimate its forage quality in a cost-effective and timely manner.

This chapter compares the performance of Merino ewes and lambs grazing on a perennial lupin pasture with similar ewes and lambs grazing on improved conventional pastures (predominantly lucerne and occasionally grass-clover pasture) at Sawdon Station. It also describes the seasonal pattern of herbage mass, composition and nutritional value of the perennial lupin pasture as indicators of sheep performance.

3.2 Materials and methods

3.2.1 Location and establishment of the perennial lupin pasture

The perennial lupin pasture was approximately 9 ha and located on a flat lower terrace beside Edward Stream (Figure 3.1), at Sawdon Station, 6.3 km south of Tekapo (44°03′54″S, 170°29′22″E and 680 m.a.s.l.). The soil type was classified as a sandy loam, Ashburton fluvial raw soil (S-Map, (Research, 2014).

The pasture was established in 2003 as follows (G. Loxton, personal communication). The resident vegetation, which was dominated by browntop and sweet vernal (*Anthoxanthum odoratum*) and produced a minimal amount of quality herbage for grazing, was sprayed with Glyphosate 360 herbicide (360 g/L glyphosate at 3 L/ha) in autumn 2003, fallowed over the winter and early spring, then sprayed again with Glyphosate 360 at the same rate in October 2003. Later that month, the area was over-sown and top-dressed with a seed mix of perennial lupin (3 kg/ha), oats (*Avena sativa*), barley (*Hordeum vulgare*) and Italian ryegrass (*Lolium multiflorum*) combined with Cropzeal 20N fertiliser (19% N, 11% P and 12% S at 200 kg/ha) and then lightly tilled with a tyne-harrow cultivator to incorporate the seed and fertiliser into the topsoil. The sowing rates of the oats, barley and Italian ryegrass were not recorded. The establishing lupin plants were allowed to flower and set seed before grazing in the first growing season (2003/2004). The cereals were harvested in autumn 2004 and, over the following years, the area was leniently grazed with sheep to allow the lupin stand to thicken. In 2010, the pasture was harvested for lupin seed (ca. 40 kg/ha), and in 2011, the area was divided into five paddocks of similar size with a central holding pen (Figure 3.2).
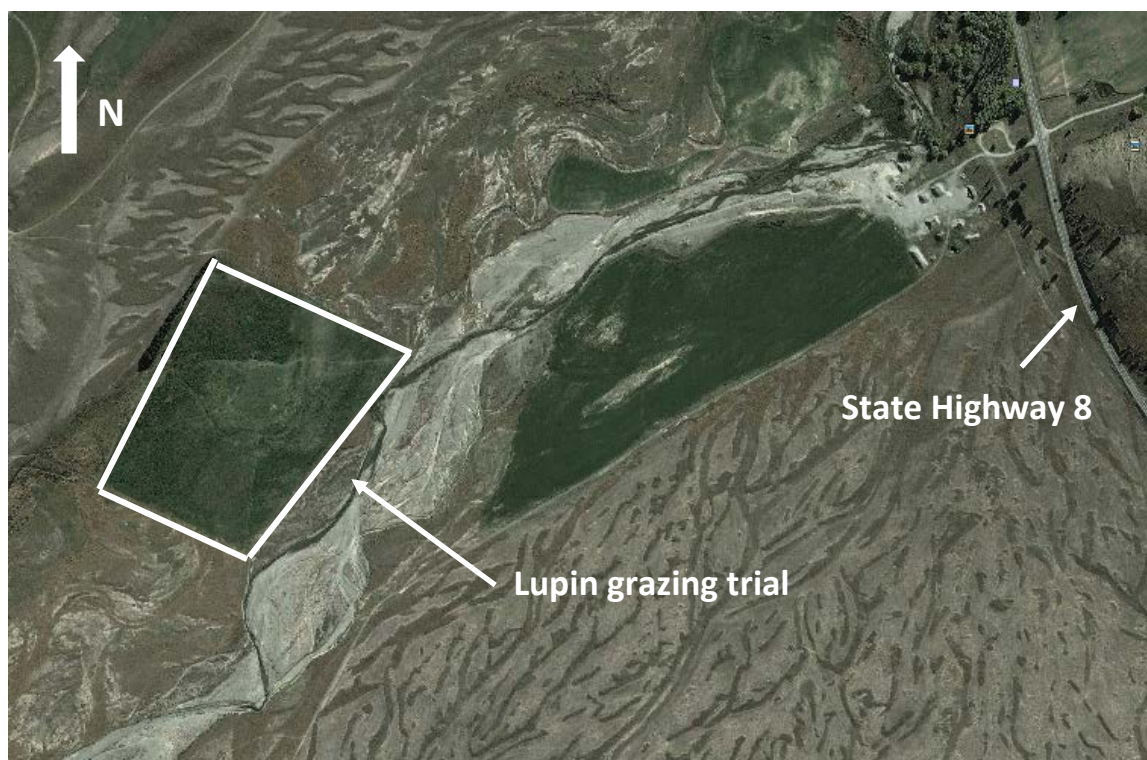
Figure 3.1 Location of the perennial lupin pasture at Sawdon Station, beside Edward Stream and 6.3 km south of Tekapo, Canterbury, New Zealand (44°03'54"S, 170°29'22"E and 680 m a.s.l.).
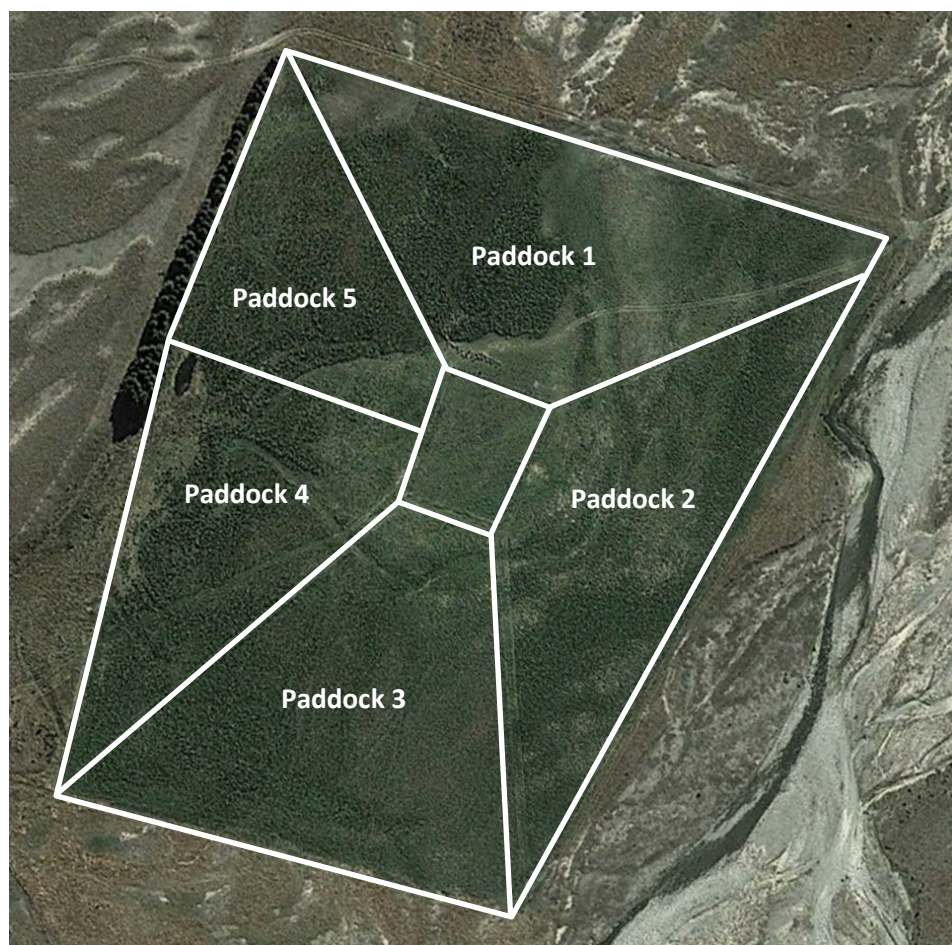
Figure 3.2 Paddock layout of the perennial lupin pasture at Sawdon Station.

While other lupin-clover-grass mixtures on Sawdon Station received modest fertiliser inputs, this lupin pasture was fertilised annually with 200-250 kg/ha of superphosphate (9% P and 12% S). In 2013, the pasture was dressed with a 200 kg/ha mix of lime (20%), superphosphate (40%) and elemental S (40%). A soil test on 23 March 2012 indicated that soil pH, Olsen P and Sulphate S were adequate and the level of exchangeable Al was low (Table 3.1).

Table 3.1 Soil test (0-7.5 cm depth) on 23 March 2012 for the perennial lupin pasture at Sawdon Station.

pH	Olsen P	Sulphate S	Ca	Mg	K	Na	Exch. Al
	mg/L	mg/kg		Me/100 g			mg/kg
6.0	24	9	4	25	13	5	<0.5

3.2.2 Animal and grazing management

On 12 December 2011, a single group of 143 two-tooth (approximately 26-months of age) Merino ewes and their 114 lambs were brought onto the lupin pasture. The group rotationally grazed around all five paddocks with fortnightly shifts. On 10 February 2012, the ewes and lambs were taken off the lupin pasture to wean the lambs and allow recovery of herbage mass. Six weeks later, on 23 March 2012, 120 ewes were put back on to the lupin pasture, and this group was rotationally grazed on all paddocks with 2-week shifts. On 18 May 2012, around five rams joined the ewes for mating. The mob was taken off the lupin pasture on 20 June 2012 for winter feeding. The ewes were shorn in September 2012.

On 11 October 2012, the same 120 ewes (now pregnant) that grazed the lupin pasture in autumn were brought back to the lupin pasture and set-stocked on four of the five paddocks at 16 ewes/ha for lambing. These animals were grouped together at tailing on 14 December 2012 for rotational grazing on all five paddocks with fortnightly shifts (Plate 3.1), starting in the paddock that was not grazed during lambing, until weaning on 18 February 2013. After a 1-month spell off the lupin pasture, 103 ewes were brought back to continue the grazing rotation of the five paddocks with 2-week shifts. About five rams joined the ewes on 20 May 2013 (Plate 3.2), all sheep were taken off the lupin pasture on 20 June 2013, and all ewes were shorn on 19 September 2013.

The third year of the experiment started on 10 October 2013, when 101 pregnant ewes that were on the lupin pasture in the previous season were set-stocked on four paddocks at about 13.5 ewes/ha for lambing. The group rotationally grazed on all five paddocks with 2-week shifts from tailing on 16 December 2013 to 27 March 2014, with lambs being weaned on 19 February 2014. After a 1-month spell for crutching, on 24 April 2014, 94 of the ewes were put back onto the lupin pasture and continued the grazing rotation. The rams joined the ewes on 19 May 2014 and all stock were taken off the lupin pasture on 23 June 2014.

Plate 3.1   Merino ewes and lambs grazing on the perennial lupin pasture at Sawdon Station, 14 December 2012.

Plate 3.2   A Merino ram with ewes on the perennial lupin pasture at Sawdon Station, 20 May 2013.

In 2011/12, a separate mob of two-tooth ewes (ca. 800-900 ewes with lambs) were measured parallel to the lupin mob and used as a "control". In the second year (2012/13), the control ewes were set-stocked in mobs of ca. 100 until tailing and then were grouped into a single mob (ca. 800-900 ewes) for rotational grazing. The same management was used in the third season (2013/14) of

measurements. The mob primarily grazed lucerne pastures and occasionally on grass/clover pasture mixes in nearby paddocks throughout each grazing season. Measurements of animal performance and major stock movements (e.g. weaning) occurred on the same date as the lupin mob.

3.2.3 Measurements

3.2.3.1 Animal performance
Live weights of the ewes in the lupin and control mobs were recorded in October, at tailing in December, weaning in February, March/April and May of each year. The lambs were also weighed at tailing and weaning each year. Lambing percentage was calculated at tailing as the number of lambs tailed relative to the number of ewes in each mob. All ewes and lambs on the lupin pasture and a sample of at least 50 ewes and 50 lambs in the control mob were weighed, and the average weights of each mob were recorded at each measurement date. On 18 March 2013, all of the 103 lupin ewes and a sample of 75 ewes in the control mob were identified with numbered tags to allow collection of individual animal data thereafter.

At shearing on 19 September 2013, the (greasy) fleece weight, staple length and fibre diameter ("micron") of the 103 tagged ewes that had been on the lupin pasture during the previous two seasons were compared with the 75 tagged ewes in the control flock. The staple length and fibre diameter of a mid-side sample of wool/ewe (Plate 3.3) were examined using a Fibrescan analyser (Eugene O'Sullivan, Pasture Measurements Ltd, Timaru, NZ).

3.2.4.2 Aboveground biomass
The above-ground biomass of the perennial lupin pasture was measured at monthly intervals during the second and third growth seasons of the experiment. At each measurement date, a strip of pasture (normally 5 × 1.15 m, but occasionally 3 × 1.15 m) was cut to a height of approximately 4-5 cm using a walk-behind sickle mower at three random sites per paddock, including paddocks sheep were grazing in (Plate 3.4). Each swath was raked, weighed and sub-sampled, and the sub-samples were taken back to Lincoln University for processing. At Lincoln University, 200-500 g of each sub-sample was dried and weighed to calculate DM content (dry weight as a proportion of fresh weight). Another 200-500 g of each sub-sample was separated into lupin lamina, lupin petiole, lupin stem, lupin flower (inflorescence), lupin pods, lupin dead material and other species (predominantly white clover, yarrow (*Achillea millefolium*), brome and Italian ryegrass (*Lolium multiflorum*), dried and weighed to estimate the proportion of each component in the sub-sample.

All samples were dried in a force-draft oven at 65°C to constant weight (at least 48 h). The DM content was multiplied by the fresh weight of the whole swath and the area of the cut swath to calculate total herbage mass. The total herbage mass was then multiplied by the proportion of each separated component in the sub-sample to estimate the botanical and morphological composition of the total herbage mass.

3.2.3.2   Nutritive value of perennial lupin

Two hundred and seventy samples of perennial lupin, collected from the lupin pasture and oven-dried during the first growing season (11 October 2012 to 22 May 2013), were ground (Cyclone mill with a 1-mm sieve) and analysed for forage quality at Riddolls Analytical Laboratory, Lincoln University. There were 68 samples of lamina, 69 of petiole, 60 of stem, 28 of flower (inflorescence), five of pod and 40 of dead material. All samples were scanned using a FOSS NIRSystems 5000 spectrometer (FOSS; Hillerod, Denmark).

One hundred and twenty-six of the scanned samples were used to develop calibration models to predict CP, Carbon (C), *in vitro* DM digestibility (DMD), acid detergent fibre (ADF), NDF, residual DM (rDM), OM and water soluble carbohydrate (WSC) content. They included samples of lamina, petiole, stem, flower, dry pod and dead material. Ninety-eight samples were used to generate (build up) the calibration equations, and 28 samples were selected as a validation set. The corresponding reference values were determined by traditional laboratory analyses. Specifically, total N and C were predicted using a Variomax CN analyser with CP calculated as N% × 6.25. DMD was determined following the pepsin-cellulase assay as modified by Clark *et al.* (1982), WSC was estimated by the Anthrone method, and ADF and NDF were determined gravimetrically (Fibre (Acid Detergent) and Lignin in Animal Feed 973.18) Official Methods of Analysis, 1990, Association of Official Analytical Chemists, 15th Edition (Association of Official Analytical Chemists, 1990). WinSIV was used for model simulation (FOSS; Hillerod, Denmark).

Equation 3.1   ME = (%DMD+3)*(OM/100*0.16)

A modified Partial Least Squares (PLS) model was selected for calibration, as advised by Riddolls Analytical Laboratory. Various scatter correction and derivation methods were compared in order to allow the PLS algorithm to find the most accurate correlation between spectral data and concentrations. The evaluation of the model was undertaken using the standard error of calibration (SEC), R-squared value (RSQ), standard error of cross validation (SECV) and one minus the Variance Ratio (1-VR). The monitor statistics for standard error of prediction (SEP) and RSQ for the validation

set were also taken into consideration. Following calibration, all samples, including those used for the calibration, were re-scanned to predict nutritive value. Nutritional data presented in this chapter includes metabolisable energy (ME: MJME/kg DM), DMD and CP, whilst the calibration regressions for all nutrition parameters are given in Appendix 1.

Plate 3.3   Example of a mid-side sample of wool collected from a Merino ewe that grazed on perennial lupin pasture at Sawdon Station, 11 April 2013.

3.2.4   Meteorological conditions

Meteorological data for the area of Sawdon Station, where the lupin and conventional pastures were located, were obtained from the National Institute of Water and Atmospheric Research (NIWA) Virtual Climate Station (VCS) network. This network produced climate data based on the spatial interpolation of actual data observations (NIWA, Auckland). Monthly rainfall and Penman potential evapotranspiration (PET) data are given in Figure 3.3 and temperature and solar radiation data are presented in Figure 3.4.

The potential soil water deficit (PSWD) for the area was estimated from a fixed NIWA climate station near Lake Tekapo (170.441°E, 44.002°S, 762 m.a.s.l.) and presented in Figure 3.5. The plant available water capacity (PAWC) for the lupin pasture was estimated to be approximately 75 mm based on information provided in the Landcare Research S-map Online soils portal (Landcare Research, 2015).
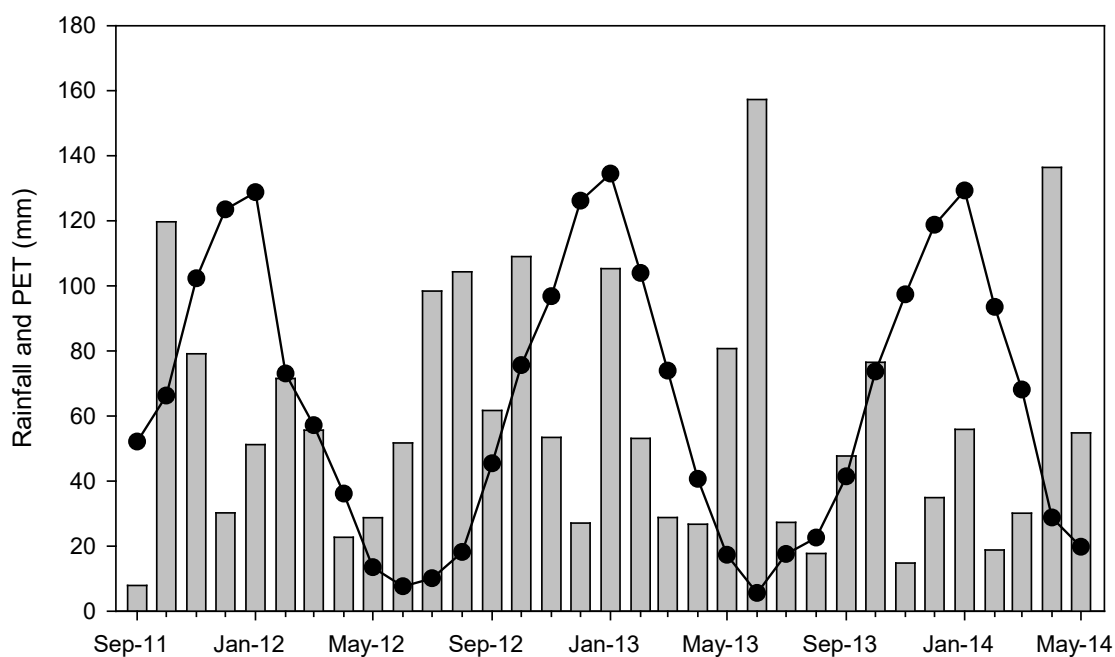
Figure 3.3 **Monthly Penman potential evapotranspiration (PET; ●) and monthly rainfall (bars) for the area of Sawdon Station, from 1 September 2011 to 31 May 2014. The data were obtained from the National Institute of Water and Atmospheric Research (NIWA) CliFlo Virtual Climate Database.**
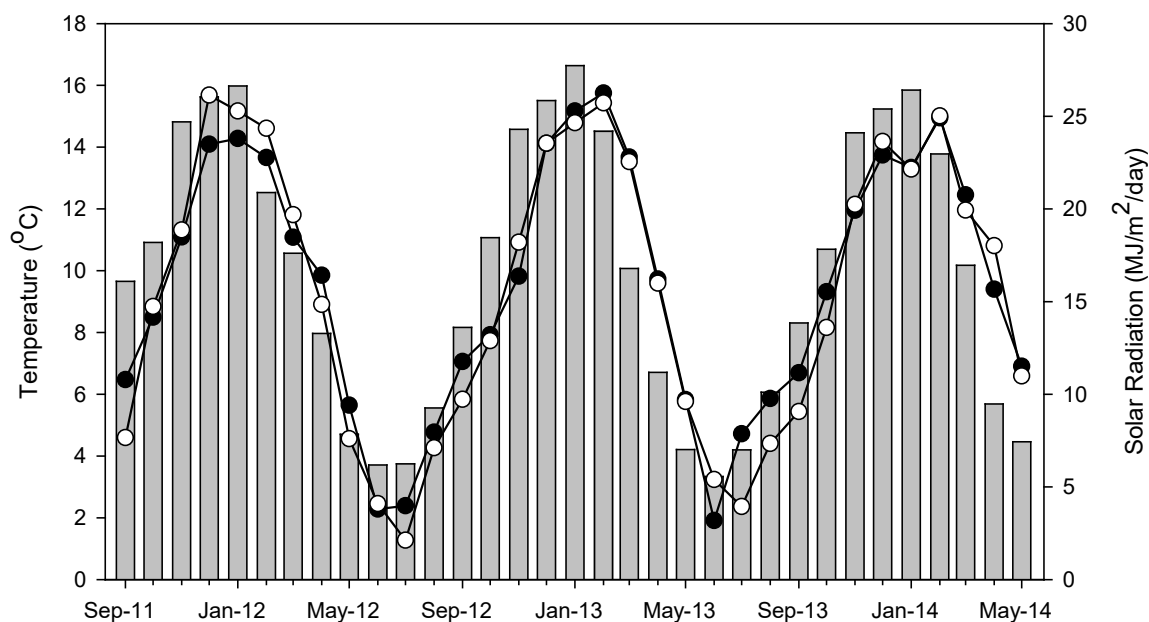
Figure 3.4 **Mean monthly air (●) and 10 cm soil (○) temperatures, and mean monthly solar radiation (bars) for the area of Sawdon Station, from 1 September 2011 to 31 May 2014. The data were estimated by the National Institute of Water and Atmospheric Research (NIWA) CliFlo Virtual Climate Database.**
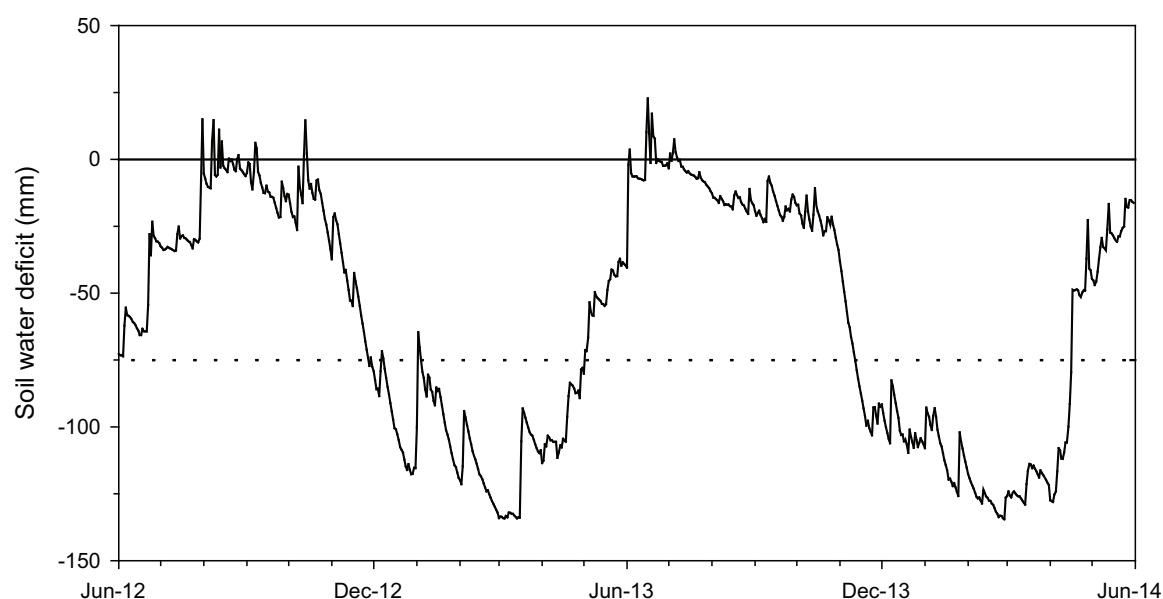
Figure 3.5 **Estimated potential soil water deficit at Sawdon Station between 1 June 2012 and 1 June 2014 at Sawdon Station, Tekapo. The data were obtained from the National Institute of Water and Atmospheric Research (NIWA) CliFlo Virtual Climate Database.**

Note: Upper line (–) represents field capacity and lower line (- -) represents soil water content at 50% depletion of potentially available water capacity (PAWC) which was estimated from the Landcare Research S-map Online soils portal (Landcare Research, 2015).

3.2.5 Data analysis

Significant (α=0.05) differences in liveweight gain and wool characteristics between the lupin and control mobs, for the dates when individual animal data were obtained, were tested by one-way analysis of variance using Genstat Ed. 16 statistical software (VSN International, 2014).

To quantify how the total aboveground biomass and botanical composition of the lupin crop changed over time, and before and after grazing, average values calculated for the whole crop and for each paddock were plotted against measurement date. The nutritive value data were also plotted over time for each lupin fraction. The mean values were normally presented with their standard errors.

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3.3 Results

3.3.1 Liveweight gain

For the first growth season, at weaning on 10 February 2012, the lambs on the lupin pasture averaged 28 kg (n=114) and had gained 150 g/day since tailing on 12 December 2011, while the lambs in the control mob averaged 31 kg and gained 217 g/day (Figure 3.6 and Table 3.2). The ewes on the lupin pasture lost an average of 3 kg over the 2-month summer period while the control ewes gained 5 kg. Between 23 March and 18 May 2012, the ewes on the lupin pasture gained 7 kg (125 g/day) and the control ewes gained 9 kg (161 g/day).

In the second season, scanning on 18 August 2012 indicated lamb percentages of 151% in the lupin ewes and 147% in the control ewes. At tailing on 14 December 2012, the lambing percentage was 103% for the lupin mob and 93% for the control mob, and the lambs averaged 20 and 21 kg, respectively (Figure 3.7 and Table 3.2). The ewes on the lupin pasture lost 8 kg during lambing while the control ewes lost 2 kg. At weaning on 18 February 2013, the lambs on the lupin pasture again averaged 28 kg (n=120) and had gained 121 g/day since tailing while the control lambs averaged 31 kg and gained 152 g/day. The lupin ewes maintained weight over the summer period (tailing to weaning) while the control ewes gained 3 kg. Between 11 April and 22 May 2013, the lupin ewes gained 2.6 kg (64 g/day) and the control ewes gained 4.9 kg (120 g/day).

In the third season, scanning on 16 August 2013 revealed 150% lambs in the ewes of both mobs. At tailing on 16 December 2013, the lambing percentage was 120% for the lupin mob and 112% for the control mob, and the lambs averaged 19 and 17 kg, respectively (Figure 3.8 and Table 3.2). The lupin ewes lost 4.3 kg during lambing whereas the control ewes gained 4.0 kg. At weaning on 19 February 2014, the lambs in both mobs averaged 30 kg after they had gained 166 g/day on the lupin pasture and 194 g/day on the control pastures since tailing. During this period, the lupin ewes gained 1.3 kg while the control ewes lost 3.6 kg. Between 24 April and 19 May 2014, the lupin ewes gained 1.7 kg (63 g/day) and the control ewes gained 2.6 kg (96 g/day).
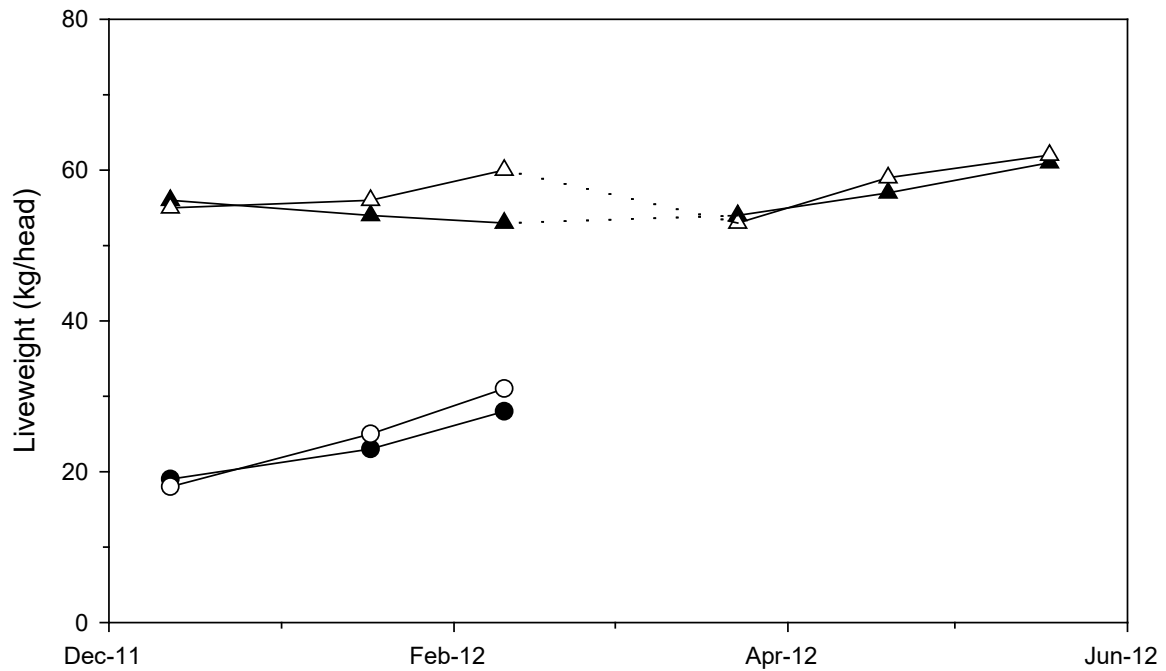
Figure 3.6 Liveweight of Merino ewes (triangle) and lambs (circle) grazing on a perennial lupin pasture (closed symbols) compared with control pastures (open symbols) at Sawdon Station from December 2011 to June 2012.

Note: Periods of grazing are represented by line type where solid lines (–) indicate grazing of respective treatments and dashed lines (– –) represent a common grazing period when sheep were maintained on grass-dominant pastures.
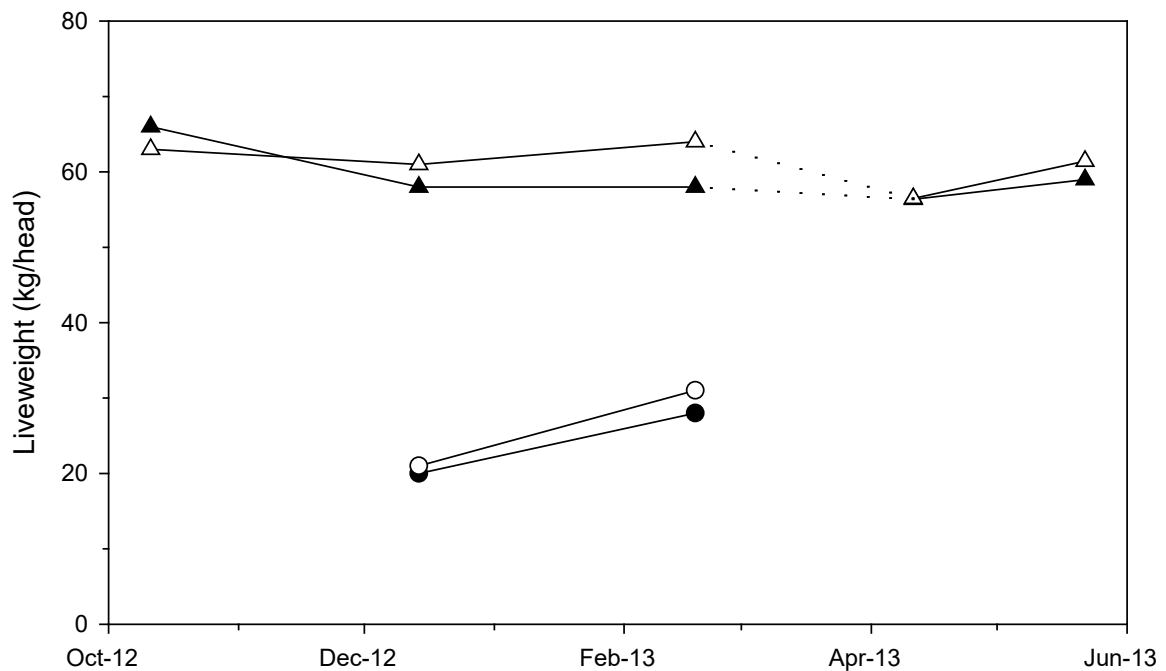
Figure 3.7 Liveweight of Merino ewes (triangle) and lambs (circle) grazing on a perennial lupin pasture (closed symbols) compared with control pastures (open symbols) at Sawdon Station from October 2012 to June 2013.

Note: Periods of grazing are represented by line type where solid lines (–) indicate grazing of respective treatments and dashed lines (– –) represent a common grazing period when sheep were maintained on grass-dominant pastures.
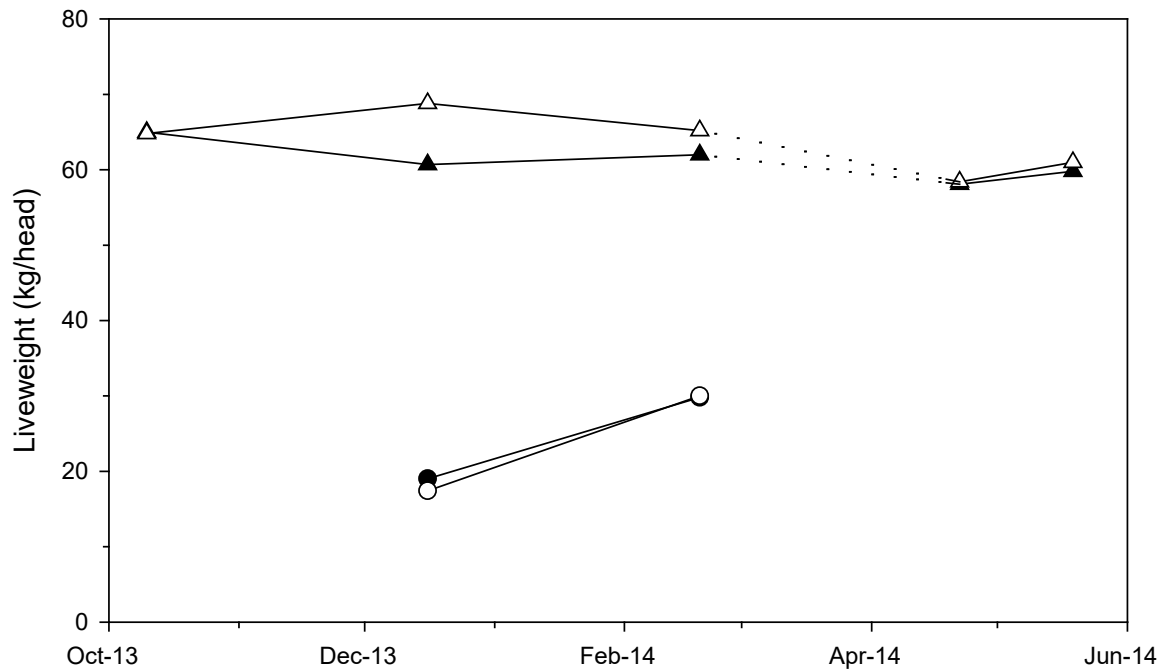
Figure 3.8 Liveweight of Merino ewes (triangle) and lambs (circle) grazing on a perennial lupin pasture (closed symbols) compared with control pastures (open symbols) at Sawdon Station from October 2013 to June 2014.

Note: Periods of grazing are represented by line type where solid lines (–) indicate grazing of respective treatments and dashed lines (– –) represent a common grazing period when sheep were maintained on grass-dominant pastures.

Table 3.2 Daily liveweight gain of Merino ewes and lambs grazing on a perennial lupin pasture compared with conventional "control" pastures at Sawdon Station for three consecutive seasons between December 2011 and May 2014.

	Lupin ewes	Control ewes	Lupin lambs	Control lambs
	g/day			
1st season (2011/12)				
Dec-Feb	-50	83	150	217
Mar-May	125	161	-	-
2nd season (2012/13)				
Oct-Dec	-125	-31	-	-
Dec-Feb	0	45	121	152
Oct-Feb	-62	8	-	-
Apr-May	64	120	-	-
3rd season (2013/14)				
Oct-Dec	-64	60	-	-
Dec-Feb	20	-55	166	194
Oct-Feb	-23	3	-	-
Apr-May	63	96	-	-

3.3.2 Wool characteristics

Wool characteristics were similar between the lupin and control grazing mobs (Table 3.3). On 11 April 2013, the mid-side wool clips indicated that staple length was 53 mm for lupin ewes compared with 49 mm for control ewes (P=0.005). Mean micron diameter was unaffected (P=0.116) by pasture type and was about 18.5 µm. At shearing on 19 September 2013, fleece weight for control ewes was 4.92 kg which was 0.28 kg greater than lupin ewes at 4.64 kg (P=0.002). Staple length was similar for both mobs (ca. 80 mm; P=0.373) and mean micron diameter remained at about 18.5 µm (P=0.664).

Table 3.3 Wool characteristics of Merino ewes that grazed on a perennial lupin pasture compared with conventional "control" pastures at Sawdon Station, at crutching on 11 April 2013 and shearing on 19 September 2013.

Characteristic	Lupin	Control	SED	P value
	At crutching on 11 April 2013			
Staple length (mm)	53	49	1.2	0.005
Mean micron (µm)	18.6	18.3	0.20	0.116
	At shearing on 19 September 2013			
Staple length (mm)	79	80	1.4	0.373
Mean micron (µm)	18.6	18.5	0.21	0.664
Fleece weight (kg)	4.64	4.92	0.090	0.002

3.3.3 Aboveground biomass of the perennial lupin pasture

Average aboveground biomass of the perennial lupin pasture during the second growth season (2012/13) is shown in Figure 3.9. On 11 October 2012, the lupin pasture had an average biomass of 2700 kg DM/ha, which had increased to 6700 kg DM/ha by 21 November. The proportion of lupin reproductive stem increased by 15% between 21 November and 14 December, whilst the proportions of other components remained unchanged. Between 14 December 2012 and 18 February 2013, herbage mass declined linearly from 7200 kg DM/ha to 5800 kg DM/ha which resulted from 10% and 8% decreases in lupin lamina and petiole proportions, respectively. Dead material became consistently more abundant after mid-February, representing 33% of herbage mass on 20 March and 90% by 22 May (Plate 3.8).
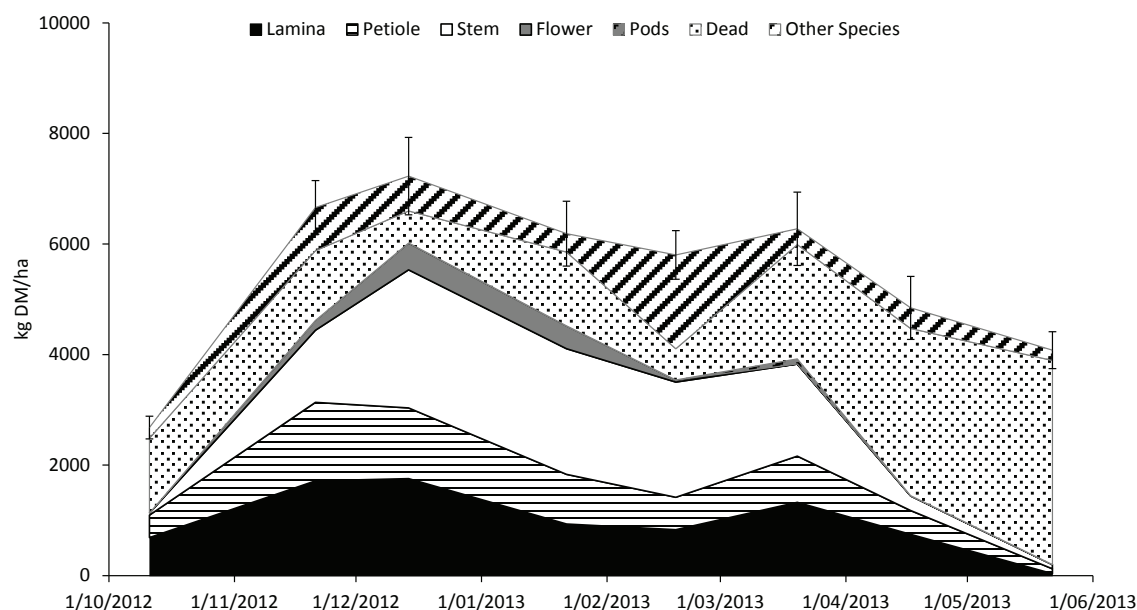
Figure 3.9 Seasonal pattern of aboveground biomass components of the perennial lupin pasture, averaged across the five paddocks, at Sawdon Station during the second growth season from 11 October 2012 to 22 May 2013. Error bars represent standard error of the mean for total biomass.

Aboveground biomass of each individual paddock throughout the second growth season is shown in Figure 3.10. On 11 October 2012, when set-stocking started on Paddocks 2-5 (see Figure 3.2 for paddock layout), total biomass across the five paddocks ranged from 2303 to 3203 kg DM/ha and composition of the herbage was around 39% lupin leaf, 52% dead material, which was mostly lupin stem from the previous season, and 7% other species (Plate 3.4). When rotational grazing started in Paddock 1 on 21 November 2012, pre-grazing biomass in that paddock was 8764 kg DM/ha with 63% green lupin, 8% dead and 30% other species, whereas post-grazing biomass of the other four paddocks averaged 6136 kg DM/ha with 72% green lupin, 23% dead and only 5% other species (Plate 3.5). The amount of green lupin stem increased in all paddocks between 21 November and 14 December 2012, and was most abundant in Paddock 1, where it represented 60% of the biomass. Lupin flowers averaged 573 kg DM/ha in Paddocks 2-5 compared with 132 kg DM/ha in Paddock 1, which had just been grazed (Plate 3.6). On 22 January 2013, there was an apparent reduction in lamina material in paddocks that had been grazed. In Paddock 4, the biomass of lupin lamina was 205 kg DM/ha, which was 4% of the herbage on offer compared to Paddocks 1 and 5 where lupin lamina was 1553 kg DM/ha and represented 20% of the herbage on offer (Plate 3.7). Between 22 January and 20 March 2013, herbage on offer did not fluctuate considerably and was 6188 kg DM/ha and 6276 kg DM/ha, respectively. However, within that period, the biomass of non-lupin species decreased from a mean of 1698 kg DM/ha on 18 February to 289 kg DM/ha in March. On 20 March 2013, lupin leaf material represented 35%, which was 55% of the total green lupin

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material on offer. The total amount of green lupin on offer had decreased to 5% by 22 May, whilst the mean proportion of dead material across all paddocks was 91%.

Plate 3.4   **An aboveground biomass of 2700 kg DM/ha in Paddock 1 of the perennial lupin pasture at Sawdon Station on 11 October 2012 when Merino ewes were put onto the pasture for lambing.**

Plate 3.5   **The average aboveground biomass of the perennial lupin pasture was 6100 kg DM/ha in the four paddocks that were set stocked for lambing, at Sawdon Station on 21 November 2012.**
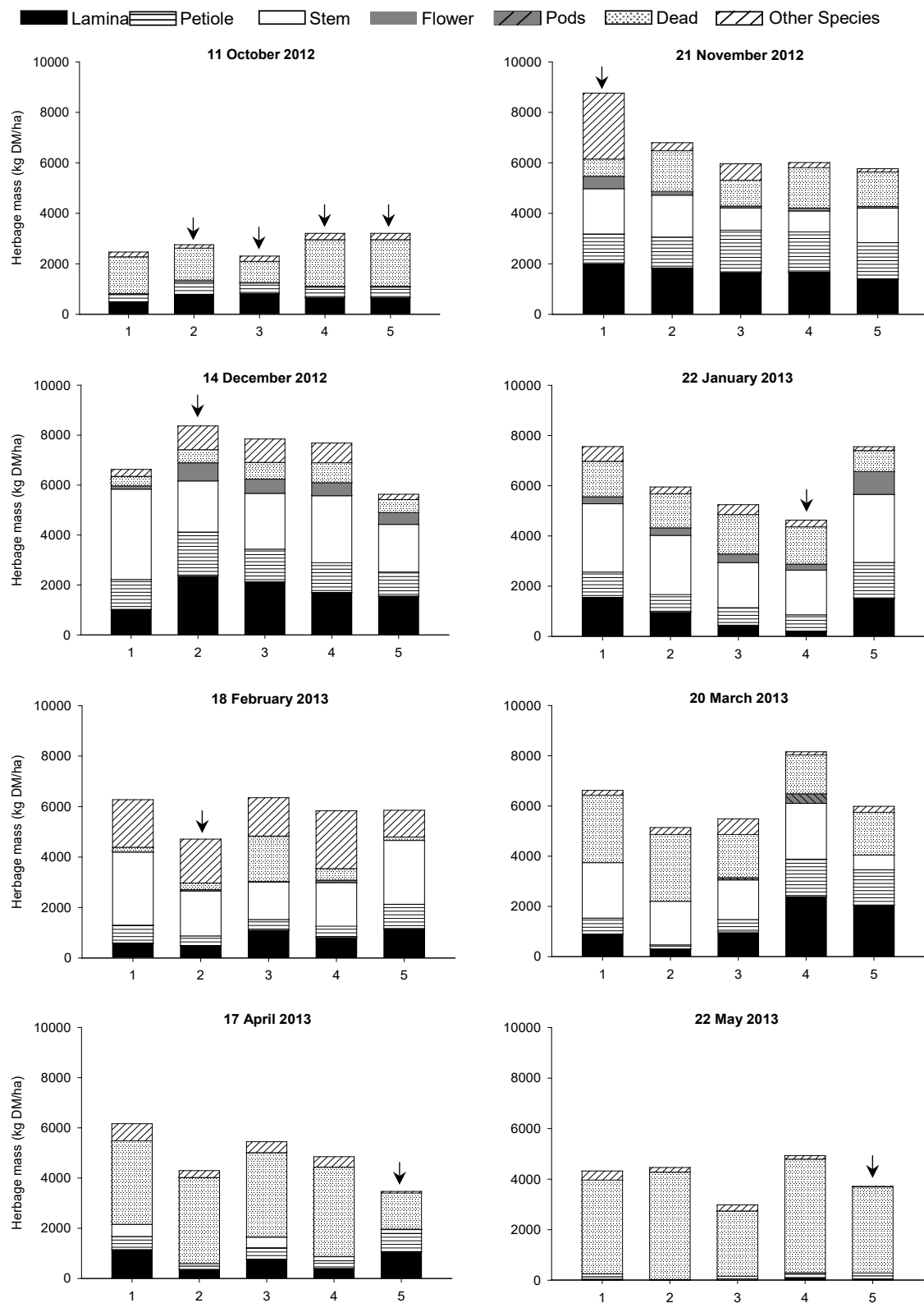
Figure 3.10 Seasonal pattern of aboveground biomass components of each of the five paddocks of perennial lupin pasture at Sawdon Station during the second growth season from 11 October 2012 to 22 May 2013. Arrows indicate paddocks that had sheep in them on each measurement date.

Aboveground biomass of the lupin pasture in the third growth season (2013/14) is shown in Figure 3.11. On 10 October 2013, the pasture had an average biomass of 3300 kg DM/ha where 51% of the material was lupin lamina and petiole, with the remainder being dead material from the previous season. The herbage mass increased to a peak of 8300 kg DM/ha on 16 December 2013, which was primarily driven by the increased proportion of lupin reproductive stem. The combined herbage mass of lupin lamina, petiole and green stem decreased from 5990 kg DM/ha on 16 December to 1773 kg DM/ha on 19 February 2014. During autumn, the proportion of dead material increased from 46% to 87%, whilst lupin lamina and petiole represented less than 30% of total herbage mass.
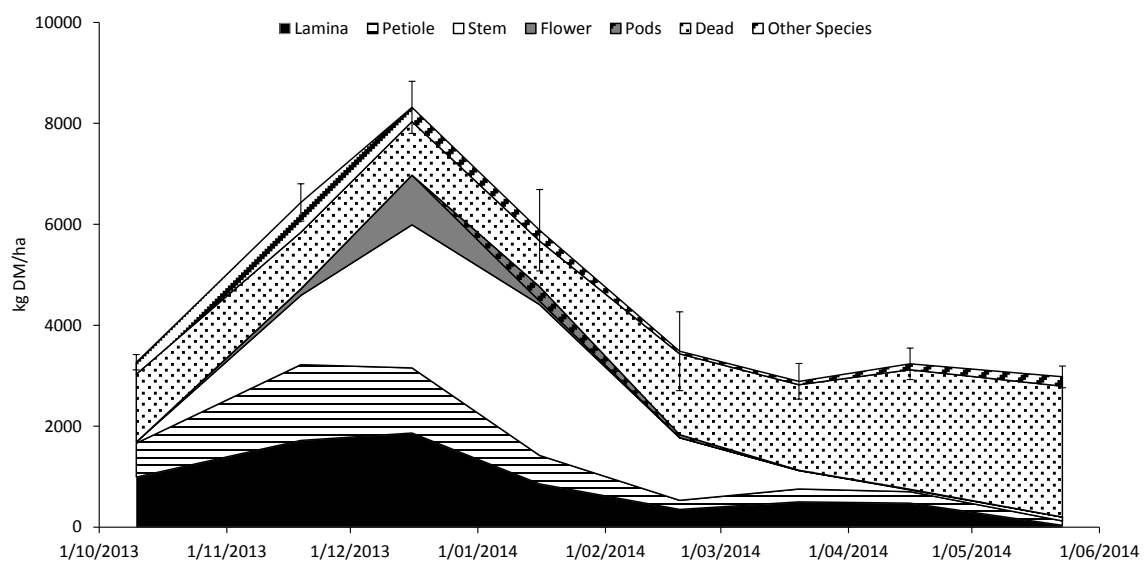
Figure 3.11 **Seasonal pattern of aboveground biomass components of the perennial lupin pasture, averaged over the five paddocks, at Sawdon Station during the third growth season from 10 October 2013 to 1 May 2014. Each bar represents one standard error of the mean for total biomass.**

The paddocks that were set-stocked during spring 2013 gained 2800 kg DM/ha between 10 October and 19 November compared with 4600 kg DM/ha for Paddock 1, which was not grazed (Figure 3.12). In paddock 1, other species represented 49% of the herbage on offer with a biomass of 2028 kg DM/ha compared with an average biomass of 1171 kg DM/ha for Paddocks 2-5. The ewes and lambs were introduced to Paddock 1 on 20 November 2013 at a herbage mass of 8715 kg DM/ha, which was comprised of 5543 kg DM/ha green lupin material and 2014 kg DM/ha of other species. In Paddocks 1, 2, 4, and 5, the mass of lupin flower increased from 123 kg DM/ha on 19 November to 1216 kg DM/ha on 16 December 2013. On 16 December, the biomass of lupin flower was 58 kg DM/ha compared with an average of 1216 kg DM/ha for paddocks that were not being grazed. Between 16 December 2013 and 16 January 2014, average pasture cover declined from 8317 kg

DM/ha to 5882 kg DM/ha. During this period, the proportion of lupin leaf decreased from 38% to 24%. On 19 February 2014, dead material represented 32% - 62% of total herbage mass, which increased to at least 86% of biomass in all paddocks by 1 May 2014. On 1 May, lupin lamina and petiole represented 13% in Paddock 1 and less than 10% in all other paddocks.

Plate 3.6   Two adjacent paddocks (paddocks 5 and 1) of the perennial lupin pasture at Sawdon Station on 14 December 2012. On the day this photo was taken, the sheep had just been shifted from Paddock 1 on the right of the photo and had eaten most of the lupin flowers.

Plate 3.7   Post-grazing biomass of 4600 kg DM/ha of mostly green and dead lupin stem in Paddock 4 of the perennial lupin pasture at Sawdon Station on 22 January 2013.
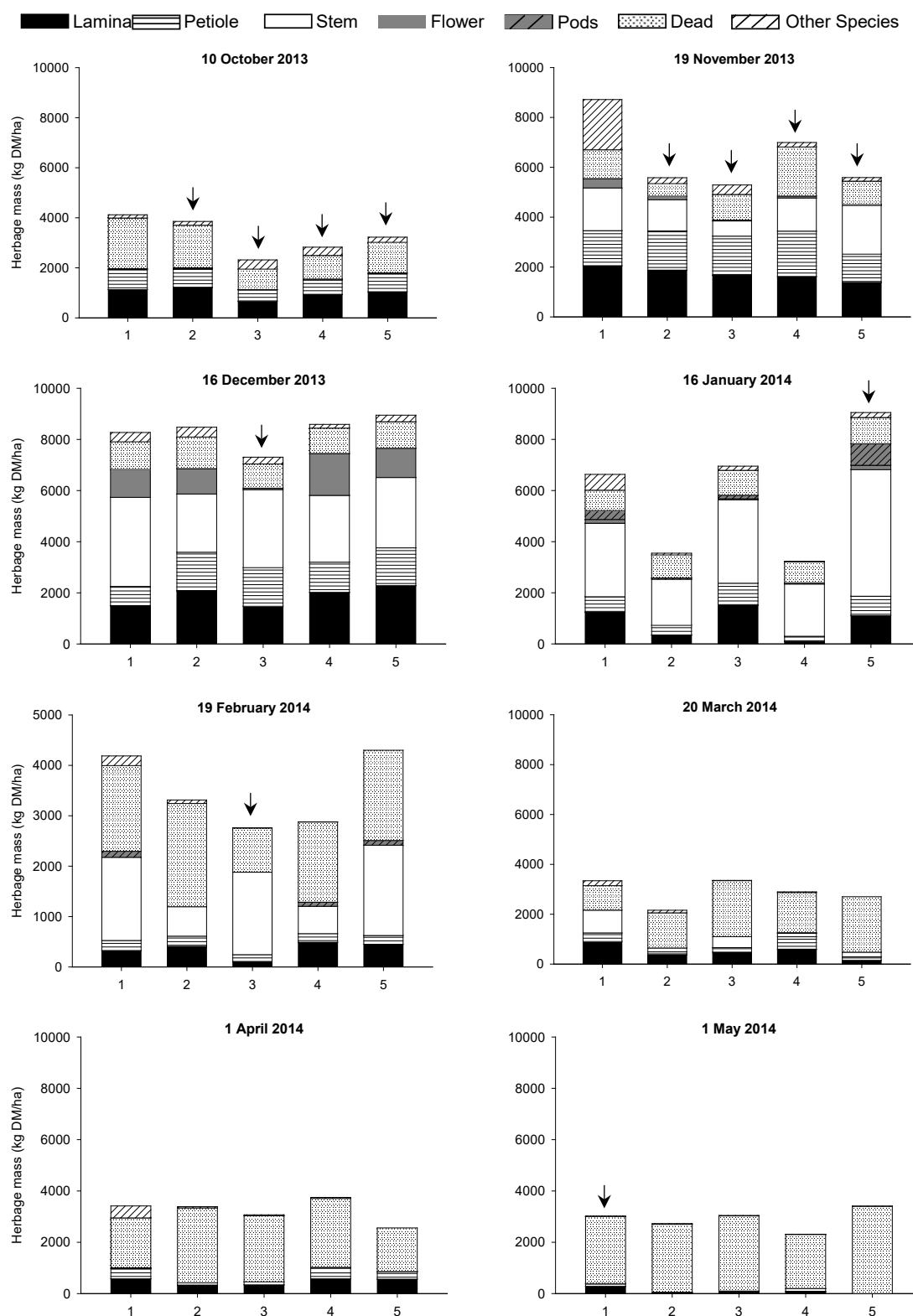
Figure 3.12 Seasonal pattern of aboveground biomass components of each of the five paddocks of perennial lupin pasture at Sawdon Station during the third growth season from 10 October 2013 to 1 May 2014. Arrows indicate paddocks that had sheep in them on each measurement date.

Plate 3.8 **Biomass of 4900 kg DM/ha of mostly dead lupin stem in paddock 4 of the perennial lupin pasture at Sawdon Station on 22 May 2013.**

3.3.4 Nutritive value of perennial lupin

Nutritive analysis indicated that the ME of lupin lamina was consistent throughout 2012/13 (Figure 3.13) and 2013/14 (Figure 3.14). In 2012/13, the mean ME of lupin lamina was 11.9 MJME/kg DM and ranged from 11.3 to 12.2 MJME/kg DM between November and May. This was similar in 2013/14, where lupin lamina had a mean ME of 12.1 MJME/kg DM and ranged between 11.8 and 12.6 MJME/kg DM. On 11 November 2012, petiole and stem both had ME of 11.9 MJME/kg DM. However, the ME of both components had declined to ~10 MJME/kg DM on 21 November 2012. Following this, the ME of petiole remained at about 9.7 MJME/kg DM until grazing ceased on 22 May 2013. The ME of stem continued to decrease after 21 November, and eventually reached 7.1 MJME/kg DM on 18 February 2013, before recovering to 8.7 MJME/kg DM on 22 May 2013. Between December 2013 and May 2014, the mean ME of lupin petiole and stem was 9.7 MJME/kg DM and 8.0 MJME/kg DM, respectively. This was similar to 9.7 MJME/kg DM for petiole and 8.3 MJME/kg DM for stem during the previous grazing season. The ME of lupin flower was about 12.1 MJME/kg DM between November and December of the 2012/13 and 2013/14 grazing seasons. However, in both seasons, this had declined to 9.7 MJME/kg DM by late January. This was followed by an increase to 12.9 MJME/kg DM and 13.7 MJME/kg DM in April for the 2012/13 and 2013/14 seasons, respectively.

The DMD of lupin lamina, petiole and stem followed a similar pattern to ME in both grazing seasons (Figure 3.13 and Figure 3.14). Lupin lamina remained at about 80% DMD from November to May in 2012/13 and 2013/14. Lupin flower also had a DMD of 80% between November and December but had decreased to 60% by mid-February. Petiole had a mean DMD of 63% between December and May in both seasons. Stem material had the lowest DMD of all components. Between December 2012 and May 2013, stem had a mean DMD of 53% (range=44-66%) which was similar to 51% (range=42-61%) in 2013/14.

Lamina had a mean CP content of 27.0% and 29.4% in 2012/13 (Figure 3.13) and 2013/14 (Figure 3.14), respectively. In both seasons, the CP content of lamina was highest in November at 33%. This steadily decreased over the growing season and was 23.7% and 24.7% in May 2013 and 2014, respectively. During November and December, the CP of flower was between 29.3 and 33.7% but had decreased to 15% by February in 2012/13 and 2013/14. The mean CP content of petiole was 11.1% in 2012/13 and 2013/14. In both growing seasons, the CP content of stem material was consistently lower than 10%.
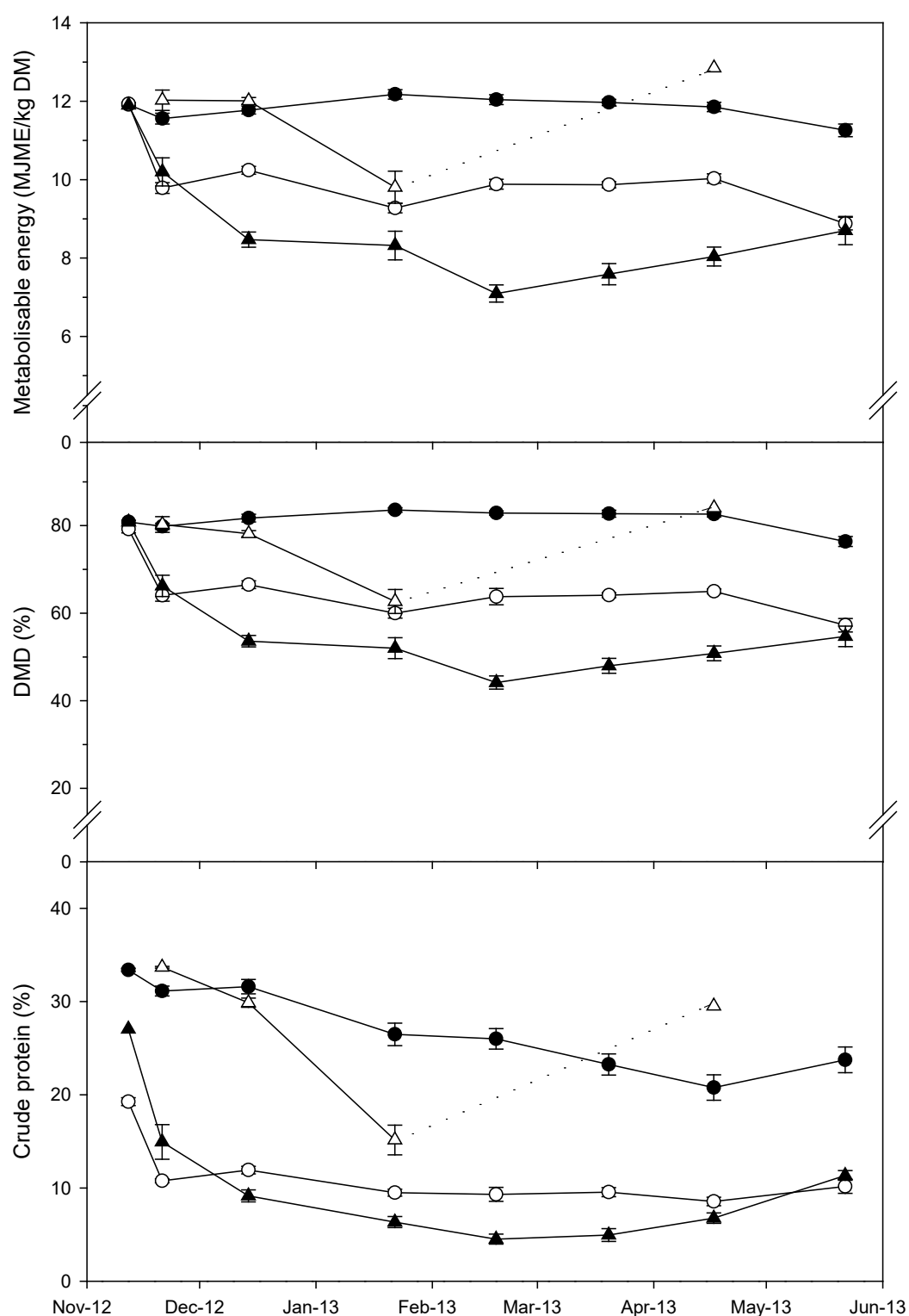
Figure 3.13 Metabolisable energy (MJME/kg DM), dry matter digestibility (DMD, %) and crude protein (CP, %) of perennial lupin lamina (●), petiole (○), stem (▲) and flower (△) at Sawdon Station from 12 November 2012 to 22 May 2013. Error bars represent standard error of the mean.
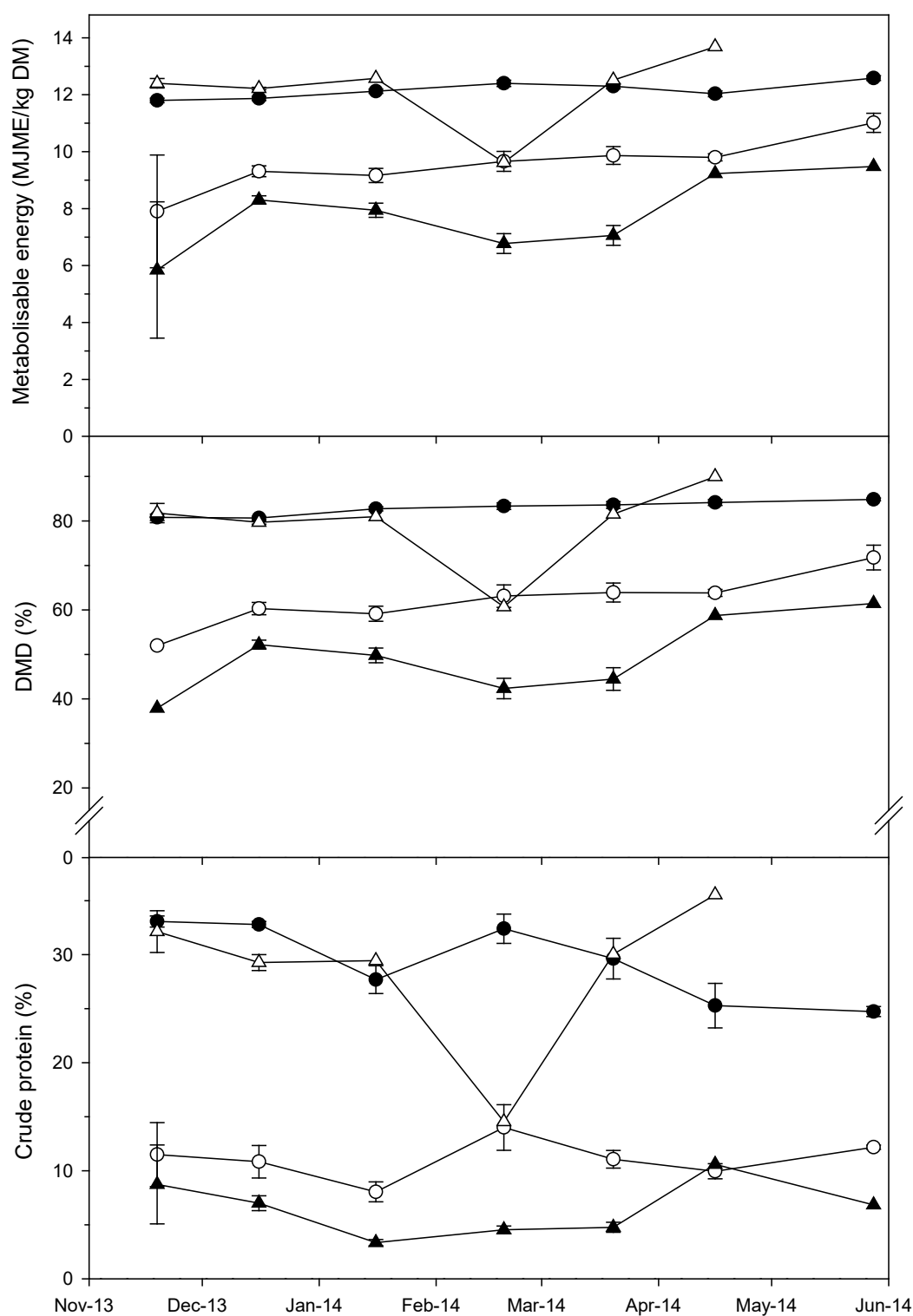
Figure 3.14 Metabolisable energy (MJME/kg DM), dry matter digestibility (DMD, %) and crude protein (CP, %) of perennial lupin lamina (●), petiole (○), stem (▲) and flower (△) at Sawdon Station from 19 November 2013 to 28 May 2014. Error bars represent standard error of the mean.

3.4 Discussion

The purpose of this chapter was to gain an understanding of the performance of a commercial Merino ewe flock that was grazing a perennial lupin-based pasture. The stand of perennial lupins evaluated in this trial had survived under sheep grazing, modest inputs of fertiliser and lime, and 600-650 mm of rain a year for 8 years prior to commencing measurements. This result, and the three decades of research at the nearby Mt John Trial Site in Tekapo (Scott, 1989, 1994, 2014), indicated that Merinos would graze perennial lupin and that the perennial lupin plants would recover after each grazing. However, the performance of ewes grazing these pastures needed to be benchmarked against those on conventional pasture options. This was then supported with monthly agronomic measurements that included nutritive values.

3.4.1 Animal performance

Ewe live weight did not experience large variability throughout for either pasture type over the three growing seasons (Figure 3.6, Figure 3.7 and Figure 3.8). For example, in 2011/12, ewes on the lupin pasture lost an average of 3 kg over the two-month summer period while the ewes in the control mob gained an average of 5 kg. In the 2012/13 and 2013/14 seasons, ewe live weight either remained unaffected or increased slightly over the summer period (Figure 3.7 and Figure 3.8). Seasonal changes in live weight are related to the balance of energy supply and demand. In practice, this is a function of the production of palatable feed and the stocking rate. In this study, the control mob was consistently shifted between different paddocks to match seasonal supply and demand. In comparison, the stocking rate of the lupin mob was not adjusted within the grazing seasons, which meant feed availability was a function of the pre-determined stocking rate and within season biomass productivity. This was reflected in the animal performance data, where reproductive performance and live weight improved as the stocking rate was reduced from 143 ewes in 2011/12 to 101 ewes in 2013/14.

The high energy demand of lactation means that losses in ewe live weight are expected under most grazing situations (Geenty and Sykes, 1986). During early lactation (September to December), ewes on the lupin pasture lost up to 12% of their bodyweight compared to 3% or less in control ewes. However, the lupin ewes maintained their live weight during the second stage of lactation (December to February) and had a mean live weight of 58 and 62 kg/head in February 2013 and 2014, respectively. In this study, the loss of live weight during lactation was minimal and did not affect ewe performance in following seasons, where differences in ewe live weight between the lupin and control mobs were minimised when both mobs grazed on a common pasture between

February and April (Figure 3.6, Figure 3.7 and Figure 3.8). Following common grazing in 2012/13 and 2013/14, ewes returned to their respective pastures for tupping, where the control ewes reached a mean tupping weight of 62 kg which was 2 kg more than ewes on the lupin pasture. Tupping weights of both mobs were similar to those reported by Anderson *et al.* (2014), near Omarama in the Mackenzie Basin, whose mixed-age Merino ewe flock had a tupping weight of 59.5 kg after autumn grazing of lucerne. Furthermore, the tupping weights of ewes on the lupin pasture were about 10% higher than the Merino NZ Benchmark Group (Table 3.4), which probably contributed to the advantage in lambing percentage seen the next spring.

Table 3.4 **Performance of mixed-age (MA) Merino ewes grazing on the perennial lupin pasture at Sawdon Station, Tekapo, compared with data from the central South Island Merino New Zealand (NZ) Benchmark Group.**

Parameter	Sawdon lupins[†]	Merino NZ Benchmark Group* (2014)
Tupping weight (MA ewes)	60.5	54.6
Lambing (%)	112	103
Lamb losses (scanning to tailing) (%)	25	25.3
Lamb weaning weight (kg; 100 day adjusted[ψ])	24.2	25.4
Lamb live weight produced/100 ewes (kg)	2710	2616
Greasy wool weight (kg)	4.64	5.3
Wool diameter (µm)	18.6	18.6

[†] = Average values of 2012/13 and 2013/14 seasons are used when available.

* = Merino NZ Benchmark Group contains 20 farmers who are South Island High Country Class 1 producers.

[ψ] = Lamb liveweight was adjusted based on average liveweight gain between the start of lambing and the liveweight measurement that was nearest to 100 days.

The live weight of lambs at tailing was similar in each of the three grazing seasons. A mean tailing weight of approximately 19 kg was observed in both pastures with differences of less than 1.5 kg in each of the seasons. However, lambs on the lupin pasture were growing at 30-70 g/day slower than those on the control pastures between tailing in December and weaning in February. This meant control lambs had reached a live weight of 31 kg while lupin lambs weighed 29 kg; similar to the lamb weaning weight of 29 kg in Anderson *et al.* (2014). The 100 day adjusted lamb live weight was 24.2 kg for lupin lambs, which was slightly lower than 25.4 kg for the Merino NZ Benchmark Group (Table 3.4).

When ewes were scanned in August, there was no evidence that the tupping weight differences of 2 kg between the lupin ewes and control ewes had affected reproductive performance, with both

mobs scanning at 150% in 2012 and 2013. At tailing, lambs on the lupin pasture appeared to have a slightly higher survival rate with a mean lambing percentage of 112% over both seasons compared with 105% for control pastures. It is possible that the lupin pasture provided greater shelter for newborn lambs than the control pastures. Lamb losses (scanning to tailing) of the lupin mob were comparable to that of the Merino NZ Benchmark Group at 25% (Table 3.4).

The ewes on the lupin pasture produced wool with similar characteristics to that produced by the ewes on the lucerne and other conventional pastures. The mean fibre diameter for ewes on the lupin pasture and control ewes was about 18.5 µm, which was the same as wool produced by Merino ewes from the Merino NZ Benchmark Group (Table 3.4). The average fleece weight of the lupin ewes was 0.3 kg lighter than the control ewes at shearing in September 2013 (Table 3.3). However, both mobs had heavier fleeces than the 4.06 kg produced by Class 1 South Island High Country farms between 2006 and 2014 (Lamb, 2015). Differences in fleece weight may have been caused by differences in forage quantity and quality between the lupin pasture and control pastures. However, there is insufficient evidence to either confirm or reject this theory.

3.4.2 Agronomic performance

The lupin pasture started growing during September each year and provided significant amounts of forage during lambing and lactation when the pasture was stocked at 13.5-16 ewes/ha. When herbage measurements were taken in the second (2012/13) and third (2013/14) growth seasons, the lupin pasture gained about 4500 kg DM/ha between the start of lambing in October and tailing in December. Paddocks that were set stocked gained an average of 3500 kg DM/ha between October and November, whilst the un-grazed paddock accumulated 6500 kg DM/ha, and had a total biomass of about 9000 kg DM/ha by November of both seasons. This compares with a nearby crop of lucerne that yielded 4500 kg DM/ha when it was cut for silage in the same month (2013, data not shown). Forage yield in the un-grazed lupin paddock (Paddock 1) was similar to those reported by Moot and Pollock (2014) at a nearby site at Glenmore Station, near Lake Tekapo, where perennial lupin-dominant plots had accumulated 9100 kg DM/ha by mid-December, which accounted for 80% of their total annual yield. That site had a pH of 5.0 and soluble Al was 5.0 mg/kg (Moir and Moot, 2014) which would be considered far less favorable than the soil pH of 6.0 and soluble Al levels of <0.5 mg/kg at Sawdon Station.

During the spring growth period, relative proportions of non-lupin species, reproductive stem, lupin lamina and petiole were the main contributors to herbage mass (Figure 3.9 and Figure 3.11). In the

un-grazed paddock, non-lupin species contributed 30% and 23% of yield by November 2012 and 2013, respectively. This was higher than the 10-20% contribution made by companion species in Moot and Pollock (2014). It is not surprising that there was a higher proportion of other species at Sawdon Station as the trial was established 8 years prior to that of Moot and Pollock (2014). Thus, it is likely that the stand would naturally progress to have a greater abundance of fine-rooted grass species which are responsive to increasing available soil nitrogen as a result of the grazed, legume-dominant pastures.

Pasture cover was highest in December of both grazing seasons (Figure 3.9 and Figure 3.11). However, cover was 13% higher in December of the second season (2013/14). Despite a higher December pasture cover, the summer grazing period reduced pasture cover to 3500 kg DM/ha by February 2014; which was 58% lower than the cover in February of the previous season. Given that stocking rate was 20% higher in the first season, this result suggests that perennial lupin maintained active growth for a longer period in the 2012/13 season and/or senescence was delayed. This is further supported by the botanical composition data, where the abundance of lupin leaf was 10% higher in February of the first grazing season. Given that mean temperature and PET remained similar in both seasons; it seems likely that improved growth resulted from the additional 140 mm of rainfall between September 2012 and February 2013 (Figure 3.3).

Between December 2012 and March 2013, sheep selectively grazed lupin lamina which resulted in consistent decreases in lamina proportion at monthly measurements (Figure 3.10). The proportion of green components comprising yield consistently decreased from March until grazing ceased in May. During this period, the mean herbage mass declined linearly from 6300 kg DM/ha to 4500 kg DM/ha, where dead material represented 90% of the herbage on offer. The senescence of lupin stem and reduced palatability of other species caused an apparent increase in consumption of green lupin leaf material whose yield contribution decreased from about 30% in March to 4% in May of both seasons (Figure 3.10 and Figure 3.12). Increased grazing preference for lupin leaf material would seem logical given the large proportion of unpalatable dead components on offer during the autumn grazing periods.

Lupin lamina and flower had the highest ME, DMD and CP values in the 2012/13 and 2013/14 growing seasons (Figure 3.13 and Figure 3.14). In both seasons, lamina had a mean CP concentration of 28.2%, whilst ME ranged between 11.6 and 12.6 MJME/kg DM. These results were

consistent with those found for Russell lupin in Kitessa (1992), where the mean ME of lamina was 12.2-12.6 MJME/kg DM between October and January. This was also similar for DMD of lamina, where the mean of 2012/13 and 2013/14 was 82% compared with 85% for Kitessa (1992). The CP content of lamina was similar to values expected for green leaves of lucerne and clovers (Brown and Moot, 2004; Halim *et al.*, 1989). For lupin flower, ME and CP were similar to lamina between November and January but had decreased substantially by February in both seasons. The decline in ME, DMD and CP was caused by the development of inflorescence and eventual hardening of seed pods (Plate 3.7). However, during autumn flowering, the nutritive value of flowers was similar to those found in spring. The consistently high nutritive value of both components helped to explain why lamina and flowers were the preferred lupin components for ewes and lambs (Figure 3.10 and Figure 3.12).

The nutritive value of lupin petiole was consistent between December and May, whilst stem material showed a general decline in ME, DMD and CP throughout the summer period (Figure 3.13 and Figure 3.14). For example, the ME of petiole was ~10 MJME/kg DM throughout both seasons, however, green stem declined from an ME of ~8.5 MJME/kg DM in December to ~6.8 MJME/kg DM in February. The decline in ME and CP of stem material appeared to be associated with the maturation of inflorescence in January. During this period, green stem began to senesce, but was not included in the dead fraction until it was brown in colour (Plate 3.8). Lupin petiole had a lower nutritive value than lamina but followed a similar pattern of disappearance, which suggested that its palatability was adequate for grazing.

Nutritive value results were similar to Kitessa (1992), which is the only other known study to have quantified the nutritive value of perennial lupin. This confirmed our confidence in the estimation values of the NIRS model (Appendix 1) so we could use it for further nutritive analysis in Chapter 4.

3.5 Summary and conclusions

Results from this study confirm that a perennial lupin pasture can provide high quality forage for Merino ewes and lambs on dryland properties in the Mackenzie Basin. The specific conclusions from the study were:

- Lupin pastures, that were set-stocked or rotationally grazed at 11 – 13 SU/ha, produced similar animal liveweights, lambing percentages and wool yields to conventional pastures.

- Rapid spring growth of lupins is suited to set-stocking during lambing that transitions to rotational grazing in late spring/summer.

- Accumulation of lupin herbage was highest between October and December, where average pasture cover reached about 8000 kg DM/ha.

- Sheep grazing the perennial lupin pasture showed preference for companion grasses and herbs during spring/early summer. However, lupin lamina and petiole was selected as other yield components and companion species matured. Sheep showed a strong preference for lupin inflorescence in December and selective grazing was evident.

- The NIRS calibration was successful for lupin components, which will make future assesments rapid and cost-effective.

- Lupin lamina maintained consistent palatiblity with a mean ME of 12.3 MJME/kg DM, DMD of 82% and CP concentration of 28%. This makes it comparable to conventional legumes and is unlikely to limit animal performance.

4 Sheep liveweight gain on dryland perennial lupin-cocksfoot and lucerne pastures at Lincoln University

4.1 Introduction

The on-farm study of Chapter 3 showed the agronomic productivity of perennial lupin-based pastures gave comparable Merino sheep performance to conventional pastures. This confirmed the potential of perennial lupin as an alternative forage legume for high-country pastures (Moot and Pollock, 2014; Scott, 1989, 2008). However, explanation of the results was limited due to the lack of experimental control. Therefore, a second grazing experiment was established at Lincoln University to quantify the relationship between animal productivity and agronomic performance (DM yield and nutritive value) of perennial lupin-cocksfoot pasture compared to lucerne pasture.

Lucerne is recognised as the greatest producer of forage under dryland conditions in Canterbury (Brown *et al.*, 2003; Mills *et al.*, 2015b; Mills and Moot, 2010; Moot *et al.*, 2008) and is used where possible in the South Island high-country (Anderson *et al.*, 2014; Stevens *et al.*, 2012). However, it is a more expensive crop to establish and maintain than perennial lupin and is not suitable in all soil types, particularly those with a high Al status. It was expected that lucerne would outperform an unfertilised mixed grass/legume pasture on the fertile Canterbury Plains at Lincoln University (Mills *et al.*, 2015a; Mills *et al.*, 2008). Therefore, the experiment described in this chapter focuses on the drivers of sheep production rather than the absolute productivity of pastures in this specific environment.

The purpose of Chapter 4 was to relate differences in sheep performance to the relative productivity of cocksfoot-lupin (cf-lupin) and lucerne pastures. Evaluation of the type and amount of feed consumed within each pasture is used to quantify the key drivers of sheep performance. They also provide a quantitative comparison of the conversion efficiency of seasonal DM production to live weight for a mixed cf-lupin pasture relative to lucerne. The results are then interpreted to explain how *L. polyphyllus* could be used on-farm to enhance productivity. The null hypothesis is that liveweight gain per unit of DM consumed is not different between the two pasture types. The supporting measurements are then used to explain the outcome and on-farm application of testing that hypothesis.

4.2   Materials and methods

4.2.1   Experimental site

The two pasture types were compared in paddock H12 at the Lincoln University Horticultural Research Area (43°38′53″S 172°27′24″E, 9 m.a.s.l.) in Canterbury (Plate 4.1). The area has minor changes (<1 m) in topography. Tall (6-8 m) poplar (*Populus deltoids × nigra*) shelterbelt trees were present along the north and south boundaries, whilst several deciduous trees grew along the eastern boundary.

Plate 4.1   Aerial view of the H12 experiment (looking south) showing the six plots of perennial cocksfoot-lupin and lucerne, each subdivided into five paddocks and rotationally grazed with a group of sheep. Plot 1 is in the foreground and the sheep were in paddock 4 of each plot at the time this photo was taken at Lincoln University, Canterbury.

The soil was a Templeton silt loam (New Zealand Classification: Typic Immature Pallic Soil (Hewitt 1998); (*Udic Haplustept*, USDA Soil Taxonomy) (Soil Survey Staff 1998) with 1-2 m of fine textured alluvial sediments overlying gravels (Cox, 1978). The profile of Templeton silt loam soils typically consists of 0.3 m of topsoil which overlies varying depths of sediments ranging from silt loams to sand textures. Templeton silt loams are moderate to free-draining and have a water holding capacity of approximately 300 mm in the top 1 m (Cox, 1978). At the time of sowing, soil test results indicated adequate fertility (Table 4.1).

Table 4.1 **Soil analysis (0-7.5 cm depth) of paddock H12 at Lincoln University, Canterbury on 5 December 2013**.

pH	Olsen P	K	Ca	Mg	Na
	(mg/L)	Me/100 g			
6.0	17	7	10	17	6

The experimental site was a 2 ha area that was previously occupied by a grass/clover pasture for 5 years. This pasture was sprayed with Roundup TRANSORB (1.08 kg/ha glyphosate), ploughed, and sown into forage oats (*Avena sativa*) in March 2013. The oats did not receive any fertiliser before they were cut and baled in October 2013. Minor regrowth from the oat crop was sprayed with Roundup TRANSORB on 13 November 2013. The area was then irrigated (50 mm), ploughed, Dutch harrowed and rolled in preparation for sowing during November 2013.

4.2.2 Experimental design and pasture establishment

The experimental area (90 × 174 m) was divided into three blocks (replicates) along its longer axis with each block containing two 29 × 90 m (0.26 ha) plots (Plate 4.1). One plot in each block was randomly selected to be sown into cf-lupin pasture and the other plot was sown into a monoculture of lucerne. Raceways were located along the north, west and south boundaries of the experiment and the west raceway connected the six plots to a small corral in the northwest corner of the experiment.

Lupinus polyphyllus seed was supplied by Rosevear & Co. Ltd, Ashburton, Canterbury, NZ. Two varieties of perennial lupin were used, one being *L. polyphyllus* and the other a crude selection of blue-flowered 'Russell' lupin, referred to as 'blue' lupin by the grower. Its exact genetic parentage is unknown. The 'blue' perennial lupin used throughout this thesis is not the annual blue lupin species (*L. angustifolius*) unless otherwise stated. The *L. polyphyllus* seed was originally derived from roadside populations in NZ and has since been multiplied by commercial seed growers.

Seeds of the two perennial lupin varieties were scarified by spinning them against a sandpaper cone for 20 seconds, and then inoculated with a peat slurry of Group G rhizobia (Becker Underwood, Australia) one day before sowing. A mix of blue lupin and cocksfoot was sown as an 8 × 80 m strip through the centre of each cf-lupin plot, with the remaining area being sown with a mix of *L. polyphyllus* and cocksfoot. Both lupin varieties were sown at 30 kg/ha with 10 kg/ha of cocksfoot. The cocksfoot cultivar was Grasslands Kara, which Rumball (1982) described as having a low tiller

density and open structure, suitable for lowland, rotationally grazed pastures. The cocksfoot was supplied by Agricom, Christchurch, NZ.

The lucerne cultivar was the European-bred SF Force 4 (Seed Force Ltd, Christchurch, NZ), which has a dormancy rating of four, meaning it is moderately winter dormant. Seed Force supplied the seed as coated and inoculated with *Ensifer melioti* rhizobia, and it was sown at 15 kg/ha.

The two pasture types were sown into the cultivated seedbed on 5 December 2013 using a Flexiseeder plot drill (Øyjoord cone type) with 150 mm row spacings and a target depth of 15 mm. After sowing, the plots were irrigated (ca. 60 mm) through January and February 2014. Then, in March, each plot was fenced with permanent sheep netting and an electric wire, plumbed with a portable water trough, and subdivided into five small (18 × 29 m, 0.052 ha) paddocks using four three-wire temporary electric fences so that each plot could be rotationally grazed by a group of sheep.

The lucerne plots were sprayed with Spinnaker (70 g/ha imazethapyr) on 8 July 2014 to control emerging broadleaf weeds, and with Gallant (130 g/ha haloxyfop-P) on 30 July 2014 to control grass weeds.

4.2.3 Meteorological data

Mean monthly air temperature and total monthly rainfall data were collected from Broadfields meteorological station located approximately 2.5 km north of the experimental site.

4.2.4 Sheep

In Year 1 (5 December 2013 – 30 June 2014), Merino ewe lambs were used to graze the pastures. They were sourced as weaned lambs from Sawdon Station, Lake Tekapo, and grazed on the two pasture types between 11 March and 19 May 2014 (69 days). However, the Merino lambs began to develop hoof problems as the ground moistened during autumn, so they were removed from the experiment on 19 May and were not used again.

In Year 2 (1 July 2014 – 30 June 2015), composite ewe hoggets were used in spring/summer and ewe lambs in autumn. These sheep were from Lincoln University's Ashley Dene flock of the Beef + Lamb New Zealand Central Progeny Test (CPT; (McLean *et al.*, 2006). They were of variable breed percentages incorporating Romney, Coopworth, Perendale, Corriedale, Texel maternal and

Growbulk. The hoggets were put onto the cf-lupin plots on 5 August 2014 and the lucerne plots on 15 September. All hoggets were shorn on 28 November 2014 and were replaced with the ewe lambs on 18 February 2015. The ewe lambs were removed from the plots on 29 May 2015.

The three different cohorts of sheep and the shearing event on 28 November created four distinct grazing periods that are reported in this chapter: GP1 (11 March 2014 – 19 May 2014), GP2 (5 August 2014 – 28 November 2014), GP3 (29 November 2014 – 17 February 2015) and GP4 (18 February 2015 – 29 May 2015).

At the start of the first, second and fourth grazing periods, 18-30 sheep were selected as 'core' animals that were used to measure liveweight gain per head per day. Those sheep were blocked on initial live weight and then one sheep from each block was randomly allocated to each plot, giving three to five core sheep per plot of the same average live weight across plots.

4.2.5 Grazing management

Each paddock was rotationally grazed in series (i.e. from paddock 1 to paddock 5 in each plot; see Plate 4.1). All groups of sheep were shifted to the next paddock in the rotation on the same day, and therefore stocking duration/paddock was the same across plots, except in spring 2014 when there were two grazing rotations for cf-lupin plots from 5 August to 20 October and one grazing rotation for lucerne from 15 September to 20 October. Stocking duration (4-15 days/paddock, mean 9 days/paddock) and stocking density (3-10 sheep/paddock) were adjusted for pasture growth rate and mass to maintain a similar herbage allowance across plots of approximately 2-3 kg DM/head/day. This required a 'Put and Take' policy where additional sheep could be added to or removed from the group of core sheep, when the group was moved to the next paddock. The plots were de-stocked in autumn of each year when herbage mass was no longer sufficient to maintain feeding levels required of the core sheep. When necessary, post-grazing residual was mown to 4-5 cm above ground level immediately after the sheep were taken out to maintain the two pasture types at a similar vegetative growth stage. All sheep were treated for parasites regularly.

4.2.6 Measurements

4.2.6.1   Sheep liveweight gain

All sheep were weighed unfasted to the nearest 0.1 kg using Gallagher Smart Scales (Hamilton, NZ) in the corral at the experiment each time they were shifted. Sheep liveweight yield (kg/ha) was calculated as the change in average live weight of the core sheep since the previous measurement multiplied by the total number of sheep in the group divided by the area of the plot. Grazing days were calculated as the total number of sheep in the group multiplied by how many days they spent in the paddock divided by the plot area. These values were summed over time within each year to calculate the annual liveweight yield and annual grazing days for each plot. The average annual daily sheep liveweight gain was calculated as annual liveweight yield divided by annual grazing days.

4.2.6.2   Herbage mass

Herbage mass was measured in all of the 30 small paddocks each time the sheep were shifted, and every 2-3 weeks when they were de-stocked for winter. For each paddock that sheep were about to be moved into, the amount of herbage on offer (DM) was determined by pre-grazing quadrat cuts. For each cf-lupin paddock, a 0.5 m^2 quadrat of herbage was sampled in the area containing *L. polyphyllus* and also in the area containing blue lupin, whilst a single quadrat of the same area was sampled in each lucerne paddock. In each quadrat, sward height was measured using a mechanical height stick (Jenquip, Feilding, NZ) and then the herbage was cut to approximately 1-2 cm aboveground using electric hand shears. Botanical composition was measured in pre- and post-grazing plots on the same day that sheep were shifted. To do this, a random subsample (ca. 50 g fresh weight) was taken from each quadrat sample and sorted into the following botanical components. Lucerne samples were dissected into leaf (i.e. lamina plus petiole), reproductive stem, flower (inflorescence), weed and dead material. Cocksfoot-lupin samples were sorted into lupin lamina, lupin petiole, lupin stem, lupin flower, cocksfoot leaf (including the leaf blade and any leaf sheath), cocksfoot reproductive stem (including any seed-head), weed and dead material before being dried and weighed. Mixed samples were weighed fresh, dried at 65°C in a forced-air oven for 48 hours and re-weighed to determine DM content. The separated samples were dried and weighed as above to determine botanical composition. For the other paddocks, herbage mass was estimated using a height stick calibrated for each pasture type and for each growth interval using the pre and post-grazing mass data. Heights and quadrats were also taken following any post-grazing topping.

Herbage yield was calculated as the change in herbage mass of a paddock since the previous measurement, assumed to be zero when the paddock was being grazed, and summed for each year. Herbage allowance was calculated as pre-grazing herbage mass multiplied by the area of the paddock (0.052 ha) divided by the number of sheep and days in the paddock. Apparent herbage intake was calculated as pre-grazing herbage mass minus post-grazing herbage mass × 0.052 divided by the number of sheep and number of days.

To determine the rate of disappearance of individual pasture components, measurements were taken on a more intensive basis whilst animals grazed Paddock 2. Paddock heights and quadrat cuts were taken every 2-3 days during the grazing of this paddock in each grazing rotation. Samples were separated and weighed in the same manner as all other quadrat cuts. During these grazing periods, two quadrats were taken from both cf-lupin and lucerne paddocks instead of the usual one quadrat/lucerne paddock.

4.2.6.3   *Nutritive value analyses*

Herbage samples were kept for nutritive analysis from Paddock 3 in Year 1 and from Paddock 2 in Year 2. The dried samples of mixed and separated herbage material were ground to pass through a 1 mm sieve using a Retch Centrifugal Mill (Dusseldorf, Germany). Nutritive samples were then scanned by NIRS (FOSS; Hillerod, Denmark) to predict the nutritive value of the pasture samples. The separated components of lupin were scanned using the NIRS calibration described in Chapter 3 (Section 3.2.3.2). The samples of mixed cf-lupin, cocksfoot and lucerne were scanned using a calibration developed for mixed grass/clover pasture. The NIRS scans predicted CP, DMD and organic matter (OM). Metabolisable energy was then calculated using Equation 3.1.

The apparent intake of ME and CP by the sheep was estimated for each pasture type. Samples for nutritional analysis were collected once at pre-grazing and once at post-grazing at each grazing of Paddock 2 (once per rotation). Individual components were stored and bulked at the end of each of the four grazing periods. Nutritive analysis was carried out on each of the bulked components and then multiplied by the amount of material that was removed during grazing throughout the entire grazing period. Values for each of the components were then summed to estimate the total amount of metabolisable energy (Equation 4.1) and crude protein (Equation 4.2) that was consumed.

Equation 4.1   ME consumption: $(DM_{tot} - DM_{re}) * ME_{conc}$

Equation 4.2   CP consumption: $(DM_{tot} - DM_{re}) * CP_{conc}$

Where DM_{tot} is the yield of an individual component that was offered to the sheep (DM kg/ha), DM_{re} is the amount of a component that remains after grazing and ME_{conc} is the ME concentration in the herbage at pre-grazing and post-grazing (MJ/kg DM). For CP consumption, ME_{conc} is replaced with CP_{conc} to represent the concentration of CP in the herbage (g/100g DM).

4.2.7   Statistical analysis

All analyses were conducted with Genstat 16 Ed. (VSN International, 2014). The experiment was analysed for significant (α=0.05) effects on the variables from cf-lupin and lucerne pastures using the general analysis of variance procedure.

To calibrate the regrowth curve of individual plots, quadrat herbage mass was plotted against height data and linear regression was applied. Linear regressions were used to determine the relationship between herbage intake and liveweight yield. Regressions were fitted to data for each plot and slopes were analysed using a one-way analysis of variance (ANOVA).

4.3 Results

4.3.1 Liveweight yield

The cumulative liveweight yield for sheep grazing cf-lupin and lucerne pastures between 11 March 2014 and 29 May 2015 is given in Figure 4.1. In the first autumn establishment period, the Merino lamb liveweight gain was 55 kg/ha for cf-lupin compared with 107 kg/ha for lucerne (*P*=0.037).

In the 2014/15 season, the annual liveweight yield was 755 kg/ha for cf-lupin compared with (*P*=0.002) 1125 kg LW/ha for lucerne (Figure 4.1). In spring, the composite hoggets grazing cf-lupin pastures gained 4.2 kg/ha/day compared with 8.4 kg/ha/day for the hoggets grazing lucerne. At the conclusion of the spring grazing period, the liveweight yield of cf-lupin was 468 kg/ha, which was 31% lower (*P*=0.012) than 614 kg/ha from lucerne. Following shearing on 28 November 2014, liveweight gain slowed for cf-lupin pastures and the hoggets gained a further 171 kg/ha over 70 days which equated to a mean rate of liveweight gain of 2.4 kg LW/ha/day. By comparison, the hoggets on lucerne gained 381 kg/ha with a mean liveweight gain of 5.4 kg/ha/day.
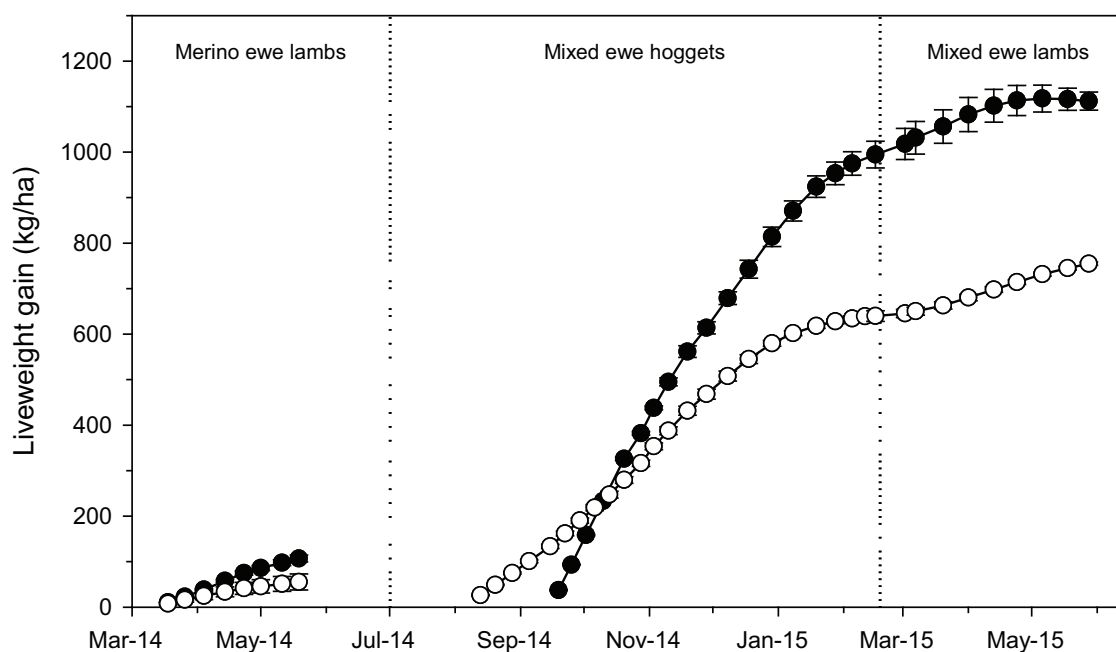
Figure 4.1 Annual liveweight yield of cocksfoot-lupin (○) and lucerne (●) pastures at Lincoln University from 11 March to 19 May 2014 (Merino ewe lambs), 5 August 2014 to 17 February 2015 (composite ewe hoggets) and 18 February to 29 May 2015 (composite ewe lambs). Error bars represent standard errors of the means. Hoggets were shorn on 28 November 2014.

Total liveweight gain was not different across each of the five paddocks for both pasture types (Figure 4.2). During the first grazing period (11 March to 19 May 2014), the liveweight yield of Paddock 1 was 35.2 kg/ha for lupin-cocksfoot compared with 57.7 kg/ha for lucerne. Liveweight yield increased as more paddocks were grazed and for Paddock 5, it was 58.1 kg/ha for cf-lupin compared with 107 kg/ha for lucerne.

Throughout the second to fourth grazing periods (5 August 2014 to 29 May 2015), inter-paddock differences were lower than during the first grazing period for both pasture types. Annual liveweight yield for each paddock ranged from 711 to 769 kg/ha for lupin-cocksfoot and 1120 to 1126 kg/ha for lucerne. Liveweight production was slow for both pasture types during the first grazing rotation in spring. However, the rate of liveweight production increased for both pasture types as sheep progressed from Paddock 1 to Paddock 5 during the first August to September rotation. When grazing concluded on 29 May 2015, there were no apparent differences in yield across the individual paddocks that could be related to the timing and number of grazings.
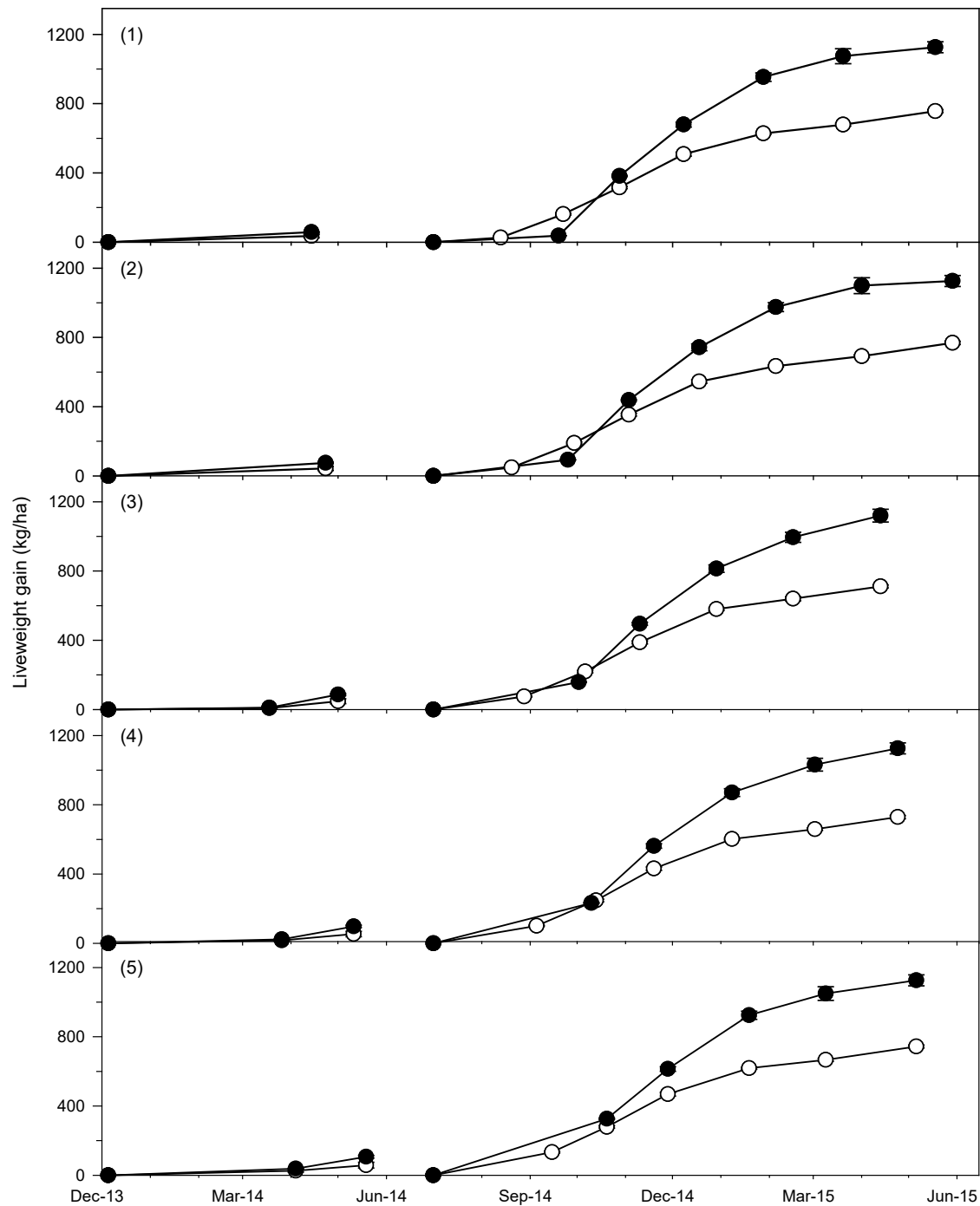
Figure 4.2 Cumulative liveweight yield of each of five paddocks of cocksfoot-lupin (○) and lucerne (●) at Lincoln University from 11 March to 19 May 2014 (Merino ewe lambs), 5 August 2014 to 17 February 2015 (composite ewe hoggets) and 18 February to 29 May 2015 (composite ewe lambs). Numbers in parentheses represent paddock number. Error bars represent one standard error of the mean.

4.3.2 Grazing days and stocking rate

In Year 1, there were 1997 grazing days/ha on cf-lupin pastures compared with (P=0.078) 1790 grazing days/ha on lucerne (Figure 4.3). At the start of Year 2, there was an extra 631 grazing days/ha on cf-lupin pastures from 5 August to when the sheep started on lucerne 41 days later on 15 September. Between 5 August and 3 November 2014, there were more grazing days on cf-lupin pastures than lucerne (P<0.05). However, both pasture types had accumulated about 3000 grazing days/ha by 30 January 2015. When grazing concluded on 29 May 2015, there were 4100 grazing days/ha on cf-lupin pastures compared with (P=0.003) 4490 grazing days/ha on lucerne.

The stocking rate of cf-lupin and lucerne pastures between 11 March 2014 and 29 May 2015 is given in Figure 4.3. Between 11 March and 19 April 2014 (Year 1), the mean stocking rate of cf-lupin pastures was 29 sheep/ha and 26 sheep/ha for lucerne. However, the mean stocking rate for all grazing rotations between 20 August 2014 and 29 May 2015 was 18.5 sheep/ha for cf-lupin pastures which was lower (P<0.001) than 21 sheep/ha for lucerne.
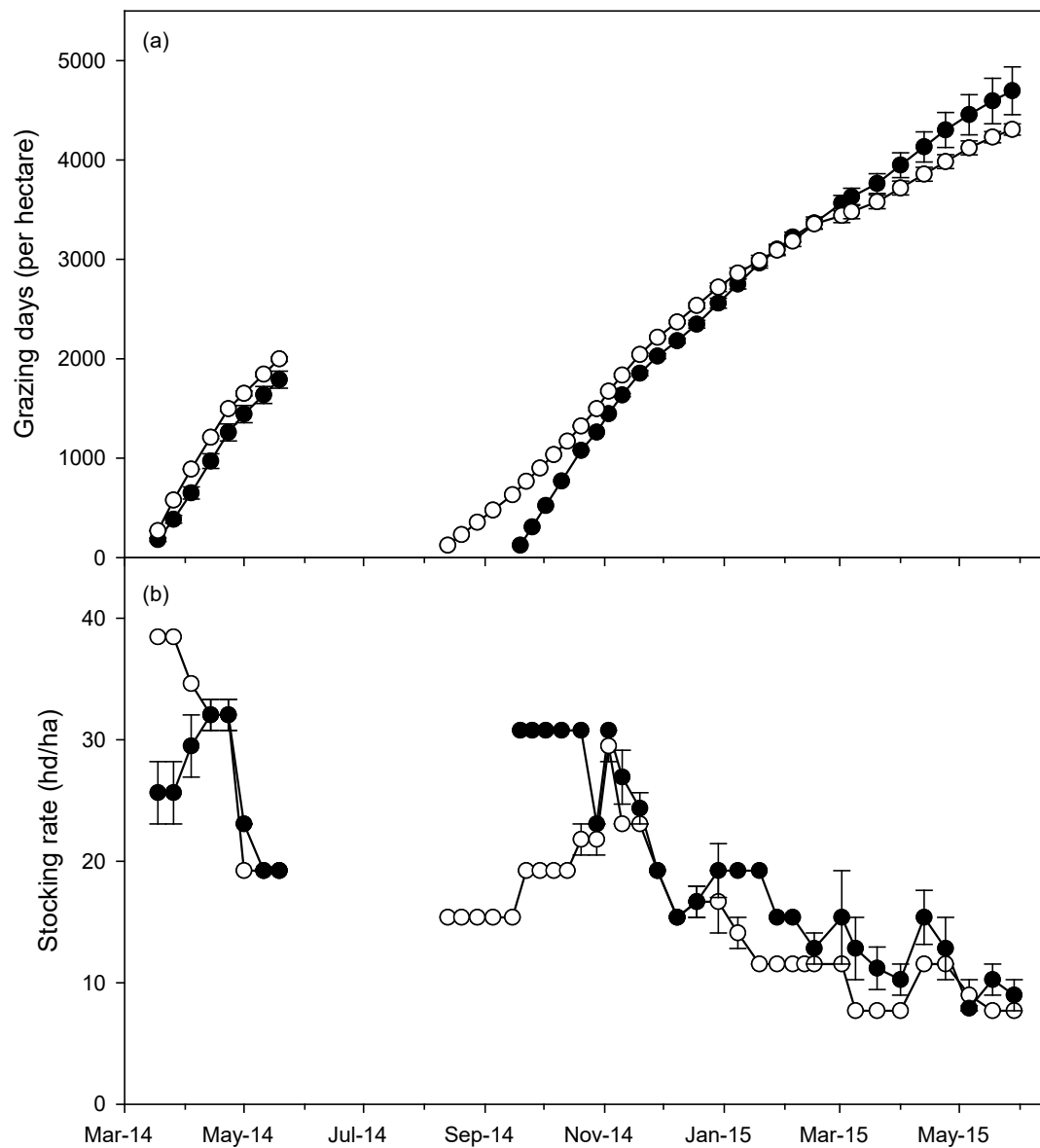
Figure 4.3 **Cumulative grazing days (a) and stocking rate (b) of cocksfoot-lupin (○) and lucerne (●) pastures at Lincoln University from 11 March to 19 May 2014 (Merino ewe lambs), 5 August 2014 to 17 February 2015 (composite ewe hoggets) and 18 February to 29 May 2015 (composite ewe lambs). Error bars represent standard errors of the means.**

4.3.3 Individual sheep live weight gain

4.3.3.1 Liveweight gain

The liveweight of sheep grazing cf-lupin and lucerne pastures is given in Figure 4.4. During Year 1, the Merino ewe lambs gained 13 g/head/day on cf-lupin and 41 g/head/day on lucerne. In the following spring, the hoggets on cf-lupin pastures gained 185 g/head/day during the first grazing cycle. On 8 December 2014, they gained a maximum of 315 g/head/day on cf-lupin and 522 g/head/day on lucerne. This was followed by a decline in daily liveweight gain for both pasture types for the rest of the growth season, with lambs gaining 127 g/head/day on cf-lupin pastures and 5.1 g/head/day on lucerne during the final month of grazing. The annual mean daily liveweight gain was 179 g/head/day for lupin-cocksfoot compared with (P=0.029) 247 g/head for lucerne from 19 September 2014 to 29 May 2015.

4.3.3.2 Sheep live weight

In Year 1, the Merino ewe lambs gained 1.9 kg and reached 31.9 kg on cf-lupin pastures compared with (P=0.009) 4.2 kg and 34.6 kg on lucerne (Figure 4.4). In spring, the composite hoggets gained 23.7 kg on cf-lupin pastures from 5 August to shearing on 28 November compared with (P=0.032) 33.9 kg on lucerne from 15 September to 28 November. After shearing, the hoggets gained less live weight on cf-lupin pastures than lucerne (P=0.004) and by 15 February, they averaged 66.4 kg on lupin-cocksfoot compared with 83.6 kg on lucerne (P<0.01). Between 18 February and 29 May 2015, the composite ewe lambs gained 12.8 kg and reached a final live weight of 47.0 kg on lupin-cocksfoot compared with (P=0.391) 11.4 kg and a final live weight of 44.6 kg on lucerne.
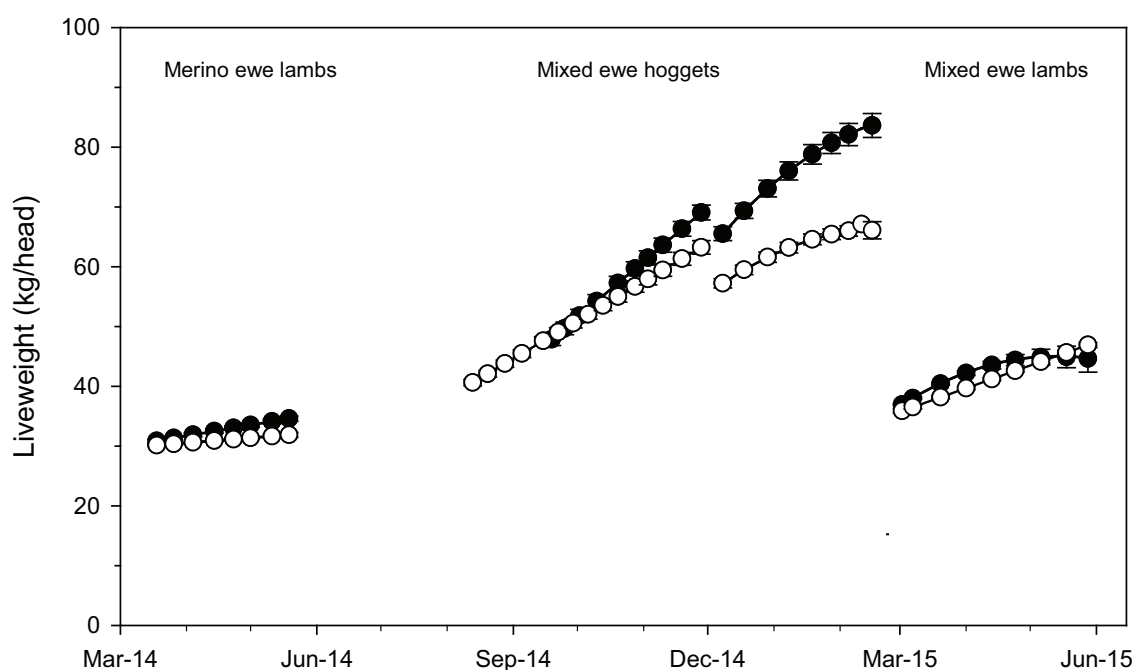
Figure 4.4 Live weight of sheep grazing cocksfoot-lupin (○) and lucerne (●) pastures at Lincoln University from 11 March to 19 May 2014 (Merino ewe lambs), 5 August 2014 to 17 February 2015 (composite ewe hoggets) and 18 February to 29 May 2015 (composite ewe lambs). Hoggets were shorn on 28 November. Error bars represent standard errors of the means.

4.3.4 Apparent intake

The apparent intake of herbage varied during grazing periods and was lowest in autumn of both years (Figure 4.5). In autumn 2014, the apparent intake by the Merino lambs was 1.0 kg DM/head/day on cf-lupin and 1.1 kg DM/head/day on lucerne (*P*=0.598). Between 5 August 2014 and 29 May 2015, the mean apparent intake was 1.3 kg DM/head/day on cf-lupin compared with (*P*<0.001) 1.9 kg DM/head/day on lucerne. During this period, apparent intake was more variable in sheep grazing lucerne (SED = 0.11) and showed three distinct peaks of at least 3 kg DM/head/day in September 2014, December 2014 and February 2015. By comparison, the apparent intakes of sheep grazing cf-lupin were less variable (SEM = 0.06) over the same period and showed one distinct peak of 2.8 kg DM/head/day in February 2015. Apparent intake declined abruptly to about 1 kg DM/head/day for both pasture types after the composite ewe hoggets were replaced with the composite ewe lambs in February 2015.
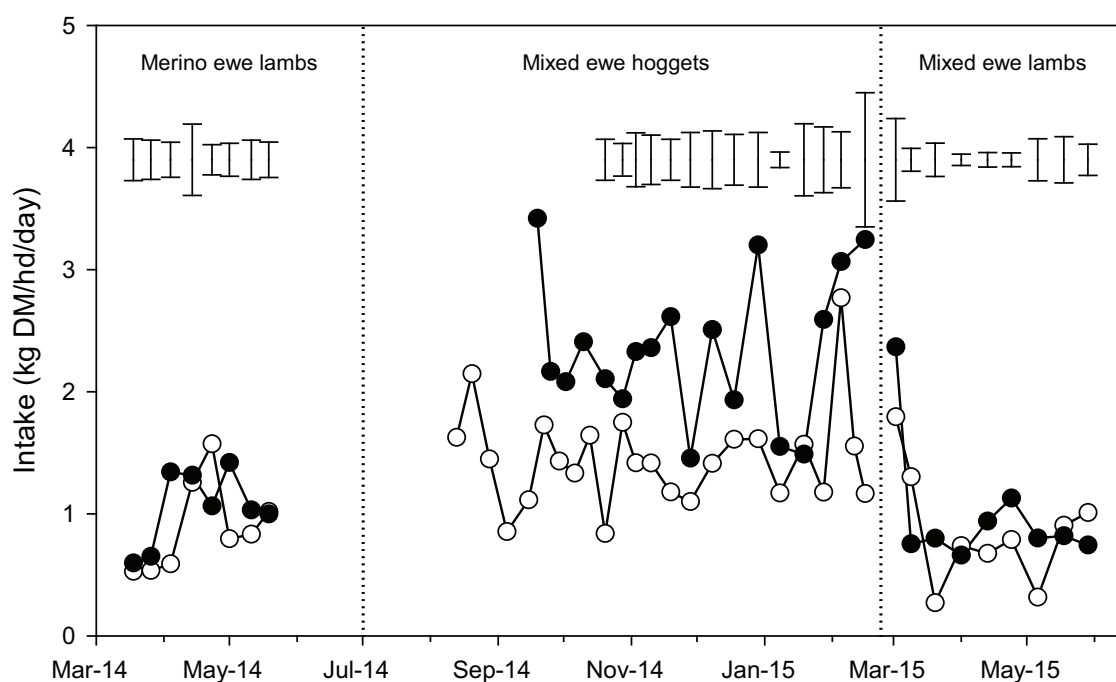
Figure 4.5 Apparent intake of sheep grazing cocksfoot-lupin (○) and lucerne (●) pastures at Lincoln University from 11 March to 19 May 2014 (Merino ewe lambs), 5 August 2014 to 17 February 2015 (composite ewe hoggets) and 18 February to 29 May 2015 (composite ewe lambs). Each error bars represents one standard error of the difference between means when measurements occurred on the same date.

4.3.5 Herbage yield

The average herbage yield of each pasture type is given in Figure 4.6 and the herbage yield of each paddock is given in Figure 4.7. Average herbage yield, on the date the first paddock was grazed (11 March 2014), was 2530 kg DM/ha for cf-lupin pastures compared with ($P<0.01$) 1730 kg DM/ha for lucerne. The rate of herbage production of both pasture types increased to about 35 kg DM/ha/day between 11 March and early April and then declined to about 10 kg DM/ha/day in May/June. From 11 March 2014, herbage yield remained about 700 kg DM/ha greater (P<0.01) for cf-lupin pastures than lucerne and by the end of June, cf-lupin had produced 4295 kg DM/ha compared with ($P<0.01$) 3520 kg DM/ha for lucerne.

In Year 2, both pasture types began herbage production at the same time in spring at the start of September when they had produced about 400 kg DM/ha (Figure 4.7). Lucerne herbage production then increased rapidly to about 9000 kg DM/ha in January (~65 kg DM/ha/day), before slowing to 10,220 kg DM/ha when the sheep were taken off the experiment on 29 May (8 kg DM/ha/day), and there was no additional production in June. Lucerne herbage production was about 2500 kg DM/ha

greater (*P*<0.001) than cf-lupin pastures in mid-December, when cf-lupin production started to slow down, and about 3800 kg DM/ha greater (*P*<0.001) from early February to the end of June, which indicated similar herbage production rates for both pasture types during this period (about 7.4 kg DM/ha/day).
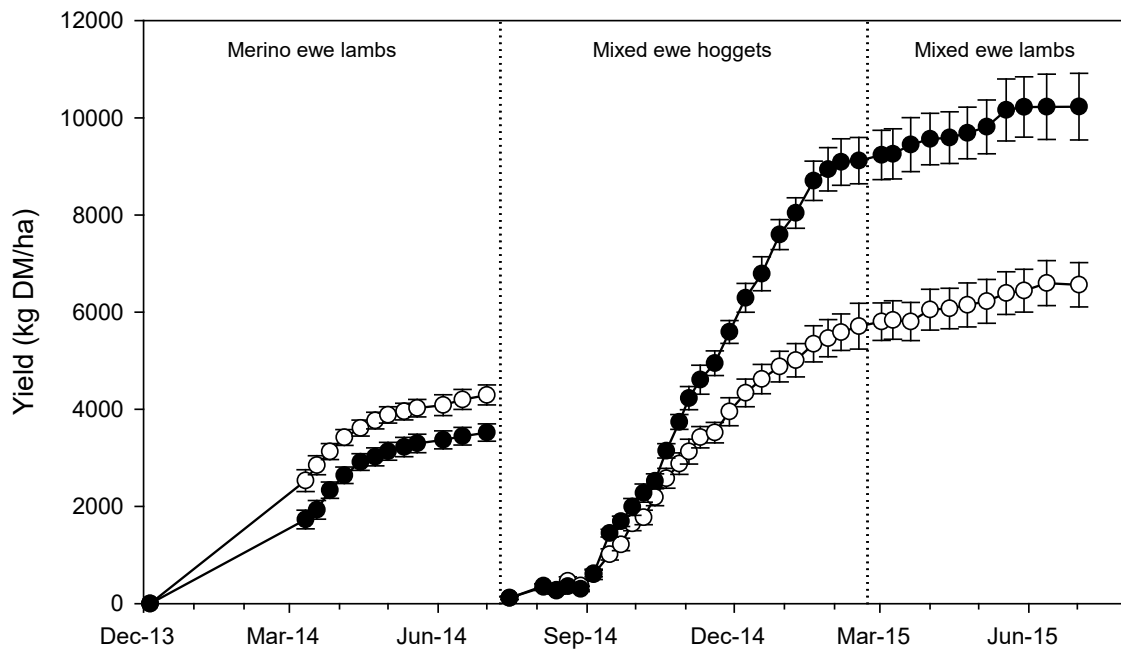
Figure 4.6 Accumulated herbage yield of cocksfoot-lupin (○) and lucerne (●) pastures at Lincoln University from 11 March to 19 May 2014 (Merino ewe lambs), 5 August 2014 to 17 February 2015 (composite ewe hoggets) and 18 February to 29 May 2015 (composite ewe lambs). Error bars represent standard errors of the means.

The DM accumulation of individual paddocks was variable for both pastures during the 2014/15 grazing season (Figure 4.7). The highest annual yield for cf-lupin pastures was 7,720 kg DM/ha produced by Paddock 4 compared with 13,620 kg DM/ha for the highest yielding lucerne pasture in Paddock 3. Both pastures produced their lowest yields in Paddock 5, where cf-lupin yielded 4,500 kg DM/ha compared with 7,800 kg DM/ha for the lucerne pasture. Yield of individual paddocks was not correlated with the timing of first grazing or their position in the grazing rotation.
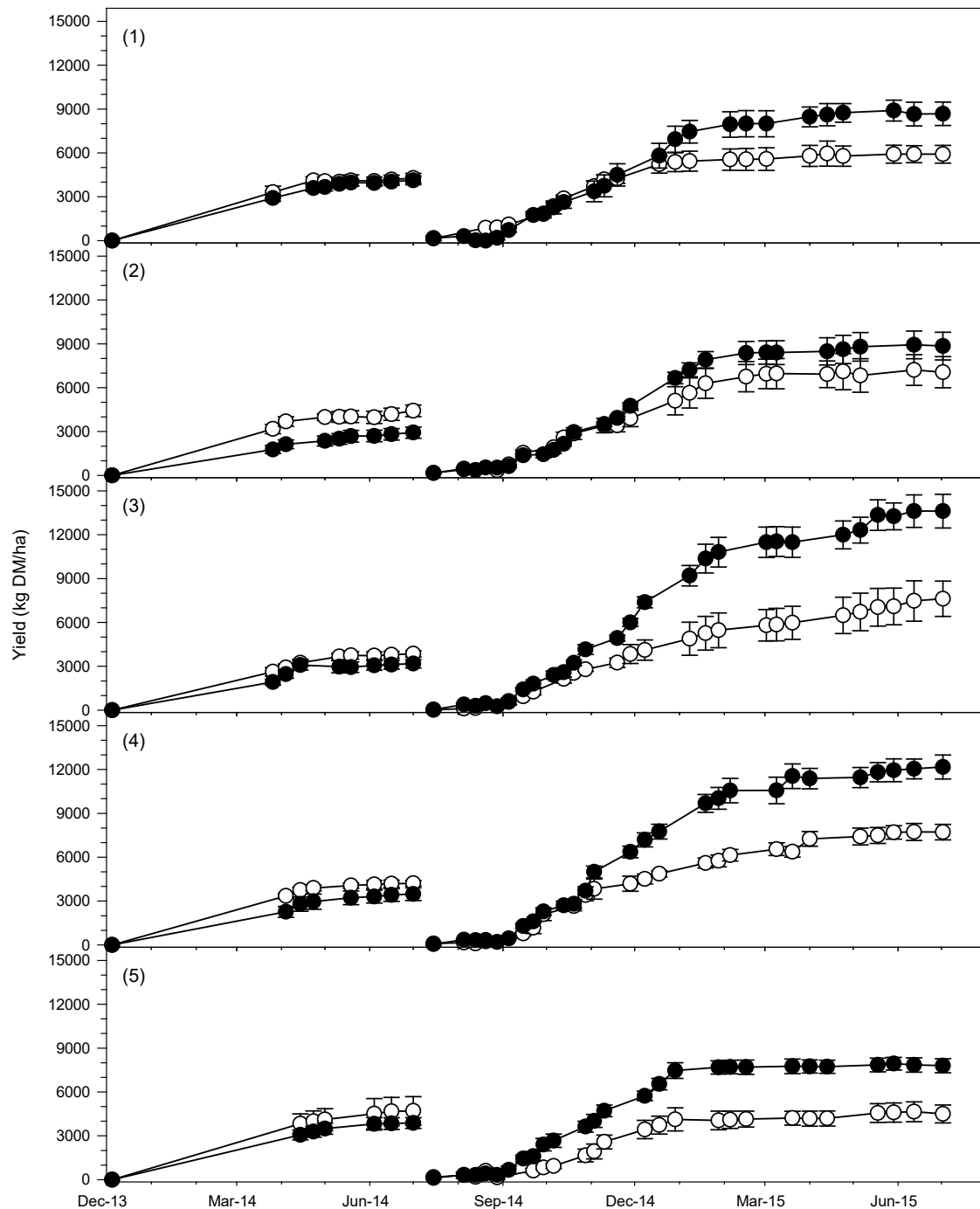
Figure 4.7 **Cumulative herbage yield (kg DM/ha) of animals grazing cocksfoot-lupin (○) and lucerne (●) pastures in a five paddock rotation between 11 March 2014 and 28 May 2015 in paddock H12 at the Horticultural Research Area at Lincoln University, Canterbury. Numbers in parentheses represent paddock number. Error bars represent the standard errors of the means.**

The DM accumulation of individual paddocks within individual regrowth cycles for cf-lupin and lucerne pastures is given in Figure 4.8. Yield accumulation was similar for both pastures in Year 1. In Year 2, the yield of both pastures followed a near linear pattern of yield accumulation throughout most regrowth periods. The slope of yield accumulation noticeably reduced in January 2015 for both pastures.
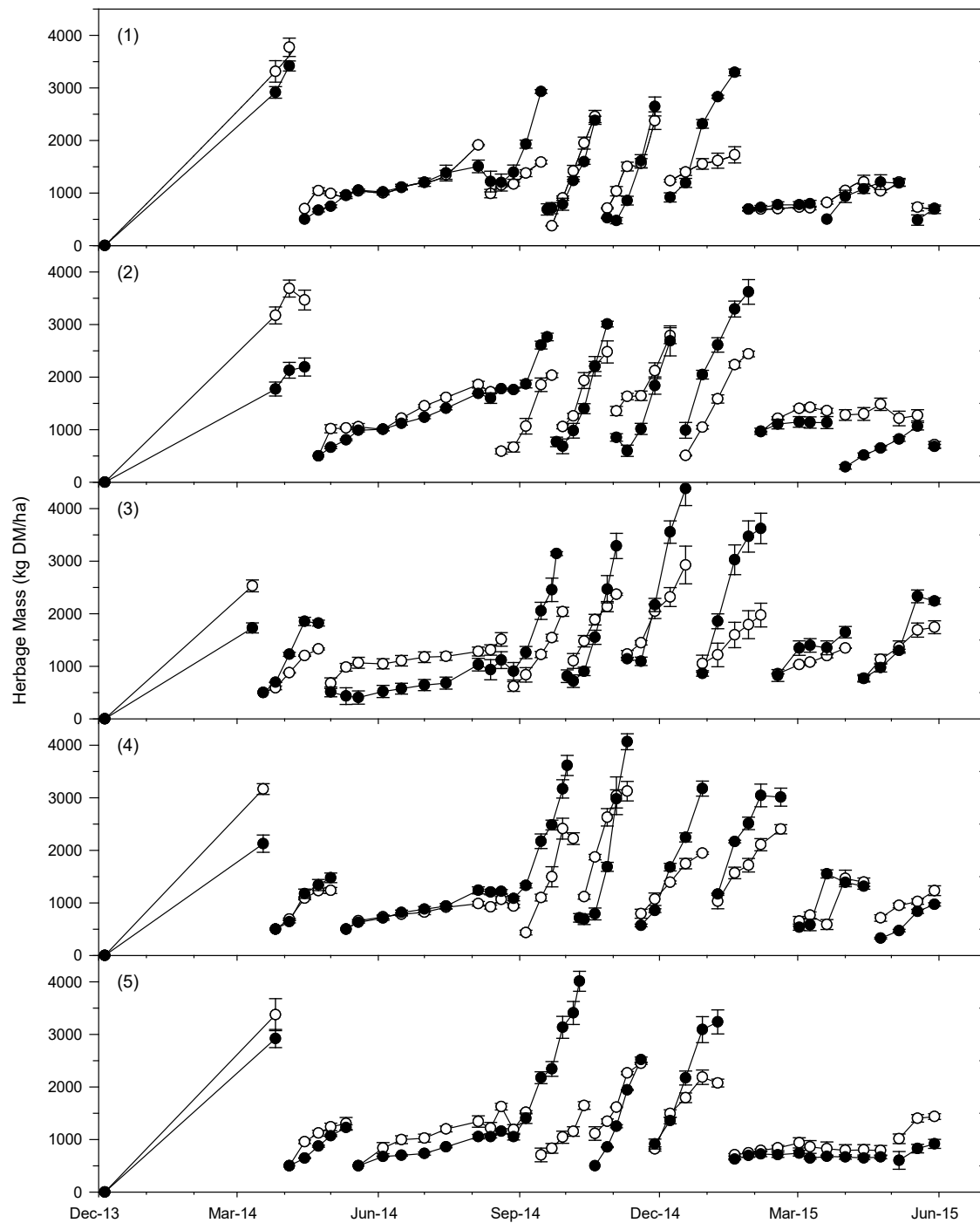
Figure 4.8 **Herbage mass (kg DM/ha) of cocksfoot-lupin (○) and lucerne (●) pastures in a five paddock rotation between 11 March 2014 and 28 May 2015 in paddock H12 at the Horticultural Research Area at Lincoln University, Canterbury. Error bars represent the standard errors of the means.**

4.3.6   Botanical composition

The proportion of each yield component was variable for cf-lupin pastures during the establishment period (Figure 4.9). When grazing commenced, unsown species contributed 50% of the herbage mass whilst the sown, lupin and cocksfoot, contributed 40% and 10%, respectively. Cocksfoot abundance increased during the establishment period and was 50% of total yield when grazing ceased on 18 May. In contrast, lupin abundance steadily declined to 20% during the establishment period.
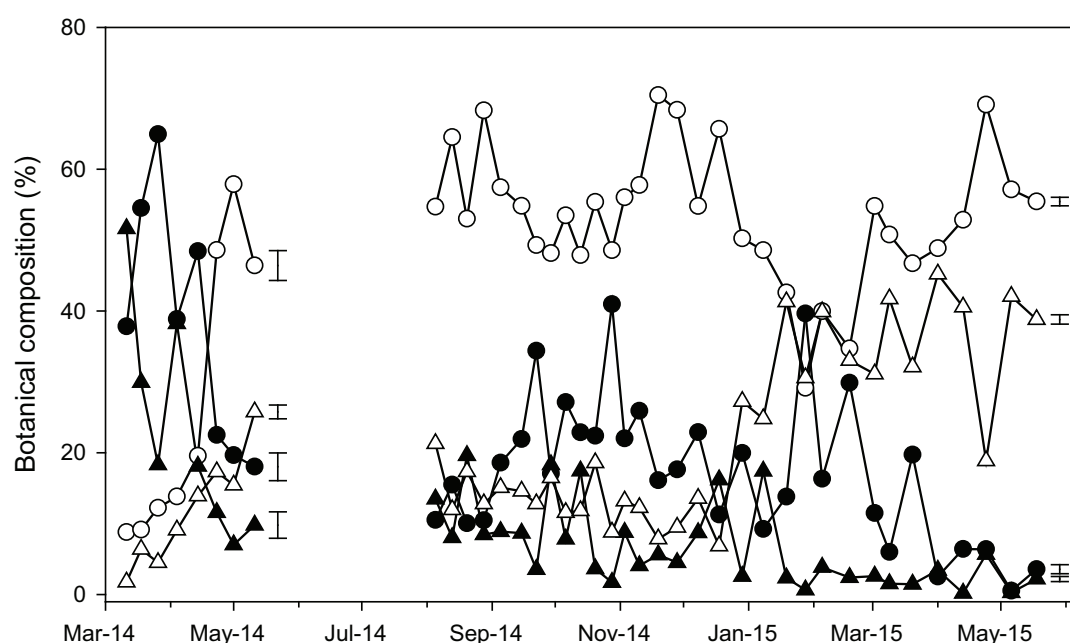
Figure 4.9   Abundance of lupin (●), cocksfoot (○), unsown species (▲) and dead material (Δ) of total pre-grazing herbage mass in cocksfoot-lupin pastures between 11 March 2014 and 29 May 2015 in paddock H12 at the Horticultural Research Area at Lincoln University, Canterbury. Error bars represent one standard error of the mean for individual components.

Lupin abundance had declined to 10% in cf-lupin pastures when grazing recommenced on 5 August 2014. Cocksfoot dominated the composition and represented approximately 60% of yield, whilst dead material and unsown species contributed smaller proportions. Lupin varied between paddocks and contributed 22% of total yield between September 2014 and February 2015. Between November and February, the proportions of dead material and lupin steadily increased whilst the abundance of cocksfoot decreased to approximately 30%. During autumn, lupin declined steadily and represented <10% of yield when grazing ceased on 29 May 2015. During the same period, cocksfoot steadily increased in abundance and represented about 55% of total DM. Similarly, the proportion of dead material rose from 15% to 40% between December 2014 and May 2015.

The morphological components of yield, for each of the sown species, are given for cf-lupin pastures in Figure 4.10. When grazing commenced in Year 1, lupin lamina represented 60% of the sown species yield. However, lupin lamina declined sharply during April 2014, which led to a final proportion of 20% on 18 May 2015. During the same period, cocksfoot leaf rose steadily and represented approximately 75% of sown species yield when grazing ceased.

During the first grazing rotation in Year 2, cocksfoot leaf represented 80% of sown species yield then slowly declined to 50% during the spring. Cocksfoot stem remained at <5% of sown species yield for most of the grazing rotations but showed a distinct increase during November and December 2014, when it represented up to 40% of the sown species. Lupin yield was primarily driven by lamina growth throughout all grazing periods and represented approximately 20% of sown species yield in Year 2.
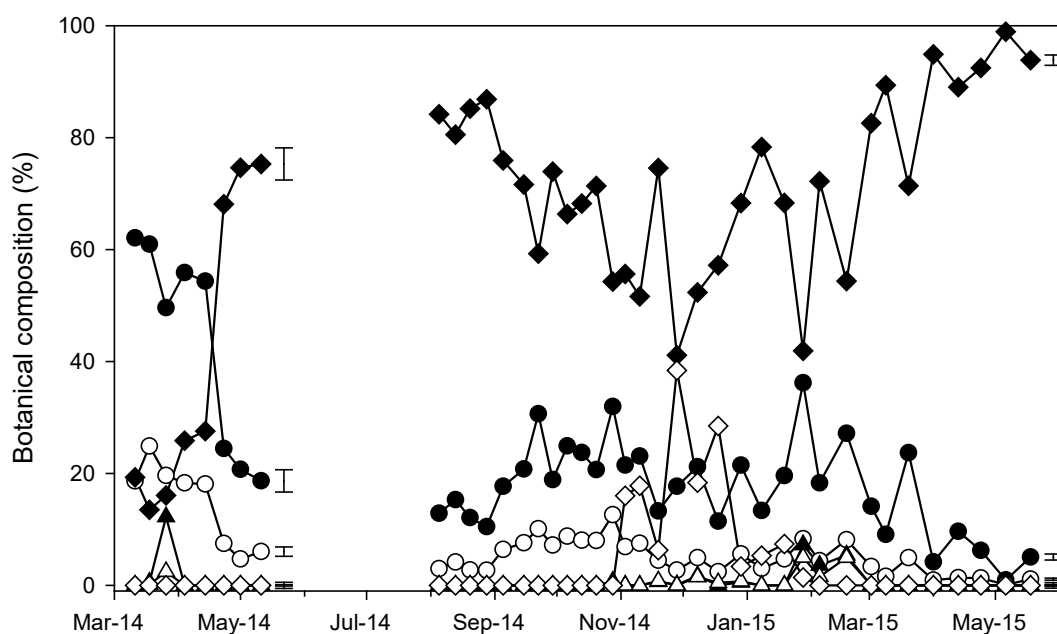
Figure 4.10 **Abundance of live, sown components, lupin lamina (●), lupin petiole (○), lupin stem (▲), lupin flower (Δ), cocksfoot leaf (♦) and cocksfoot stem (◊), in the pre-grazing herbage mass of cocksfoot-lupin pastures between 11 March 2014 and 29 May 2015 in paddock H12 at the Horticultural Research Area at Lincoln University, Canterbury. Values presented are proportions of sown species yield only. Error bars represent one standard error of the mean for individual components.**

During establishment in Year 1, the composition of lucerne pastures was variable and contained up to 50% of unsown species (Figure 4.11). However, after the first grazing cycle, unsown species were less abundant and >80% of the pasture on offer was lucerne. When grazing re-commenced in

spring, lucerne represented 75-80% of the total yield on offer. Lucerne abundance continued to increase during spring and peaked at 95% in mid-December 2014. Lucerne steadily declined in abundance as dead material increased during January and February 2015. From 1 March to 29 May 2015, lucerne pastures were stable at approximately 60% lucerne and 40% dead material. At the conclusion of grazing, lucerne pastures had maintained a mean legume proportion of 77% which was higher ($P<0.001$) than the 18% for cf-lupin pastures.
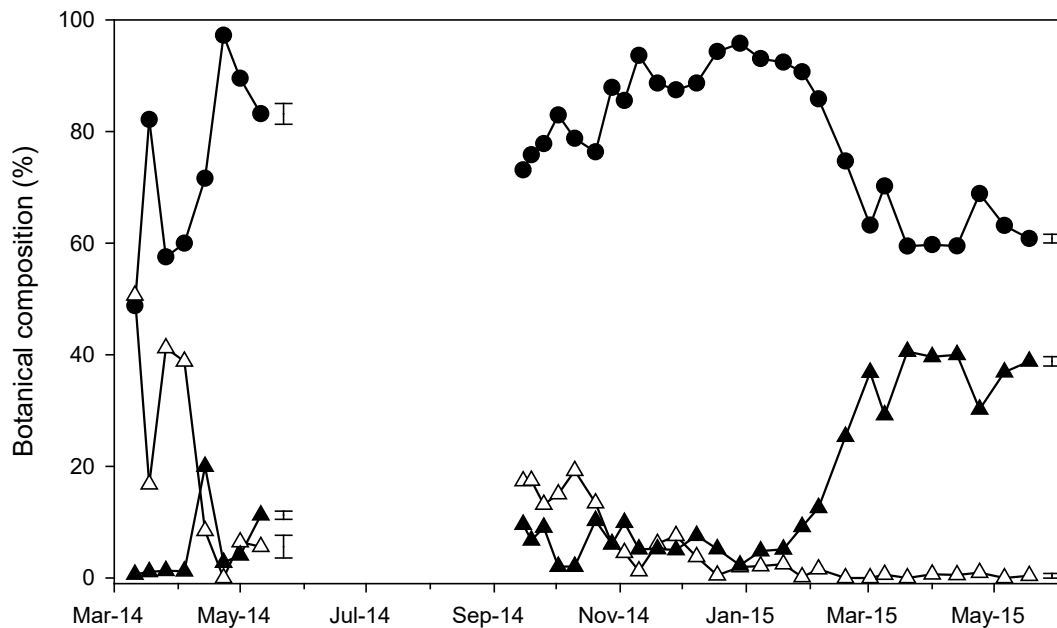
Figure 4.11 Abundance of lucerne (●), unsown species (Δ) and dead material (▲) in the total pre-grazing herbage mass in lucerne pastures between 11 March 2014 and 29 May 2015 in paddock H12 at the Horticultural Research Area at Lincoln University, Canterbury. Error bars represent one standard error of the mean for individual components.

Lucerne maintained a leaf proportion of about 60% during Year 1 and in spring of Year 2 (Figure 4.12). Stem material increased by 16% between November and mid-December 2014, whilst leaf declined from 62% to 43% during the same period. During summer and autumn, stem material steadily declined and represented 25% of sown species yield on 29 May 2015. In contrast, leaf material had increased to 75% of yield at the conclusion of grazing.
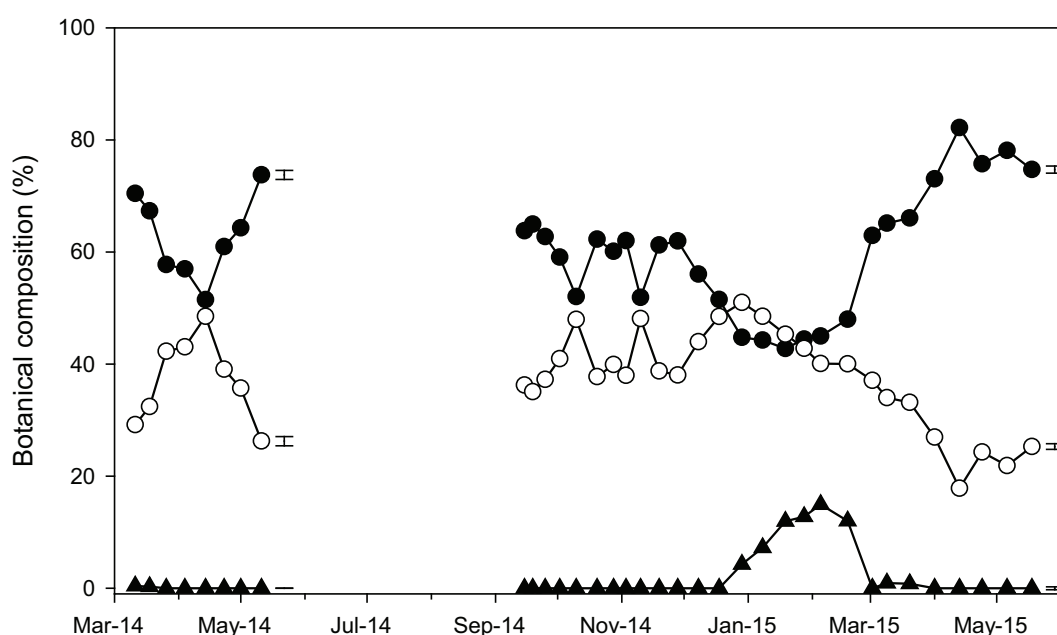
Figure 4.12 Abundance of lucerne leaf (●), lucerne stem (○) and lucerne flower (▲) expressed as percentages of total live lucerne in lucerne pastures between 11 March 2014 and 29 May 2015 in paddock H12 at the Horticultural Research Area at Lincoln University, Canterbury. Values presented are proportions of sown species yield only. Error bars represent one standard error of the mean for individual components.

4.3.7 Nutritional value

The whole-crop nutritional value of cf-lupin and lucerne pastures is presented in Figure 4.13. The DMD of both pasture types was approximately 70% between 11 March and 28 November 2014. The nutritional value of both pasture types declined during summer, where cf-lupin had a mean DMD of 64% compared with 58% for lucerne. This trend continued in autumn when DMD became increasingly variable, with a range of 58-71% for cf-lupin pastures and 26-63% for lucerne.

The CP content of the mixed lupin-cocksfoot herbage was consistently lower than lucerne between 11 March 2014 and 29 May 2015, with a mean CP of 13.7 g/100 g compared to 18.4 g/100 g, respectively. Crude protein declined for both pasture types between September 2014 and January 2015 but recovered during the autumn grazing period to 14.2 g/100 g for cf-lupin pastures and 18.7 g/100 g in lucerne, despite having increased variability in the final month of grazing.

The ME level ranged from 10.1 to 11.1 MJ/kg DM between 11 March and 28 October 2014, with both pastures having a mean of 10.6 MJ/kg DM over the same period. Cf-lupin pastures had a mean of 10.3 MJME/kg DM compared with 7.9 MJME/kg DM for lucerne. The ME of lucerne pastures

decreased to 9 MJME/kg DM in December, where it remained until declining to 4.7 MJME/kg DM on 7 May 2015. This extremely low value was most likely caused by the large abundance of dead material present, as there was little to no growth of leaf material at this time.
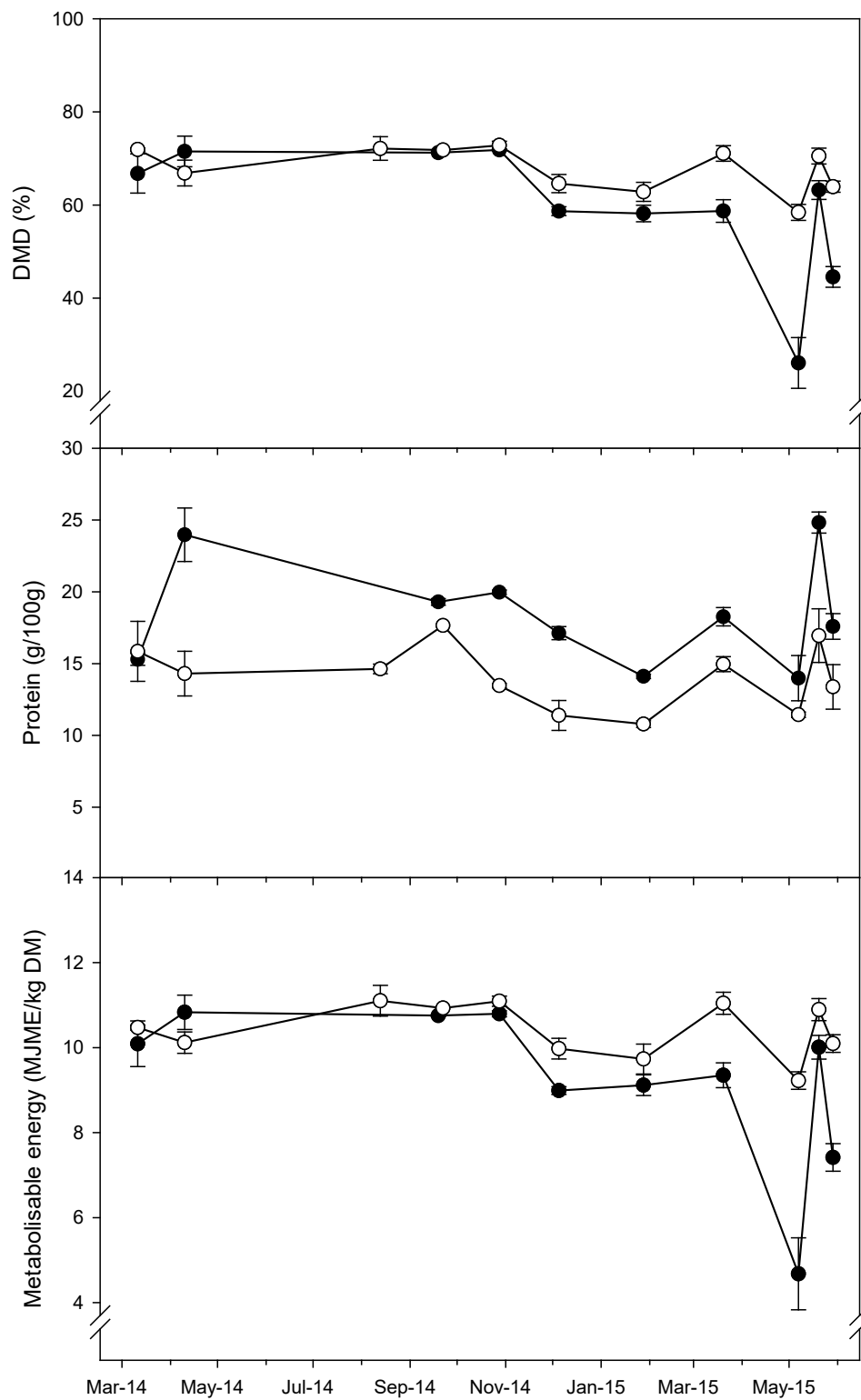
Figure 4.13 Dry matter digestibility (DMD), crude protein and metabolisable energy of lucerne (●) and cocksfoot-lupin (○) pastures between March 2014 and May 2015 at Lincoln University, Canterbury. Each error bar represents standard errors of the means.

The DMD, CP and ME content of various yield components of cf-lupin and lucerne pastures is given in Table 4.2. Lupin lamina had a mean DMD of 81.1%, and consistently high ME which ranged between 11.7 and 12.4 MJME/kg DM. The CP of cocksfoot leaf was lower than lucerne leaf and lupin lamina at all measurements, and had a mean concentration of 16.5 g/100 g. The mean ME of leaf for lucerne and cocksfoot was 11.6 MJME/kg DM for the 2014/15 grazing period. The DMD of lucerne stem was lowest between December 2014 and February 2015 at 48.6% but increased to 64.8% in autumn. Structural components were lower in CP than leafy material and were generally variable in both pastures; ranging between 9.8 and 16.5 g/100 g for lucerne stem and 6.8 and 13.9 g/100 g for lupin petiole.

Table 4.2 **Nutritive value of various plant components from lucerne and cocksfoot-lupin pastures between 11 March 2014 and 29 May 2015 at Lincoln University, Canterbury. Values in parentheses indicate standard error of the mean.**

	Period	Lucerne		Lupin-cf		
		Leaf	Stem	Lamina	Petiole	Cf leaf
DMD (%DM)	Mar - Apr 14	77.0 (0.76)	55.9 (2.44)	79.7 (1.67)	65.1 (1.54)	67.4 (0.97)
	Aug - Nov 14	76.0 (0.46)	66.5 (3.53)	78.9 (0.59)	76.4 (1.7)	76.5 (1.36)
	Dec 14 - Feb 15	76.9 (0.42)	48.6 (1.52)	83.4 (0.55)	64.9 (2.66)	72.2 (0.37)
	Mar - May 15	78.3 (0.67)	64.8 (2.78)	83.8 (0.46)	60.4 (12.15)	75.1 (2.24)
Grand Mean		76.9 (0.34)	58.4 (1.98)	81.1 (0.55)	68.5 (1.87)	73.1 (0.96)
Crude Protein (g/100g)	Mar - Apr 14	25.8 (1.06)	13.0 (1.58)	24.2 (0.86)	10.8 (0.96)	16.3 (0.82)
	Aug - Nov 14	25.9 (0.34)	15.8 (0.52)	27.0 (0.8)	13.9 (0.85)	17.8 (0.99)
	Dec 14 - Feb 15	24.5 (1.24)	9.8 (0.75)	22.6 (1.35)	11.6 (0.89)	14.5 (0.33)
	Mar - May 15	25.6 (1.59)	16.5 (2.71)	22.8 (1.78)	6.8 (1.97)	17.0 (0.43)
Grand Mean		25.5 (0.49)	13.5 (0.85)	24.5 (0.66)	11.7 (0.66)	16.5 (0.44)
Metabolisable energy (MJME/kg DM)	Mar - Apr 14	11.6 (0.14)	8.7 (0.3)	11.7 (0.22)	10.2 (0.23)	10.2 (0.15)
	Aug - Nov 14	11.4 (0.06)	10.1 (0.22)	11.9 (0.08)	11.8 (0.28)	11.7 (0.24)
	Dec 14 - Feb 15	11.6 (0.08)	7.8 (0.38)	12.3 (0.09)	10.1 (0.38)	11.3 (0.09)
	Mar - May 15	12.0 (0.14)	10.2 (0.5)	12.4 (0.15)	9.3 (1.98)	11.8 (0.31)
Grand Mean		11.6 (0.07)	9.1 (0.27)	12.1 (0.07)	10.6 (0.29)	11.3 (0.16)

4.3.8 Herbage disappearance

The disappearance of pasture components during the September grazing of cf-lupin pastures is shown in Figure 4.14. Sheep consumed lupin lamina and cocksfoot leaf at a similar rate during the first two days of grazing. However, grazing preference of cocksfoot leaf appeared to increase between 24 and 26 September, which was followed by a plateau in disappearance. Grazing remained relatively consistent on lupin lamina throughout the period and sheep had consumed 270 kg DM/ha of the 520 kg DM/ha offered. A similar pattern of disappearance was seen during the next grazing period (Figure 4.15). However, utilization of lupin lamina was 76% compared with 52% in the first grazing period. All other components of yield showed minor fluctuations and there was no measureable apparent intake by sheep.

During the first (Figure 4.14) and second (Figure 4.15) grazings of lucerne in Paddock 2, sheep showed a strong preference for lucerne leaf material. During the first three days of the September grazing, sheep consumed 550 kg DM of leaf before they started to show an apparent preference for stem material. When the first grazing ceased on 25 September, 1170 kg DM of lucerne leaf had been removed of the 1350 kg DM/ha on offer whilst 300 kg DM/ha of stem had been consumed. The trend was similar during the 28 October to 3 November grazing, but the shorter grazing period meant that the pasture components declined in abundance more rapidly.

The proportion of stem in lucerne pastures had increased to 39% at the commencement of the December grazing (Figure 4.16). Lucerne leaf remained a highly preferred yield component and showed a linear decline from 1350 kg DM/ha to 250 kg DM/ha during the 10 day grazing period. During the same period, sheep grazing cf-lupin pastures appeared to show a strong preference for cocksfoot leaf (Figure 4.16). Sheep consumed 520 kg DM/ha of cocksfoot leaf during the first four days of grazing which continued to an eventual consumption of 900 of the 1150 kg DM/ha offered. Sheep showed minimal preference for lupin petiole during the first four days of grazing. However, sheep had consumed 340 kg DM/ha of petiole when grazing ceased after 10 days on 18 December.
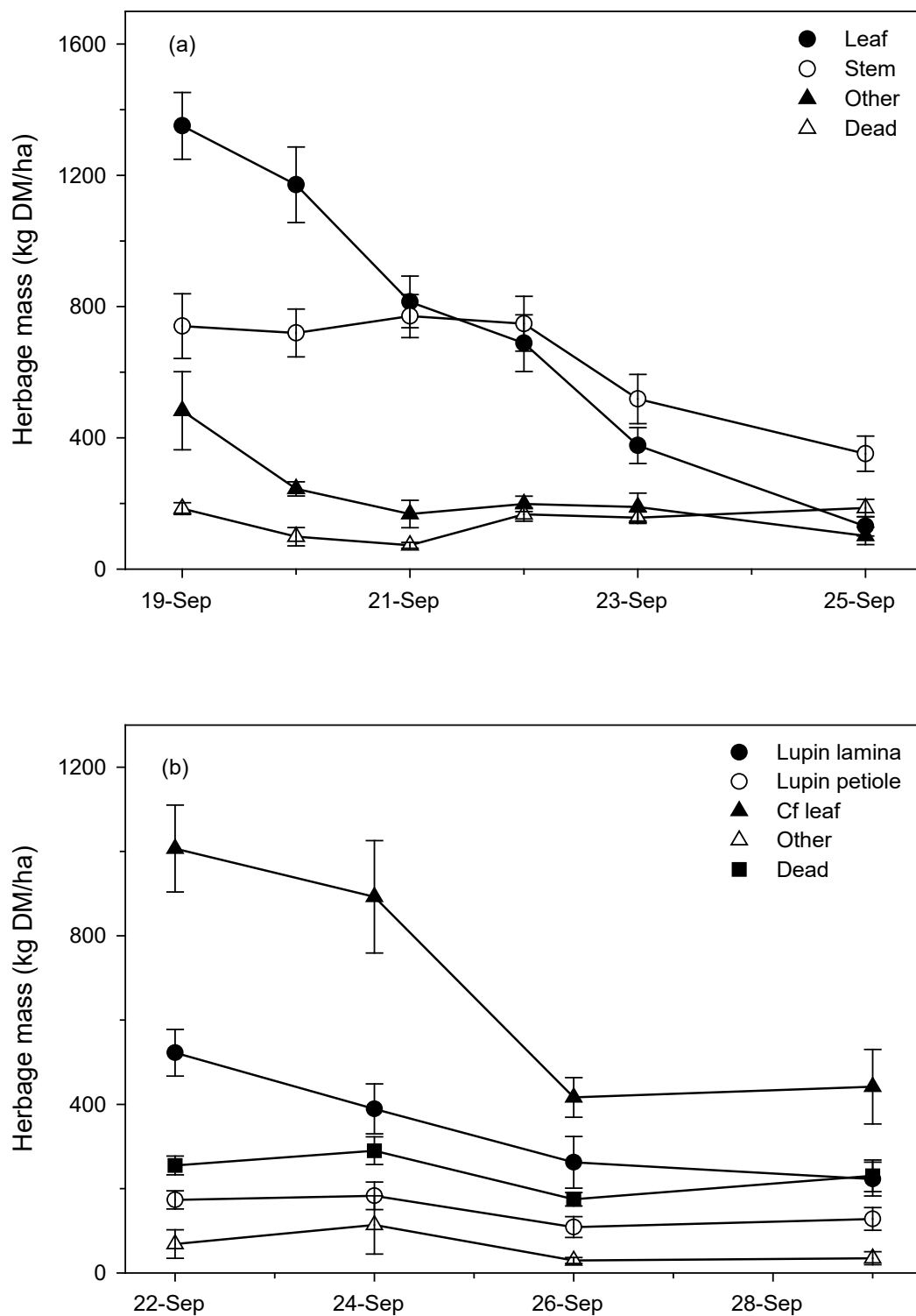
Figure 4.14 Disappearance of pasture components for sheep grazing (a) lucerne from 19 to 25 September 2014 and (b) cocksfoot-lupin from 22 to 29 September 2014 at Lincoln University, Canterbury. Error bars represent standard errors of the means.
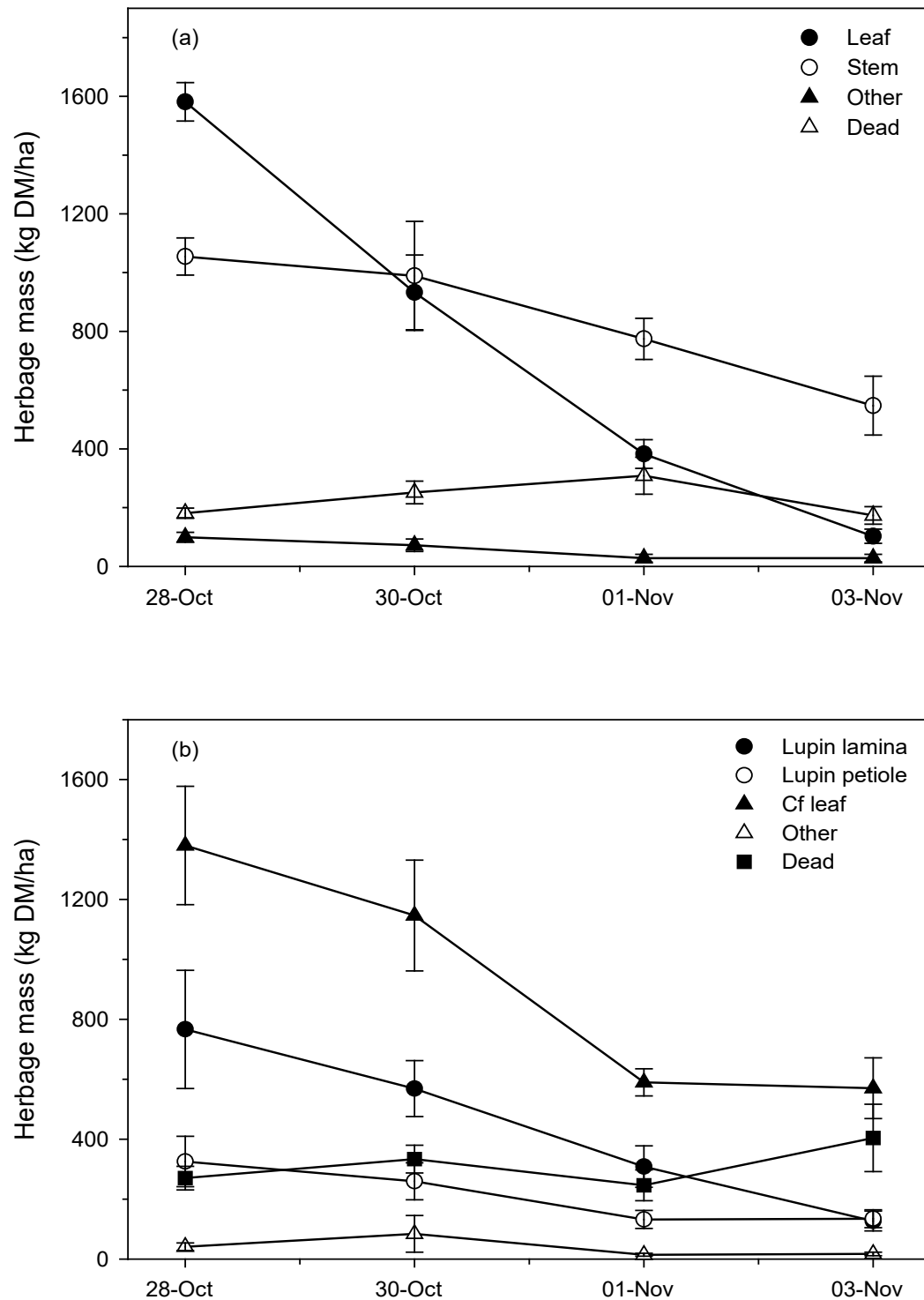
Figure 4.15 Disappearance of pasture components for sheep grazing lucerne (a) and cocksfoot-lupin (b) pastures between 28 October and 3 November 2014 at Lincoln University, Canterbury. Error bars represent standard error of the mean.
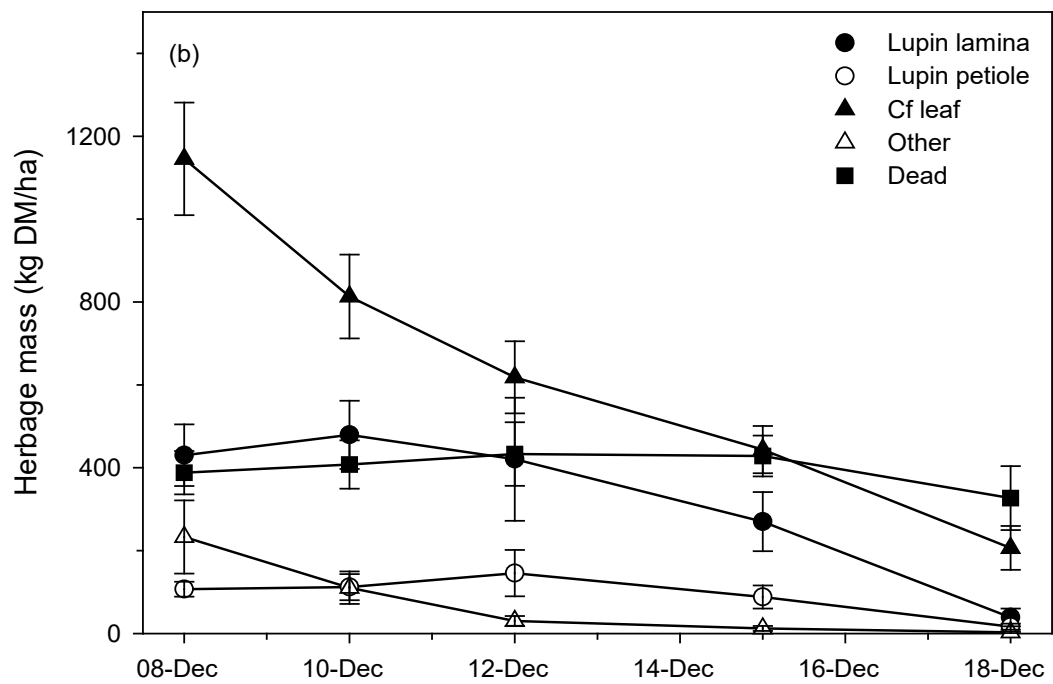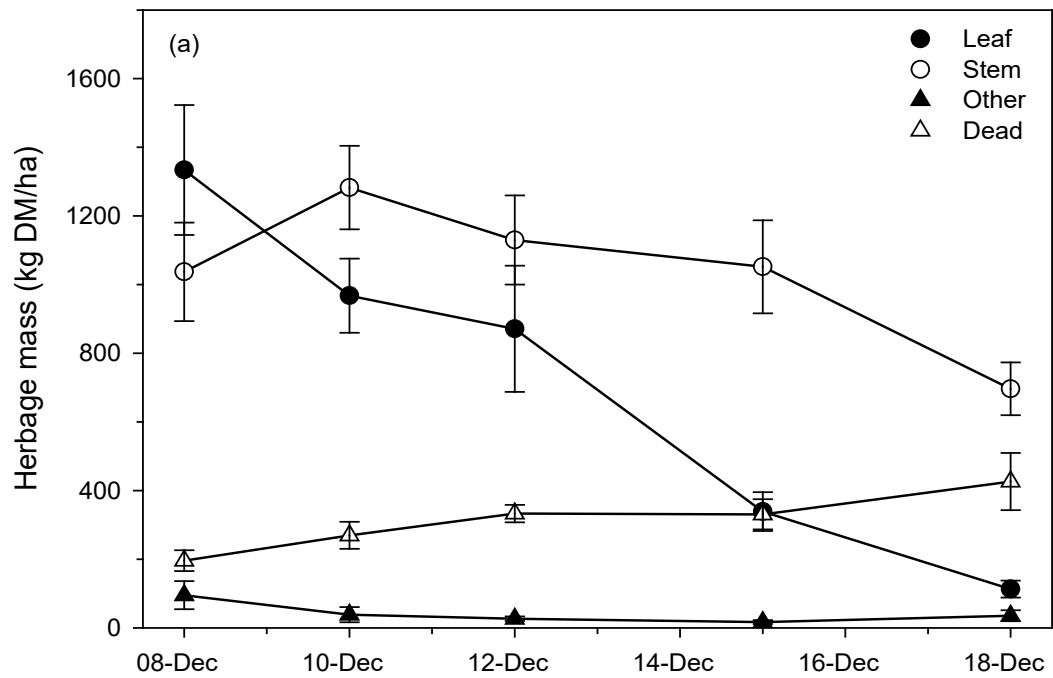
Figure 4.16 Disappearance of pasture components for sheep grazing lucerne (a) and cocksfoot-lupin (b) pastures between 8 and 18 December 2014 at Lincoln University, Canterbury. Error bars represent standard error of the mean.

Between 28 January and 5 February 2015, intake during the first two days of grazing appeared to be minimal on cf-lupin pastures (Figure 4.17). However, sheep eventually consumed small amounts of lupin lamina and cocksfoot leaf whilst all other components appeared to be rejected. Sheep showed a strong preference for lucerne leaf and did not appear to consume any of the other yield components (Figure 4.17). Sheep utilised 69% of the 700 kg DM/ha of lucerne leaf that was offered.

By the fifth grazing rotation, which began on 20 March 2015, preference for cocksfoot leaf had apparently declined compared with previous grazings (Figure 4.18). However, sheep consumed the majority of lupin lamina offered and removed 76% of the material during the grazing period. The grazing period of 12 days was longer than all previous grazings. Thus, it is possible that the apparent increase in dead material was caused by senescence/trampling throughout the grazing period. The proportion of live material on offer had declined substantially when the fifth grazing of lucerne pastures began on 20 March 2015 (Figure 4.18). Dead material had increased since the previous grazing and now represented 36% of the herbage on offer. Lucerne leaf remained as the preferred component and 330 kg DM/ha of the 470 kg DM/ha on offer had been consumed when grazing ceased on 1 April.

During the final grazing period, cocksfoot leaf was the primary component offered to sheep in the cf-lupin pastures (Figure 4.19). Given the high proportion of dead material and minor amounts of lupin (<100 kg DM/ha) offered to sheep, cocksfoot leaf was the most highly preferred component with sheep consuming 380 kg DM/ha of the 720 kg DM/ha offered. In contrast, sheep were offered 480 kg DM/ha of lucerne leaf and had consumed 295 kg DM/ha when they were removed on 29 May (Figure 4.19). Lucerne stem and dead material appeared to be variable during this final period with sheep showing some preference for the stem on offer.
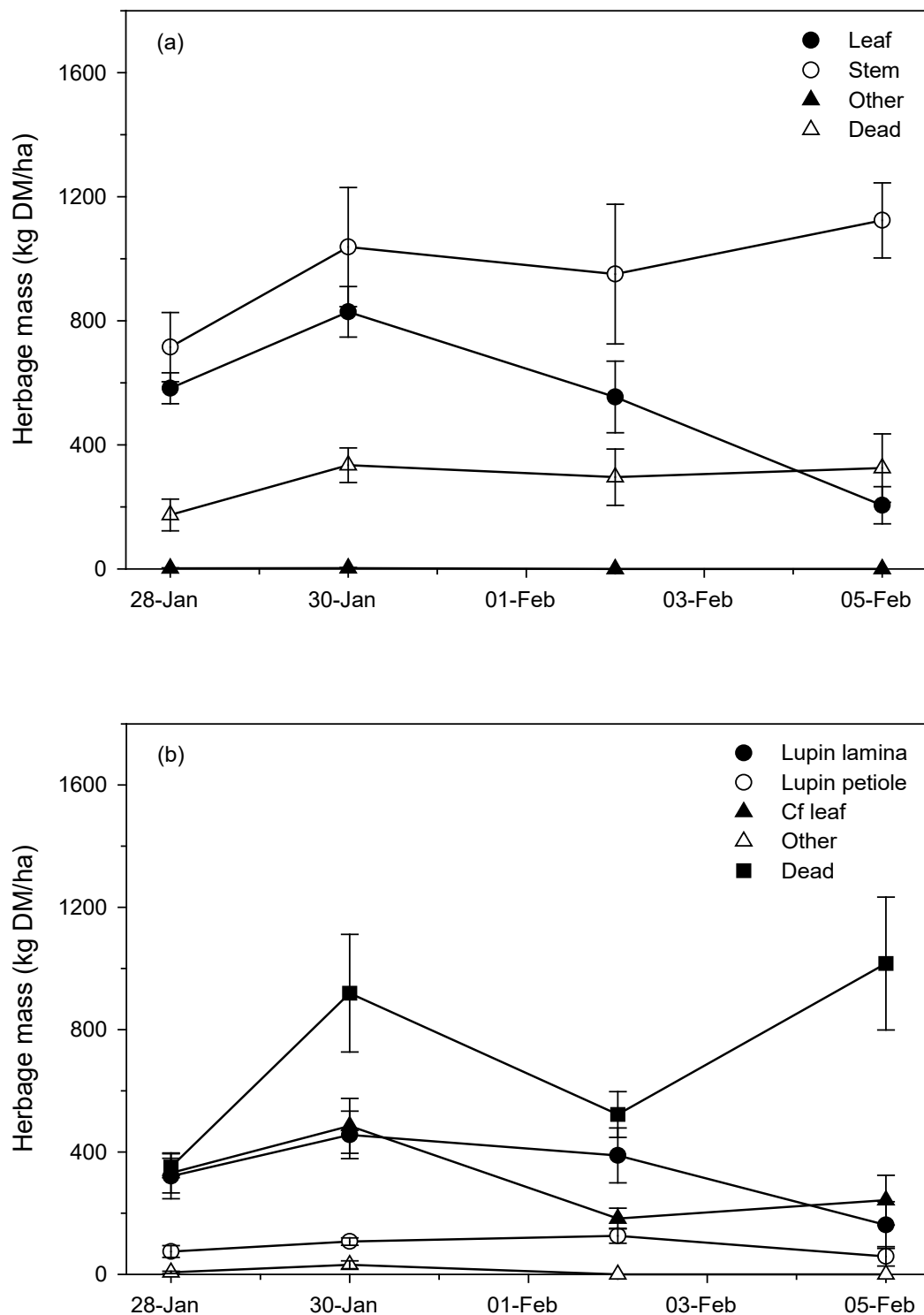
Figure 4.17 Disappearance of pasture components for sheep grazing lucerne (a) and cocksfoot-lupin (b) pastures between 28 January and 5 February 2015 at Lincoln University, Canterbury. Error bars represent standard error of the mean.
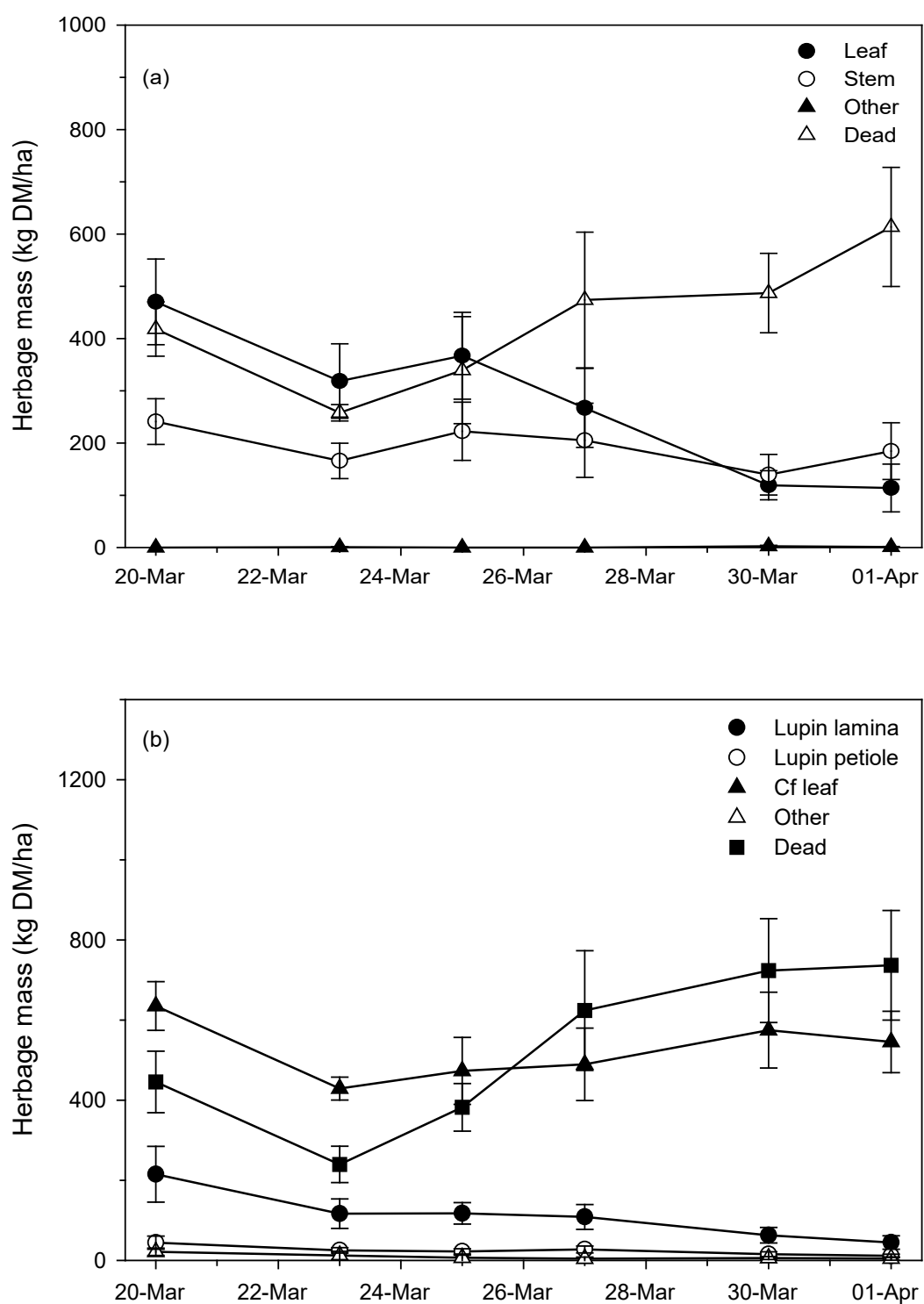
Figure 4.18 Disappearance of pasture components for sheep grazing lucerne (a) and cocksfoot-lupin (b) pastures between 20 March and 1 April 2015 at Lincoln University, Canterbury. Error bars represent standard error of the mean.
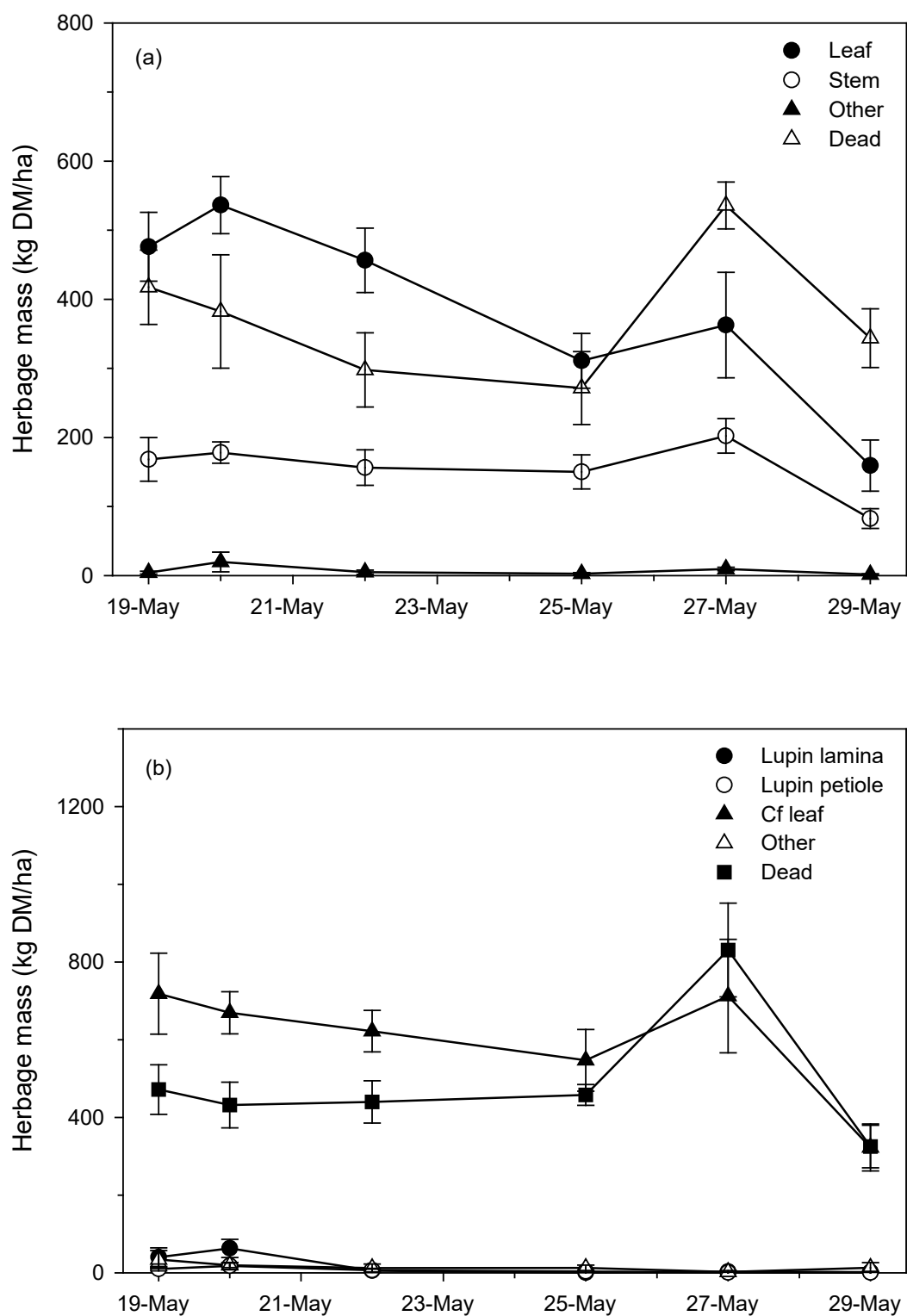
Figure 4.19 Disappearance of pasture components for sheep grazing lucerne (a) and cocksfoot-lupin (b) pastures between 19 and 29 May 2015 at Lincoln University, Canterbury. Error bars represent standard error of the mean.

4.3.9 Herbage, metabolisable energy and crude protein consumption

Combined botanical composition and DM yield data showed considerable differences between the amount and type of herbage that sheep were offered on cf-lupin and lucerne pastures (Table 4.3). During the first grazing period, 3636 kg DM/ha was offered to sheep grazing cf-lupin pastures which was not different (P=0.298) from the 3218 kg DM/ha offered to those on lucerne. However, sheep grazing cf-lupin pastures consumed 941 kg DM/ha of legume compared with 1580 kg DM/ha of legume on lucerne (P=0.035). Of the lupin components, lupin lamina contributed the greatest yield at 1237 kg DM/ha and also had a utilisation of 61% compared with 23% for petiole. Utilisation of sown pasture components was 54% in cf-lupin pastures, whilst 63% of the unsown species material was refused.

The earlier commencement of grazing for cf-lupin pastures, in grazing period two, did not increase (P=0.233) the total amount of herbage that was offered to sheep when compared with lucerne. Sheep showed a preference for lupin lamina during this period consuming 798 kg DM/ha, which led to a utilization of 82%. However, this was lower (P=0.003) than the 2897 kg DM/ha of legume leaf consumed by sheep on lucerne pastures, which resulted in a utilization of 94%. Sheep showed some preference for the unsown species in both pastures, but total consumption remained at <14% of the total diet.

Observed differences between the two pasture types were most apparent during the third grazing period. Sheep grazing cf-lupin pastures were offered 2159 kg DM/ha less (P=0.047) than the 4992 kg DM/ha offered to sheep on lucerne. This led to the consumption of 1669 kg DM/ha compared with 3212 kg DM for sheep on lucerne (P=0.029). Sheep grazing cf-lupin pastures showed a strong preference for the minor lupin flower component and continued to show preference for lupin lamina which had a utilization of 84%. Cocksfoot leaf was still the predominant yield component in cf-lupin pastures at 1465 kg DM/ha during GP3. Cocksfoot stem was strongly rejected by sheep and there was no apparent intake during GP3. Sheep showed a strong preference for lucerne leaf material, whilst the utilization of stem material had declined from 50% in GP2 to 33% in GP3.

During the final grazing period, cf-lupin and lucerne pastures produced 1455 and 1521 kg DM/ha, respectively (P=0.586). Despite similar amounts of DM being offered, sheep on cf-lupin pastures consumed 813 kg DM which was lower (P=0.027) than the 1195 kg DM consumed by sheep on lucerne. Utilisation remained high for lupin components, but the total contribution to the diet was 209 kg DM/ha which was lower than the 589 kg DM/ha of cocksfoot leaf that was consumed.

Table 4.3 Herbage offered (kg DM/ha) and consumed by sheep grazing cocksfoot-lupin and lucerne pastures during four consecutive grazing periods between 11 March 2014 and 29 May 2015 in paddock H12 at the Horticultural Research Area at Lincoln University, Canterbury. Grazing periods were: GP1 (11 March 2014 - 19 May 2014), GP2 (5 August 2014 – 28 November 2014), GP3 (29 November 2014 – 17 February 2015) and GP4 (18 February 2014 – 29 May 2015). Differences were considered significant when *P*≤0.05.

	GP 1		UTIL	GP 2		UTIL	GP 3		UTIL	GP 4		UTIL
	Offered	Consumed	(%)	Offered	Consumed	(%)	Offered	Consumed	(%)	Offered	Consumed	(%)
Lupin lamina	1237	749	*61*	977	798	*82*	495	432	*87*	198	143	*72*
Lupin petiole	436	99	*23*	336	181	*54*	111	79	*71*	49	36	*73*
Lupin stem	81	81	*100*	7	7	*100*	41	15	*37*	16	16	*100*
Lupin flower	12	12	*100*	5	5	*100*	31	29	*96*	14	14	*100*
Cf leaf	805	454	*56*	3163	1915	*61*	1465	1015	*69*	1130	589	*52*
Cf stem	0	0	-	178	-228	-	404	-88	-	0	0	-
Unsown	1066	395	*37*	508	380	*75*	287	186	*65*	48	14	*30*
Grand Total	**3636**	**1790**	***49***	**5174**	**3058**	***59***	**2833**	**1669**	***60***	**1455**	**813**	***56***
Lucerne leaf	1471	1141	*78*	3093	2897	*94*	2342	2107	*90*	982	838	*85*
Lucerne stem	920	437	*47*	2148	1068	*50*	2216	734	*33*	478	310	*65*
Lucerne flower	2	2	*100*	0	0	-	322	320	*99*	55	55	*99*
Unsown	825	364	*44*	709	607	*86*	112	51	*45*	6	-7	-
Grand Total	**3218**	**1944**	***60***	**5950**	**4571**	***77***	**4992**	**3212**	***64***	**1521**	**1195**	***76***
P legume leaf	0.146	0.188	-	0.002	0.003	-	<0.001	<0.001	-	0.006	0.011	-
P total legume	0.078	0.035	-	0.003	0.002	-	0.003	0.002	-	0.009	0.005	-
P grand total	0.298	0.714	-	0.233	0.055	-	0.047	0.029	-	0.586	0.027	-

Energy intake (GJME) was consistent for sheep grazing cf-lupin and lucerne pastures during the first two grazing periods (GP1, *P*=0.966; GP2, *P*=0.248) (Table 4.4). At the conclusion of GP3, sheep grazing cf-lupin pastures had consumed 23.3 GJME/ha compared with 38.6 GJME/ha for lucerne (*P*=0.051). Protein consumption remained lower (*P*=0.009-0.024) for cf-lupin pastures throughout GP2, GP3 and GP4 when compared with lucerne. During GP3, sheep grazing cf-lupin pastures consumed 289 kg CP/ha which was less than half (*P*=0.009) the 664 kg CP/ha for sheep on lucerne.

Table 4.4 **Metabolisable energy and crude protein consumption of sheep grazing cf-lupin and lucerne pastures at four consecutive grazing periods between 11 March 2014 and 29 May 2015 in paddock H12 at Lincoln University, Canterbury. Grazing periods were GP1 (11 March 2014 – 19 May 2014), GP2 (5 August 2014 – 28 November 2014), GP3 (29 November 2014 – 17 February 2015) and GP4 (18 February 2015 – 29 May 2015).**

		GP1	GP2	GP3	GP4
Metabolisable energy (GJME/ha)	Cf-lupin	23.1	48.8	23.3	12.7
	Lucerne	22.9	57	38.6	14.7
	P	0.966	0.248	0.051	0.061
Protein (kg/ha)	Cf-lupin	346	630	289	141
	Lucerne	418	1032	664	251
	P	0.403	0.024	0.009	0.018

Linear regressions were calculated for LWY against DM intake (DMI), ME intake (MEI) and CP intake (CPI) for both pasture types. The relationship between liveweight yield (LWY) and feed intake between 5 August 2014 and 29 May 2015 is given in Figure 4.20. Sheep grazing cf-lupin pastures converted DMI to LWY at a rate of 132 g LWY/kg DM (R^2=0.91) which was not different (*P*=0.627) from the 126 g LWY/kg DM (R^2=0.98) for those on lucerne. Sheep grazing lucerne consumed an extra 3430 kg DM/ha which gave the increased LWY of 1125 kg LW/ha above the 755 kg LW/ha for cf-lupin pastures. The pattern of MEI versus LWY was not different (*P*=0.274) for both pasture types, with conversion efficiency values of 8.7 kg LWY/GJME/ha (R^2=0.92) for cf-lupin pastures and 10.3 kg LWY/GJME/ha (R^2=0.98) for lucerne (Figure 4.21). Liveweight yield showed a strong linear response to CP intake for cf-lupin pastures (R^2=0.94) with a conversion efficiency of 0.69 kg LWY/kg PrI compared with 0.59 kg LWY/PrI (R^2=0.98) for lucerne (*P*=0.201).
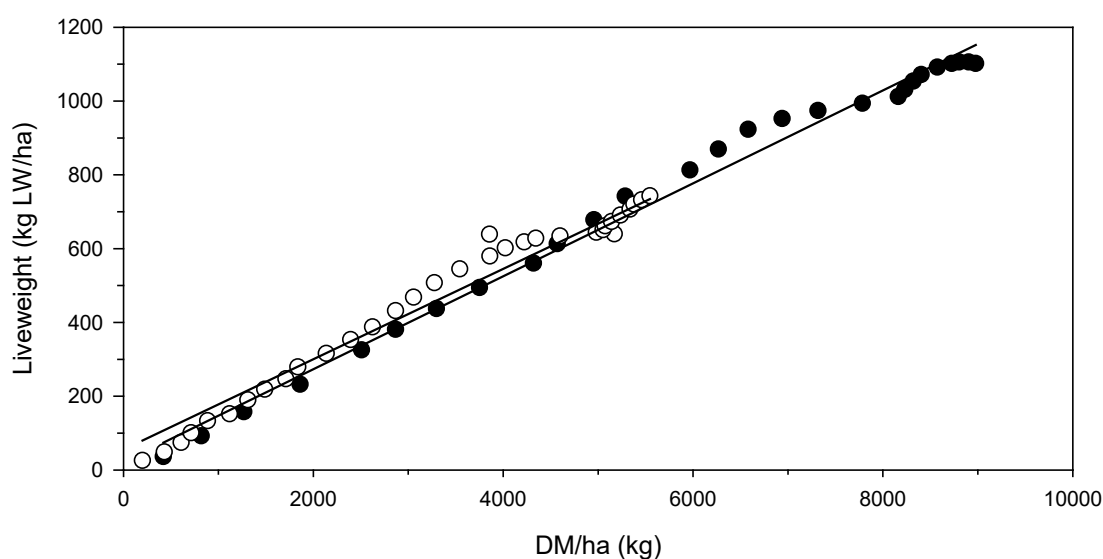
Figure 4.20 **Liveweight yield (kg LW/ha) plotted against apparent herbage intake (kg DM/ha) for sheep grazing cocksfoot-lupin (○) and lucerne (●) pastures between 5 August 2014 and 29 May 2015 in paddock H12 at Lincoln University, Canterbury.**

Note: Linear regressions; Cf-lupin: y = 0.132x + 24 (R^2=0.985), lucerne: y = 0.126x + 18.9 (R^2=0.99)
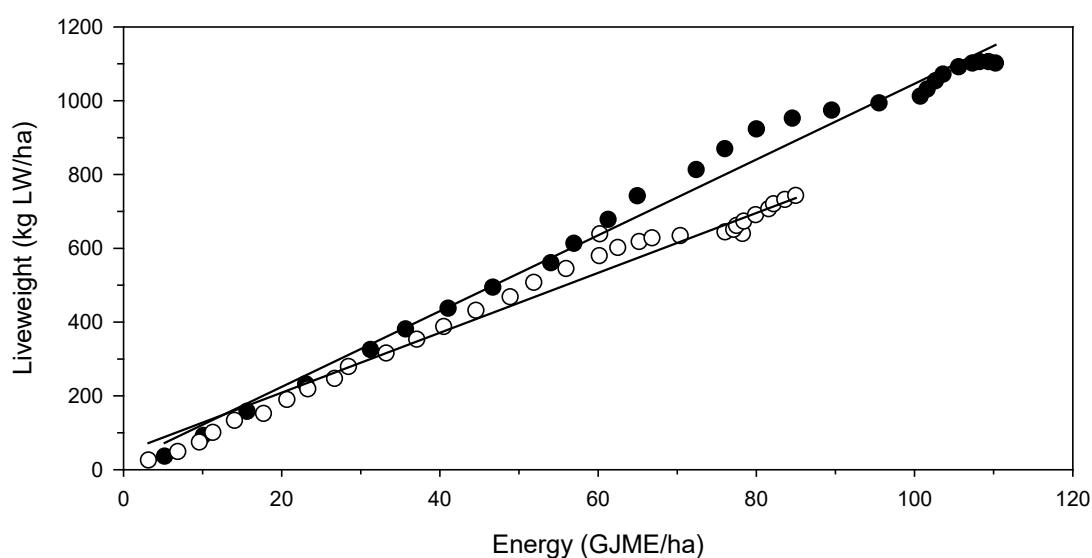
Figure 4.21 **Liveweight yield (kg LW/ha) plotted against energy intake (GJME/ha) for sheep grazing cocksfoot-lupin (○) and lucerne (●) pastures between 5 August 2014 and 29 May 2015 in paddock H12 at Lincoln University, Canterbury.**

Note: Linear regressions; Cf-lupin: y = 8.71x + 17.1 (R^2=0.989), lucerne: y = 10.31x + 16.7 (R^2=0.989)

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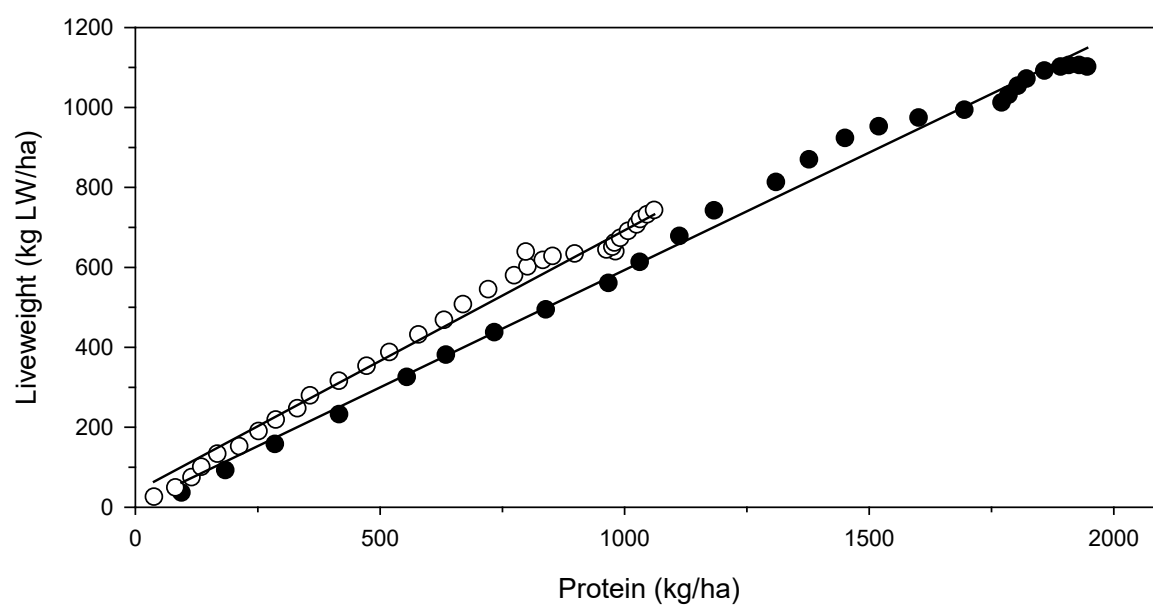
Figure 4.22 Liveweight yield plotted against crude protein intake for sheep grazing cocksfoot-lupin (○) and lucerne (●) pastures between 5 August 2014 and 29 May 2015 in paddock H12 at Lincoln University, Canterbury.

Note: Linear regressions; Cf-lupin: y = 0.69x + 19.1 (R²=0.992), lucerne: y = 0.59x + 5.1 (R²=0.993)

4.3.10 N content and consumption

Nitrogen content was derived from the CP content of pasture components and is presented in Table 4.5. Lupin lamina had the highest N concentration in cf-lupin pastures with a range of 2.9-4.5% and a mean value of 3.9%. Lupin petiole and stem had lower N content than all other components with mean values of 1.8 and 2%, respectively. Cocksfoot leaf contained less N than lupin leaf with a mean of 2.6%. Lucerne leaf maintained a consistently high N content of 3.8% and was less variable than the stem proportion which had a mean of 2.2%.

Table 4.5 **Nitrogen concentration (%N) of yield components of cocksfoot (cf)-lupin and lucerne pastures between 11 March 2014 and 29 May 2015 in paddock H12 at the Horticultural Research Area at Lincoln University, Canterbury. Values in parentheses represent standard error of the mean.**

Date	Cf-lupin						
	Lupin lamina	Lupin petiole	Lupin stem	Lupin flower	Cocksfoot leaf	Cocksfoot stem	Unsown
11/03/14	3.7 (0.19)	1.4 (0.15)			2.7 (0.26)		
14/04/14	4.1 (0.15)	2.0 (0.11)			2.5 (0.08)		
13/08/14	3.9 (0.24)	3.0 (-)			2.9 (0.07)		2.5 (0.26)
22/09/14	4.3 (0.05)	2.1 (0.09)			3.2 (0.16)		3.2 (-)
28/10/14	4.5 (0.18)	2.0 (0.01)			2.4 (0.21)		2.7 (-)
8/12/14	4.1 (0.17)	1.1 (0.57)	1.7 (-)	3.8 (-)	2.3 (0.05)	1.3 (0.13)	
28/01/15	2.9 (0.09)	2.0 (0.20)	2.3 (0.2)	4.0 (0.08)	2.4 (0.09)	1.2 (-)	2.7 (-)
20/03/15	3.3 (0.18)	1.4 (-)			2.7 (0.07)		
18/05/15	3.4 (0.82)	0.8 (-)			2.7 (0.14)		

Date	Lucerne			
	Leaf	Flower	Stem	Weed
11/03/14	3.8 (0.15)		1.6 (0.12)	
14/04/14	4.5 (0.03)		2.6 (0.17)	
19/09/14	4 (0.05)		2.7 (0.07)	2.5 (0.04)
28/10/14	4.3 (0.03)		2.4 (0.07)	3.4 (0.04)
8/12/14	2.8 (1.4)		1.8 (0.15)	
28/01/15	3.7 (0.3)	3.1 (0.15)	1.4 (0.10)	
20/03/15	3.8 (0.05)		2.2 (0.20)	
18/05/15	3.4 (1.5)		3.9 (-)	4.2 (0.87)

The combination of herbage intake and N content meant that animals on lucerne consumed the same amount of N during the establishment phase (P=0.340) but an additional (P=0.019) 130 kg N/ha during Year 1 (Figure 4.23). During GP1, the amounts of N consumed at each measurement date were not different (P=0.07-0.938). During spring grazing (GP2), the rate of N consumption was not different (P>0.05) for both pasture types for 50% of the measurement dates. However, the N consumption of sheep on lucerne exceeded (P≤0.05) those on cf-lupin pastures on all other dates during spring.

Differences in N consumption between the two pasture types was not as evident during the summer period (GP3), where the N consumption of sheep on lucerne was higher (*P*=0.035) on one occasion. Total N consumption of pastures slowed during GP4. During this period, animals consumed more (*P*≤0.05) N on lucerne pastures on 33% of the measurement dates.
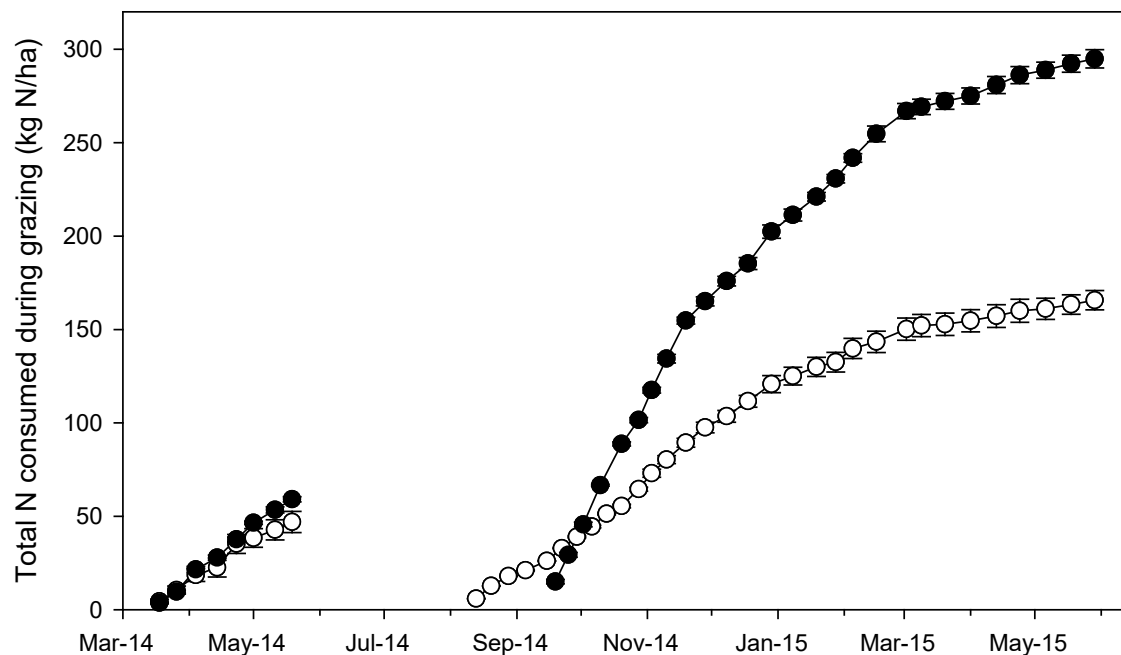
Figure 4.23 **Total N consumed (kg N/ha) by sheep grazing cocksfoot (cf)-lupin (○) and lucerne (●) pastures between 11 March 2014 and 29 May 2015 in paddock H12 at Lincoln University, Canterbury. Error bars represent standard errors of the mean.**

4.4   Discussion

The null hypothesis was accepted, that sheep liveweight gain per unit of DM consumed was not different between pasture types (Figure 4.20) but the amount of pasture grown (Figure 4.6) and consumed was lower for cf-lupin pastures. This discussion highlights when and why these differences occurred. The results of each grazing period are discussed in chronological order. Lucerne is recognized as the highest quality feed we have available for dryland pasture production. However, in regions where lucerne cannot be grown, such as those with acidic soils with high levels of exchangeable Al, cocksfoot and lupin pastures can provide a significant improvement over resident vegetation. Therefore, the aim of this discussion is to benchmark the performance of sheep grazing cf-lupin against those on lucerne.

4.4.1   Measurement period one – 11 March 2014 to 30 June 2014

Total liveweight gain for Merino ewe lambs grazing cf-lupin pastures was 55 kg/ha compared with 107 kg/ha for lucerne during the first autumn grazing period (Figure 4.1). This result was surprising because the lucerne had a lower carrying capacity over this period. Thus, the difference was caused by greater animal liveweight gain of lambs on lucerne (Figure 4.4). The cf-lupin pastures accumulated 18% more herbage than lucerne, so differences in animal performance are attributed to a higher utilization and consumption of about 600 kg DM/ha more legume in the lucerne (Table 4.3). This would be expected given the tendency of livestock to select legumes over grass components (Cosgrove *et al.*, 1999; Freer and Jones, 1984). The lower herbage productivity of lucerne during this establishment period was also expected due to its priority of partitioning DM to roots and crowns during its establishment phase (Morot-Gaudry *et al.*, 1987; Teixeira *et al.*, 2008, 2009).

The lower per head performance of animals on cf-lupin pastures was unexpected given the greater herbage yield accumulation (Figure 4.6). Both pastures maintained similar stocking rates during this period, which suggests that herbage allowance was higher for cf-lupin pastures. The implication is that the lower per head performance of these animals was due to a lower abundance of legume on offer and associated lower herbage quality of the grass (Table 4.2). Legume abundance in cf-lupin pastures represented up to 65% of the herbage on offer during the establishment period (Figure 4.9). However, slow regrowth and the increasing dominance of cocksfoot meant that the mean legume proportion was 38% compared with 74% for lucerne. This is the most likely explanation for differences in liveweight performance.

4.4.2 Measurement period two – 1 July 2014 to 30 Jun 2015

In Year 2, the cf-lupin pastures produced 755 kg of live weight/ha compared with 1125 kg/ha for lucerne (Figure 4.1). For both pasture types, over 80% of total liveweight gain was produced in spring. Both pasture types supported a feed conversion efficiency of 0.131 kg of liveweight gain/kg of DM consumed (Figure 4.20). However, cf-lupin pastures produced less total live weight due to animals consuming less (3430 kg DM/ha). The total annual sheep liveweight productivity in this experiment was comparable to that reported by Mills *et al.* (2015a) at a similar location, where annual sheep liveweight gain was 1156 kg/ha for dryland lucerne compared with 443-903 kg/ha for dryland cocksfoot-dominant pastures.

Seasonal patterns of pasture production led to differences in the management of each pasture type during this period. For cf-lupin, earlier spring growth meant they were initially stocked 41 days before the lucerne plots. When grazing commenced, cf-lupin had a pasture mass of 1900 kg DM/ha (Figure 4.8) and was stocked at 17 sheep/ha (Figure 4.3). The spring flush of pasture production meant that the stocking rate had to be continually increased until mid-November. Despite the increased stocking rate, low utilisation suggests that these pastures were understocked for some of this period. For example, cocksfoot leaf represented more than 60% of yield during this period but had 61% utilisation. Experimentally, the cf-lupin pastures were managed in unison with the lucerne. However, this may not have maximised pasture utilisation and animal performance during the spring period. Had the stocking rate been higher, to consume 70% of the cocksfoot leaf, cf-lupin pastures would have produced an additional 40 kg LW/ha. This suggests that in a commercial situation, cf-lupin pastures could have produced up to 80% of the yield potential of lucerne.

During spring, the quality of both pastures was comparable (Figure 4.13). Grab samples from both pastures had an ME of approximately 10.8 MJ/kg DM and a DMD of 70%, which explains the similarities in sheep growth rates at this time. This suggests the lower CP of the cf-lupin pastures did not impact liveweight gain. The grab samples represented the herbage on offer as the sheep entered each paddock. However, disappearance measurements taken once in each grazing cycle gave a more realistic indication of the components that were being consumed. Figure 4.14 shows that lamina of all species was consumed in preference to stem material during the first grazing cycle of spring. This equated to the consumption of 850 kg DM/ha for cf-lupin and 1750 kg DM/ha for lucerne pastures. Sheep showed a similar preference during the second grazing cycle. However, sheep consumed 650 kg DM/ha of the 800 kg DM/ha of lupin lamina on offer, despite their being an abundance of cocksfoot leaf.

Between shearing and mid-February, cf-lupin pastures produced 2.4 kg of liveweight gain/ha/day which resulted in a total liveweight gain of 171 kg/ha. This was 45% of the liveweight yield of lucerne over the same period despite both pastures having a utilisation of about 60%. Therefore, the difference in annual sheep productivity was largely the result of the difference in the amount and quality of feed. During this period, cf-lupin pastures offered 2070 kg DM/ha of palatable feed compared with about 2700 kg DM/ha for lucerne. This reduced the carrying capacity of cf-lupin pastures by 24%. It is also likely that the quality of feed consumed was lower on the cf-lupin pastures. Over this period, sheep consumed the lupin lamina and cocksfoot leaf, which had mean ME values of 12.3 and 11.3 MJ/kg DM, respectively. For the lucerne, it appears that sheep selected the palatable leaf and top third of the stem which had an ME of about 12.0 MJ/kg DM (Brown and Moot, 2004).

The increasing abundance of less palatable components caused sheep to become highly selective in their diet. For both pasture types, the utilisation of leaf material remained between 75-90% due to its high grazing preference, whilst stem and dead material were mostly refused (Figure 4.16 and Figure 4.17). Because stocking rate was determined by the total amount of feed on-offer, the reduction in palatable material led to reduced liveweight gain (Figure 4.4). However, the consumption of an additional 600 kg DM/ha of leaf material on lucerne was responsible for the additional liveweight production during this period. This agrees with Brown (2004) where herbage intake remained the largest determinant of liveweight production despite vast differences occurring in the abundance of yield components across several different pastures.

As expected, liveweight gain of mixed age lambs was limited by pasture availability during autumn. Both pastures grew at 6-8 kg DM/ha/day, which reduced sheep carrying capacity to about 11 lambs/ha (Figure 4.3). This combination meant that autumn liveweight gain was about 15% of annual sheep liveweight production. This highlights the importance of maximising DM production during spring in dryland systems.

The abundance of legume on offer directly impacted the amount of nitrogen that was consumed and potentially mobilised. Sheep on cf-lupin pastures consumed 160 kg N/ha which was about half of that consumed by those on lucerne (Figure 4.23). The lupin leaf had a mean N concentration of 3.7%, which was 90% of the leaf N content maintained by lucerne over the season. However, lupin leaf represented 30% of the diet of animals on cf-lupin compared with a diet that was 65% leaf material on lucerne. This would have reduced total CP available for animal growth and also the total amount of N mobilised for cocksfoot growth.

4.4.3 On-farm implications

The locality, site fertility and management of this experiment, due to the need to conduct it at Lincoln University, were more conducive to the productivity of lucerne. This maintains data integrity but can reduce its relevance to farmers. Therefore, this section highlights how the individual and combined effects of these results might be used to inform on-farm productivity in a high-country environment.

The initial contribution of lupin to herbage yield suggests that the sowing rate and establishment techniques were adequate for lupin survival. Lupins contributed about 50% of herbage yield during the first grazing rotation of cf-lupin pastures. However, this steadily declined to represent <10% of yield at the conclusion of grazing on 29 May 2015. The sowing rate of 30 kg/ha in this experiment was several times higher than the 3-8 kg/ha that was found to be ample for the establishment of successful lupin stands in other experiments (Black *et al.*, 2014b; Moot and Pollock, 2014; Scott, 2008). Additionally, Kitessa (1992) found that perennial lupin had a yield potential of 12 t DM/ha at a similar site near Lincoln to the current experiment. However, the considerable abundance of cocksfoot in the seed mix and frequent grazing of plots in the current experiment were probably detrimental to the survival of *L. polyphyllus*.

The strong competition from cocksfoot was likely caused by its high sowing rate. In comparison, Moot and Pollock (2014) established a high-country cf-lupin pasture that maintained lupin abundance of more than 80% of total herbage yield over two years with a sowing rate of 2 kg/ha cocksfoot. Therefore, to maximise the contribution of perennial lupin to pasture growth, the sowing rate of companion species should be conservative and grazing should be carefully managed to prevent over-grazing. This is of particular importance for *L. polyphyllus*, which is sensitive to heavy grazing (Scott, 2008) and inter-species competition (Scott, 2012) during establishment. These combined results suggest that in an on-farm production, sowings should generally contain 10 kg/ha of lupin, 2-3 kg/ha of cocksfoot and 2-3 kg/ha of another legume.

The time of grazing commencement in spring did not affect annual productivity in any paddock for either pasture type (Figure 4.7). However, there was considerable variability in productivity between each of the paddocks. Cf-lupin pastures ranged between 4500 and 7720 kg DM/ha/year compared with lucerne, which produced between 7800 and 13,620 kg DM/ha/year. This agrees with Moot *et al.* (2016) who found that, despite yield variability across paddocks, there was no link between annual yield and time of first grazing for lucerne. Therefore, productivity differences were most likely driven by variation in soil moisture availability (Sim *et al.*, 2012) and this will be explored in the next chapter.

The implication is that productivity will be maximised in areas that are most suitable for each legume species.

The amount of palatable feed on offer affected the quantity of N consumed by the sheep on both pasture types. Palatable components retained their quality throughout the season. Therefore, differences in N consumption were primarily driven by yield as can be seen by the similarity of curves between total yield and N accumulation. Thus, the importance of maximising palatable components is evident. Sheep showed a preference for legumes which is consistent with previous work (Cosgrove *et al.*, 1999; Parsons *et al.*, 1994). However, Cosgrove *et al.* (2002) found that sheep showed a several-fold improvement in preference for grass when its N concentration was increased from 3.2% to 4.5%. Therefore, improved N nutrition of grass species should be a priority for dryland pastures, as this will improve their palatability. However, improvements in palatability must also be balanced against the associated increase in competitiveness, and its resultant effect on legume persistence within mixed pastures.

Increased N availability to cocksfoot, either as fertiliser or through N fixation, would have reduced the difference between cf-lupin and lucerne pastures. Cocksfoot had a mean herbage N concentration of 2.7% across the season (Table 4.2). This suggests that it was photosynthesising at approximately 70% of its maximum (Peri *et al.*, 2002). Mills *et al.* (2009) found that the radiation use efficiency of cocksfoot could be doubled when N fertiliser was applied to dryland swards. This effectively doubled annual pasture growth with the majority of yield being attained during spring and autumn growing periods (Mills *et al.*, 2006). Under high-country conditions, Fasi *et al.* (2008) found that the growth rate of cocksfoot was tripled with a spring application of 150 kg N/ha. This highlights the ability of cocksfoot to tolerate moderate fertility whilst also being highly responsive to N inputs (Smith *et al.*, 1998). Thus, grazing management to maximise the lupin content is also likely to improve total yield and utilisation.

Increased presence of other legumes in high-country pastures could also lead to greater herbage productivity. Annual clovers have been shown to offer a considerable contribution to annual DM production of grass-dominant pastures. Ates *et al.* (2010) showed the addition of an annual clover increased annual yield of cocksfoot-based pastures by 62%. Alternatively, the addition of Caucasian clover at establishment may be beneficial in a high-country environment due to its tolerance of drought (Black and Lucas, 2000) and ability to survive under low fertility conditions (Allan and Keoghan, 1994; Black *et al.*, 2014a), particularly in soils with high Al .

4.5   Summary and conclusions

- Cf-lupin pastures produced 70% of the annual sheep liveweight yield that lucerne produced under dryland conditions. This demonstrated that lucerne was a more suitable pasture for maximising sheep performance when soil fertility and climate conditions are favourable.

- Both pastures produced 80% of their total liveweight during spring.

- Lupins represented <20% of annual yield in cocksfoot-lupin pastures during 2014/15. This implied that they have limited adaptation to intensive rotational grazing, particularly in early spring, and were vulnerable to competition from cocksfoot

- Sheep grazing the pastures converted feed to live weight at the same efficiency. Therefore, differences in liveweight yield were driven by the amount of DM consumed on each pasture type.

- The growth rate of cf-lupin pastures was 21 kg DM/ha/day over summer, which was 50% of that achieved by lucerne.

- The most palatable components (higher ME) were consumed first. In lucerne crops, leaf material was highly preferred and utilised which negated the increasing abundance of stem during summer. In cf-lupin pastures, only moderate preference was shown for cocksfoot leaf which limited liveweight productivity.

Lucerne produced 3780 kg DM/ha more than a grass-dominant pasture in this experiment, but there were differences amongst individual plot yields not attributable to differences in the time of initial grazing. Under dryland conditions, greater productivity will be achieved through improved water use efficiency, access to a larger volume of water or a combination of both. These factors would have influenced the results of the current chapter and will now be examined in Chapter 5.

5 Analysis of seasonal dry matter production and water extraction of dryland lupin-cocksfoot and lucerne pastures

5.1 Introduction

In Chapter 4, cf-lupin pastures produced 6440 kg DM/ha/year compared with 10,220 kg DM/ha/year for lucerne. Lucerne pastures grew more rapidly in the spring and produced herbage at twice the rate of cf-lupin over the summer period. These differences are potentially due to higher water use efficiency (WUE) or greater extraction of soil moisture by lucerne (Brown, 2004; Dardanelli *et al.*, 1997; Mills *et al.*, 2006).

In pastoral situations, water demand is driven by evapotranspiration, so is usually consistent among species and is independent of yield (Black and Murdoch, 2013; Brown *et al.*, 2003). Therefore, under limited water supply in dryland situations, it is important to convert available moisture to biomass as efficiently as possible. A consistent thread throughout several studies is the notable improvement in WUE as N nutrition of the pasture improves either through increased use of legumes (Moot *et al.*, 2008) or application of N fertiliser (Black and Murdoch, 2013; Mills *et al.*, 2009).

The point at which moisture becomes limiting will 'break' the linearity of non-moisture limited growth (Mills *et al.*, 2015b). This is the relationship between the volume of soil occupied by the root system and the amount of plant available moisture in the profile. Growth generally becomes limited once plant available water reaches 50% or less (Mills *et al.*, 2006). Therefore, when grown on the same soil, pasture species that can exploit a greater volume of soil will generally exhibit a longer period of linear growth before moisture becomes limiting.

The aim of Chapter 5 was to explain the differences in yield between cf-lupin and lucerne pastures reported in Chapter 4. The first objective was to quantify the linear growth rate of cf-lupin and lucerne pastures. This defines the period of non-limited pasture growth and also indicates the onset of moisture stress. The second objective was to determine the water use efficiency of both pasture types between August 2014 and June 2015. Following this, the third objective was to determine the pattern and seasonality of water extraction for both pasture types using the 'Monteith framework' (Monteith and Greenwood, 1986). This framework has been previously validated for perennial crops such as lucerne (Brown *et al.*, 2003; Sim *et al.*, 2017) and cocksfoot (Mills, 2007). However, its application has not been tested in mixed-pastures where variability in root dynamics and seasonality of growth may affect its application.

5.2 Materials and methods

5.2.1 Installation of neutron probe access tubes

To measure soil moisture, a steel spike ($\acute{\emptyset}$ = 50 mm) was forced into the soil in the centre of each plot in Paddock 2 in July 2014 (Figure 4.1). Following this, 47 mm ($\emptyset$) aluminium neutron probe access tubes were installed to a depth of 2.3 m. A set of stainless steel 4 mm rods were also installed to allow for soil moisture measurements, via Time Domain Reflectometry (TDR), to a depth of 0.2 m. These were located within 20 cm of the neutron probe access tubes.

5.2.2 Measurements

Volumetric soil water content (Θ, mm^3/mm^3) was measured to a depth of 2.3 m in 22 increments at 8-20 day intervals. Measurements were taken on the same day that sheep were shifted and every two weeks during winter when plots were destocked. The top layer (0-0.2 m) was measured with TDR (Trase, Soil Moisture Equipment, Santa Barbara, California, USA), which gives an average value over the 0.2 m depth. The neutron probe (Troxler Electronic Industries Inc, Research Triangle Park, North Carolina, USA) was used to measure each soil layer between 0.2 and 2.3 m in 0.1 m increments, with measurements taken at the mid-point of each depth.

The neutron probe was calibrated for water content, measured gravimetrically, of a Templeton silt loam at Lincoln University (range = 0.07-0.37 mm^3/mm^3, R^2=0.99).

5.2.3 Calculations

Total soil water content (SWC) of the profile at each measurement date was determined using Equation 5.1.

Equation 5.1 $\qquad$ **SWC = $\sum_{bot}^{top} \Theta * d$**

Where d is the depth of the layer, **top** is the 0-0.2 layer, **bot** is the 2.2-2.3 layer, and Θ is the volumetric water content. Soil water content at each measurement date could then be used to calculate total water use (WU) (Equation 5.2).

Equation 5.2 $\qquad$ **WU = P_R – (SWC_E – SWC_S)**

Where P_R is the sum of rainfall for the period and SWC_S and SWC_E are the actual soil water content at the start and end of the period, respectively. The values for WU were accumulated and used to

determine soil water deficit in Equation 5.3. The equation assumes that there is no drainage or surface runoff during the period. This was acceptable for this experiment, where rainfall did not exceed the soil moisture deficit at any point during the growing season.

Equation 5.3 $SWD = SWD_i + WU_{daily} - R$

Where SWD_i is the previous days soil water deficit, WU_{daily} is the daily water use and R is rainfall during that period.

5.2.4 Analysis of water extraction patterns

All analyses were conducted with Genstat 16 Ed. (VSN International, 2014).

5.2.4.1 *Plant available water capacity (PAWC)*

PAWC was determined for each individual soil layer in every plot as the difference between the drained upper limit (D_{UL}) and lower limit (LL). The D_{UL} of volumetric water content was measured in August 2014 after the access tubes had been installed. The soil had received 450 mm of moisture in the three months leading up to the measurements and was considered to be fully recharged. The LL was based on the lowest measured volumetric water content in the summer, which is calculated when there was no change between consecutive readings.

5.2.4.2 *Model for plant water extraction*

The 'Monteith framework' was used to describe the pattern of water extraction for each soil layer in each plot. Brown *et al.* (2009) validated this model for dryland lucerne whilst Mills (2007) validated the model for cocksfoot monocultures. Both studies used deep silt loam soils at Lincoln University.

The model uses a broken-stick analysis to describe changes in soil water content over time (SWC_t) (Equation 5.4). For any given layer, the initial linear phase represents a constant SWC prior to the start of extraction. In most cases, this is assumed to be the upper limit (UL) of the layer. Once extraction begins, the model switches (t_c) to an exponential decline in SWC_t, which continues until the lower limit (LL) is reached. The rate of extraction (curvature of the exponential function) is described by the extraction decay constant (-kl). The model is described in Figure 5.1.

Equation 5.4 $SWC_t = LL + PAWC \exp(-kl(t-t_c)S_c)$ $S_c = 0$ if $t \le t_c$
                                                                                                        $S_c = 1$ if $t > t_c$
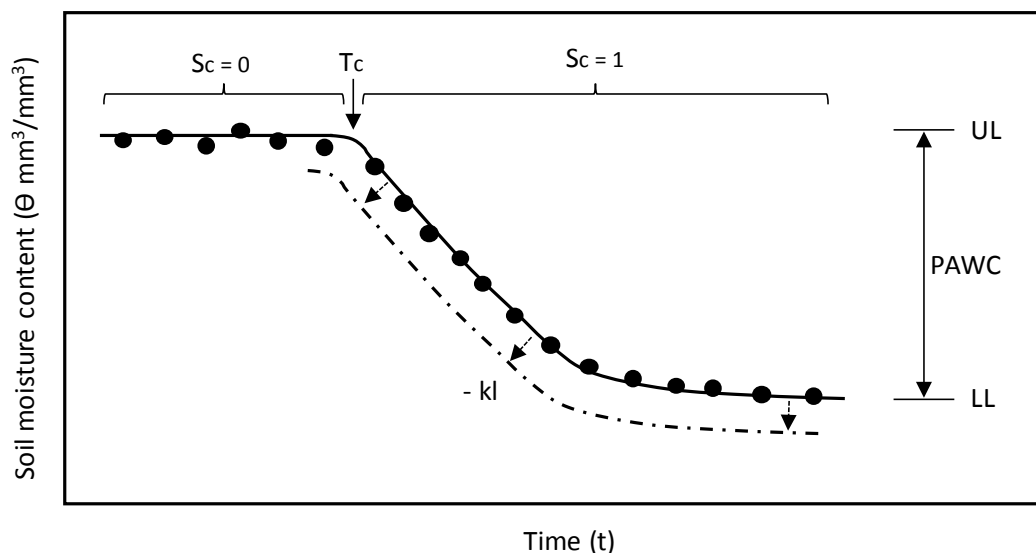
Figure 5.1 Parameters that are used in the 'Monteith framework' model for soil moisture extraction. UL is the upper limit, LL is the lower limit, PAWC is the plant-available water capacity, Sc = 0 is the time (days) prior to extraction, Sc = 1 is the phase of extraction and -kl is the extraction decay constant. Trigger points (T_c;↓) are combined for all soil layers to give the extraction front velocity (EFV). Adapted from Monteith and Greenwood (1986).

5.2.4.3 Extraction front velocity

During crop growth, most notably under dryland conditions, it is expected that water extraction will begin in the uppermost layers and will progressively move down the profile as the soil dries (Monteith 1986). To describe this downward movement, the t_c for each soil layer (0.2-2.3 m) was plotted against depth and a linear regression applied. The slope of the linear regression quantifies the extraction front velocity (EFV; mm/day).

5.2.4.4 Water use efficiency

For both pasture types, shoot DM yield was plotted against crop water use to calculate water use efficiency (WUE; kg DM/ha/mm of water extracted). A split-line regression was then used to determine the relationship between water use and shoot yield.

5.2.4.5 Statistical analysis

A one-way analysis of variance (ANOVA) was used to compare maximum SWD, PAWC and –kl of cf-lupin and lucerne pastures. Grouped linear functions were used to compare the slope of WUE and extraction front velocity regressions, whilst a non-linear function was used for SWC_t (Equation 5.4). Non-linear models for SWC_t were considered accurate when $R^2 \geq 0.85$.

5.3   Results

5.3.1   Dry matter yield response to thermal time

Figure 5.2 shows that cumulative herbage yield (kg DM/ha) regressed against cumulative thermal time (^oCd, T_b=0^oC) was initially linear. The split-line regression gave a robust description for cf-lupin (R^2 = 97.8-99.0) and lucerne pastures (R^2 = 0.98.9-99.5). During the initial linear growth phase, lucerne accumulated 3.8 kg DM/ha/^oCd which was greater (P=0.012) than 2.9 kg DM/ha/^oCd for cf-lupin pastures. Cf-lupin pastures had accumulated 6612 kg DM/ha compared with 8122 kg DM/ha for lucerne (P=0.029) when their respective break points occurred at 2383^oCd and 2366^oCd (P=0.338). For cf-lupin pastures, this meant that the period of linear growth ended on 24 January 2015 compared with 22 January 2015 for lucerne. Following the break, lucerne accumulated 0.41 kg DM/^oCd compared with 0.19 kg DM/^oCd for cf-lupin pastures (P=0.682).
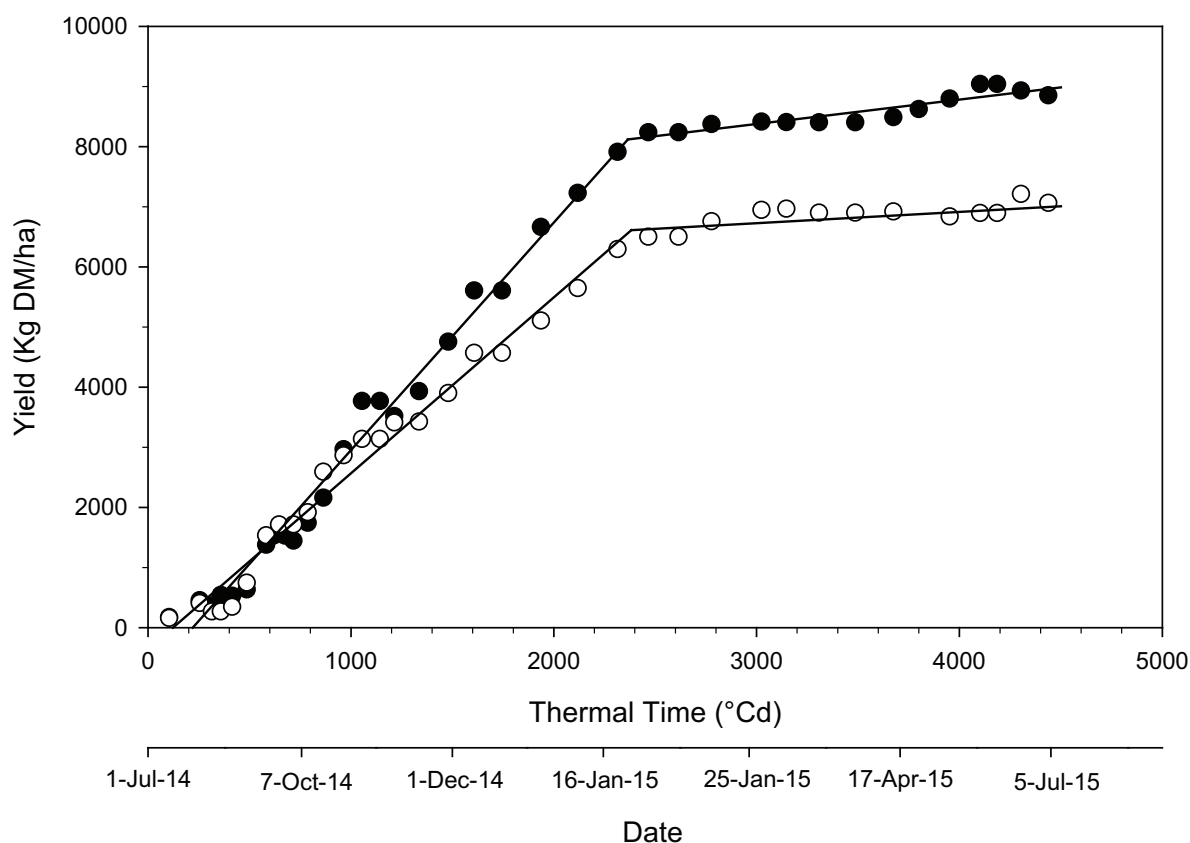
Figure 5.2 Cumulative herbage yield (kg DM/ha) regressed against thermal time (^oCd, T_b=0^oC) for cocksfoot-lupin (○) and lucerne (●) pastures grown between 20 August 2014 and 29 May 2015 at Lincoln University, Canterbury.

Note: Linear regressions (–); Lucerne, Phase 1 - y = 3.79×, Phase 2 - y=0.406×, R^2 = 0.94. Lupin-cf, Phase 1 – y = 2.93×, Phase 2 – y = 0.188×, R^2 = 0.83.

5.3.2 Soil water deficit, water use and dry matter yield

5.3.2.1 Soil water deficit

The SWD increased steadily between 20 August and 29 December 2014 and was approximately 190 mm for cf-lupin and lucerne pastures at that point (Figure 5.3). However, the rate of soil moisture extraction for cf-lupin pastures slowed ($P<0.001$), compared with lucerne, from 30 December 2014 onwards. Non-limited growth ended on 24 and 22 January 2015 for cf-lupin and lucerne, respectively (Figure 5.2). At this point, the SWD of cf-lupin and lucerne pastures was 219 mm and 280 mm, respectively. The SWD of lucerne increased to a maximum of 330 mm on 8 April 2015, which was 57 mm greater ($P<0.05$) than the maximum SWD of cf-lupin pastures.
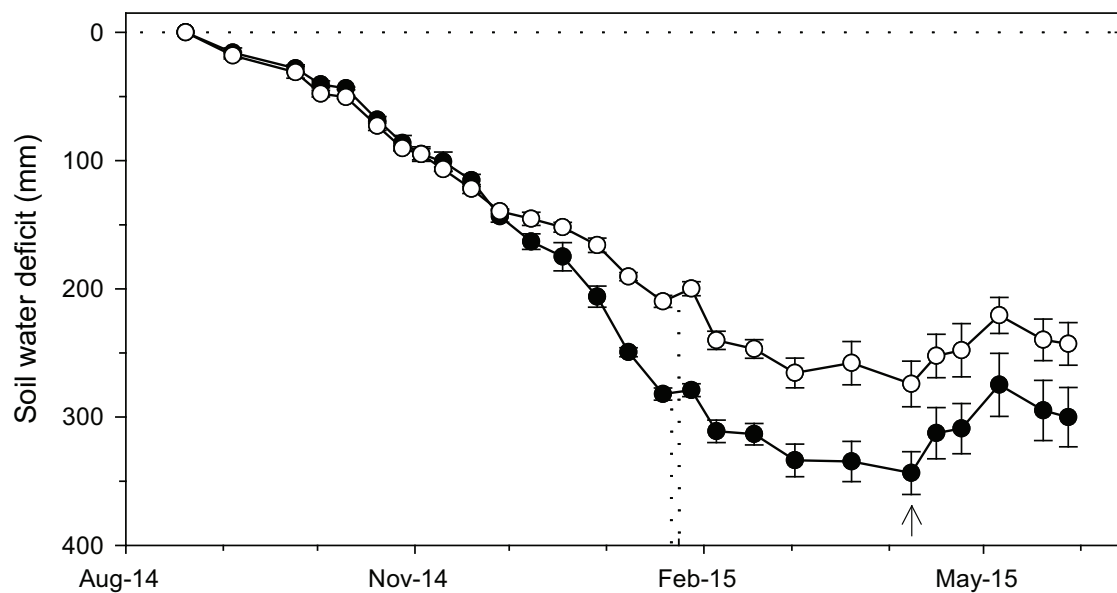
Figure 5.3 Total soil water deficit of the profile to 2.3 m for dryland cocksfoot-lupin (○) and lucerne (●) pastures from the start of extraction on 20 August 2014 until the final measurement on 29 May 2015 at Lincoln University, Canterbury.

Note: Arrow marks the date of maximum soil water deficit. Error bars represent SEM either side of the mean. Dotted line (--) represents the break-point of pasture growth.

5.3.2.2   Cumulative rainfall, water use and potential evapotranspiration

Between 20 August 2014 and 29 May 2015, cumulative water use of cf-lupin pastures was 613 mm compared with 647 mm for lucerne (*P*=0.386) (Figure 5.4). During the same period, rainfall was 268 mm, which was 41% lower than the LTM of 452 mm. Total potential evapotranspiration (PET) was 942 mm which was 6.1% higher than the long-term mean (LTM).
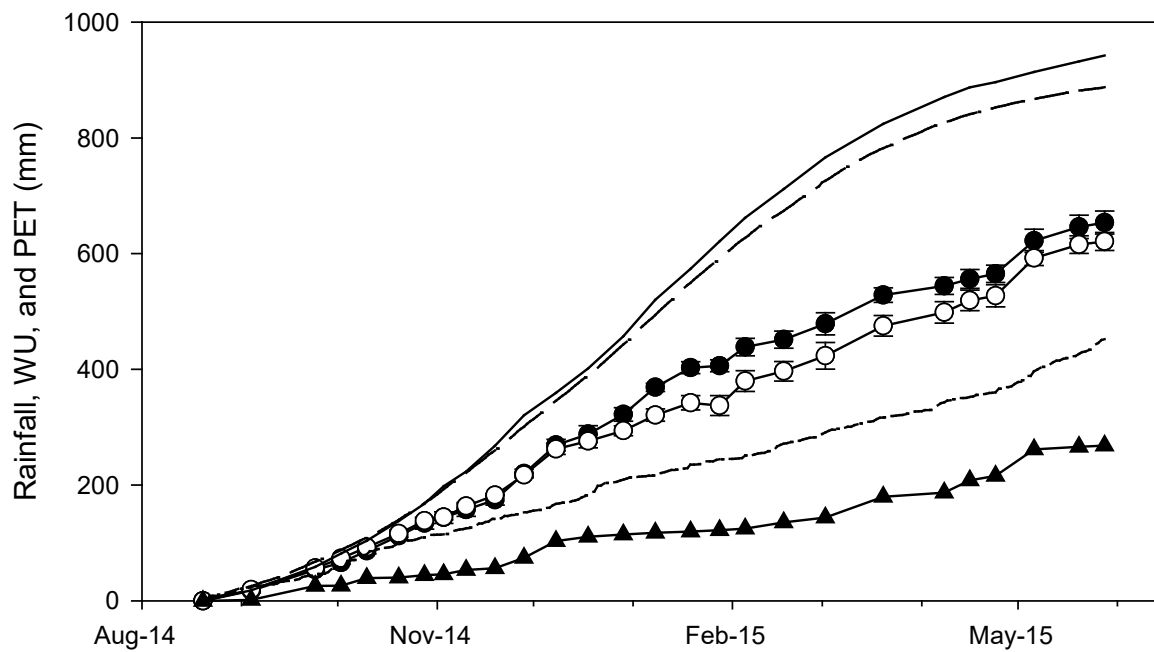
Figure 5.4 Cumulative rainfall (▲), potential evapotranspiration (PET) (−) and actual water use (WU) of cocksfoot-lupin (○) and lucerne (●) pastures between 20 August 2014 and 28 May 2015 at Lincoln University, Canterbury.

Note: Broken line represents the Long Term Mean (LTM; 2003-2013) for PET (– –) and LTM (2003-2013) for rainfall (- -). Data were collected from the Broadfields meteorological station.

5.3.2.3   Water use efficiency

Figure 5.5 shows the relationship between DM yield and WU. Dry matter yield increased linearly with WU in both pasture types and was accurately represented by split-line regression for cf-lupin (R^2=97.9-98.6) and lucerne (R^2=97.0-99.7) pastures. During the first growth phase, cf-lupin pastures produced 6510 kg DM/ha (±950 kg DM/ha) and used 350 mm (±6.7 mm) of water before the break-point on 24 January 2015, which gave a WUE of 18.6 kg DM/ha/mm (R^2=0.91). Dry matter production declined sharply after January, and the WUE of cf-lupin pastures declined to 0.29 kg DM/ha/mm as a result of continued WU with low herbage production. This resulted in an annual WUE of 11.8 kg DM/ha/mm. In comparison, lucerne had used 404 mm (±5.4 mm) of water at the break-point on 22 January 2015 but had produced 8444 kg DM/ha (±295 kg DM/ha), which resulted in a WUE of 20.9 kg DM/ha/mm (R^2=0.96). After the break-point, the WUE of lucerne declined to 3.3 kg DM/ha/mm. This meant that lucerne had an annual WUE of 13.8 kg DM/ha/mm.
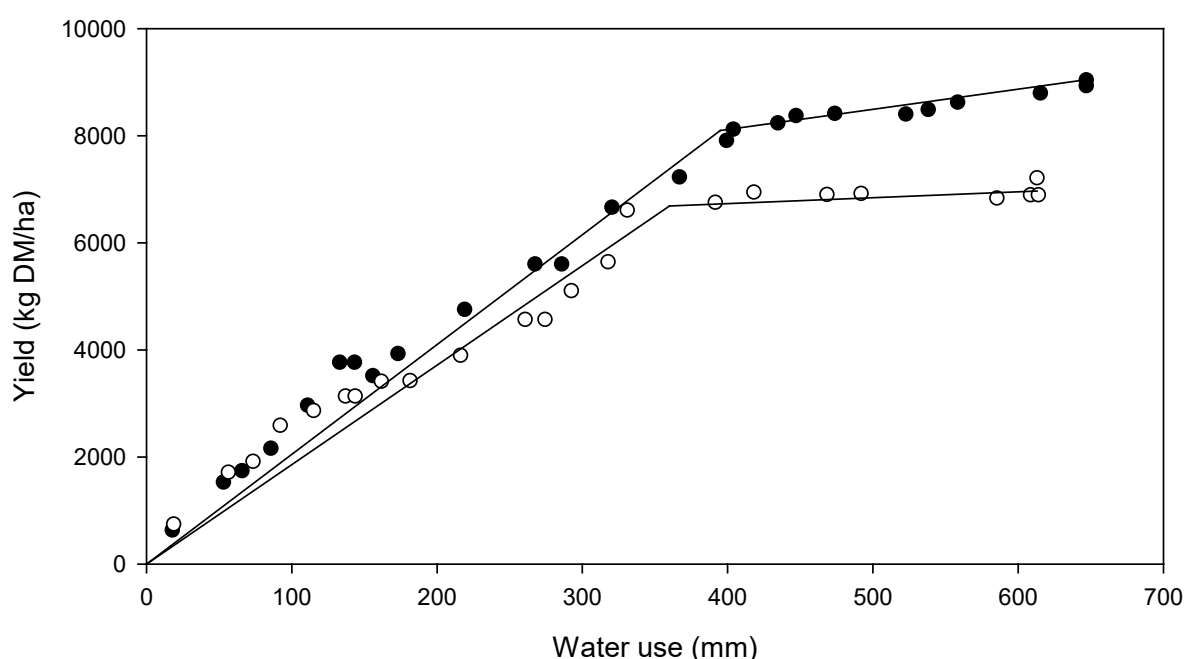
Figure 5.5 Herbage yield (kg DM/ha) plotted against water use (mm) for cocksfoot-lupin (○) and lucerne (●) pastures between 20 August 2014 and 28 May 2015 at Lincoln University, Canterbury.

Note: Linear regressions: cf-lupin – Line 1 (–) Y=18.59× (R^2=0.91), Line 2 (- -) Y=0.29×+6787 (R^2=na), Lucerne – Line 1 (–) Y=20.9× (R^2=0.96), Line 2 (- -) Y=3.3×+8146 (R^2=0.91).

5.3.3 Water extraction patterns of cf-lupin and lucerne pastures

The 'Monteith' model for water extraction was successfully applied to most soil layers between 25 and 185 cm for both pasture types. The model could not be applied to the uppermost soil layer (0-20 cm) due to constant rewetting from rainfall. Below a depth of 165 cm, fitted models became less accurate at describing extraction patterns (Appendix 3 and Appendix 4) and could not be successfully applied to depths of 175 cm and 185 cm for one of the lucerne paddocks. For cf-lupin pastures, soil layers between 175 cm and 225 cm had a mean PAWC of 5.4% (range = 1.8-13% PAWC) and a mean LL of 6.5% (range = 3.5-9.9%). Similarly, lucerne had a mean PAWC of 5% (range = 2.5-9.9% PAWC) and LL of 7.7% (range = 3.8-11) for soil layers between 175 and 225 cm. Many of the measurements taken below 165 cm fell outside the calibration range of the neutron probe (Appendix 2), which may have induced error and limited the application and accuracy of the extraction model. The outcomes of the model will be used to describe the seasonal pattern of moisture extraction in the following sections.

5.3.3.1 *Seasonal extraction pattern (SWC$_T$)*

The model was fitted to 15 soil layers (25-165 cm) within each replicate, which meant a total of 45 curves were fitted to each pasture type. The model accurately described SWC$_t$, with a mean R^2 of 0.96 (0.79-0.99) for cf-lupin pastures and 0.97 (0.88-0.99) for lucerne. Examples of the seasonal extraction pattern for different soil layers are given for cf-lupin pastures in Figure 5.6 and for lucerne in Figure 5.7. During initial model fitting in the upper soil layers (25-65 cm), rainfall-induced fluctuations in SWC caused the model to underestimate PAWC due to an elevated LL. Therefore, any significant increases in SWC were removed and layers were re-analysed.
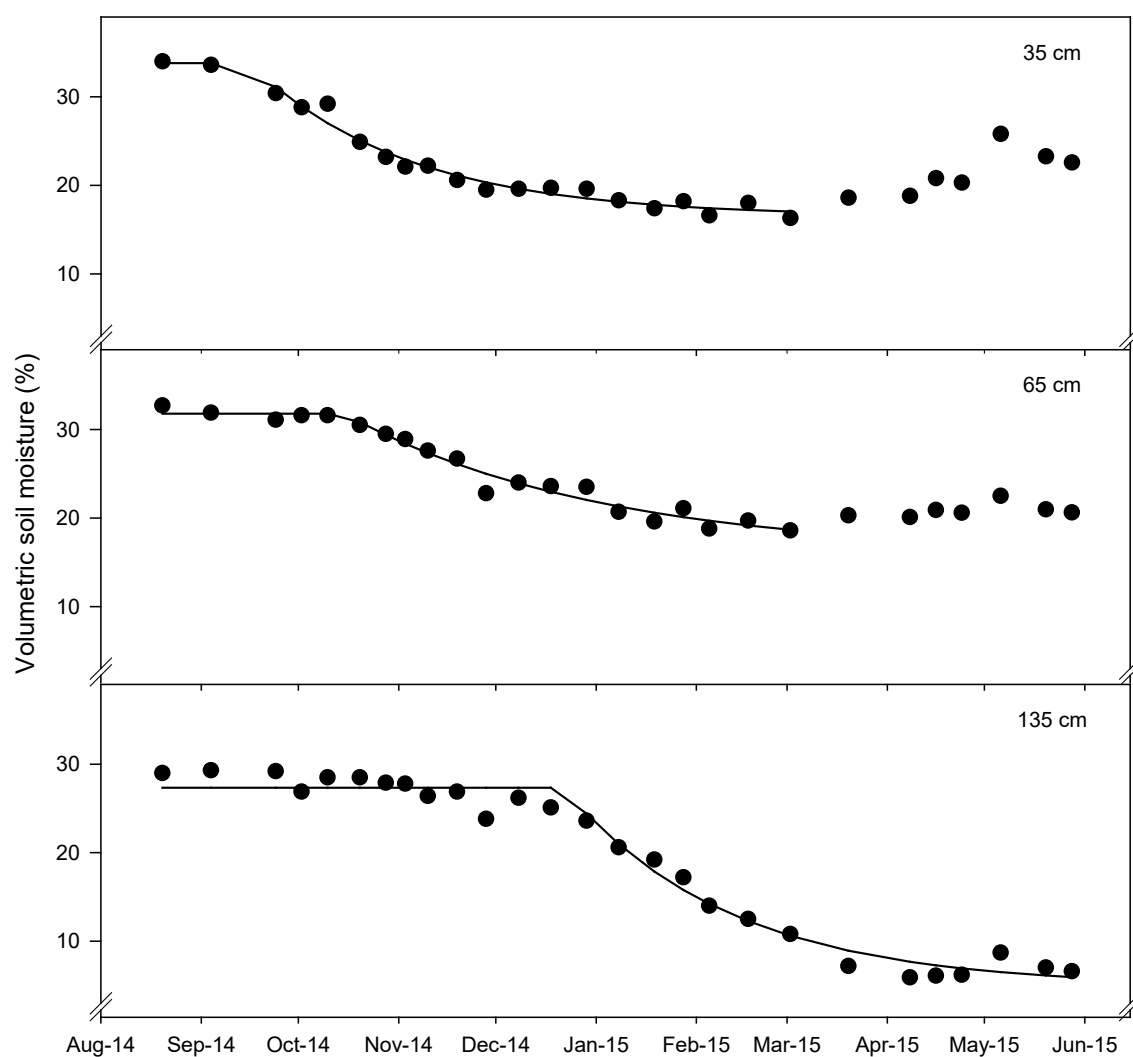
Figure 5.6 An example of seasonal water extraction patterns at different depths for a dryland cocksfoot-lupin pasture grown in paddock H12 at Lincoln University, Canterbury.
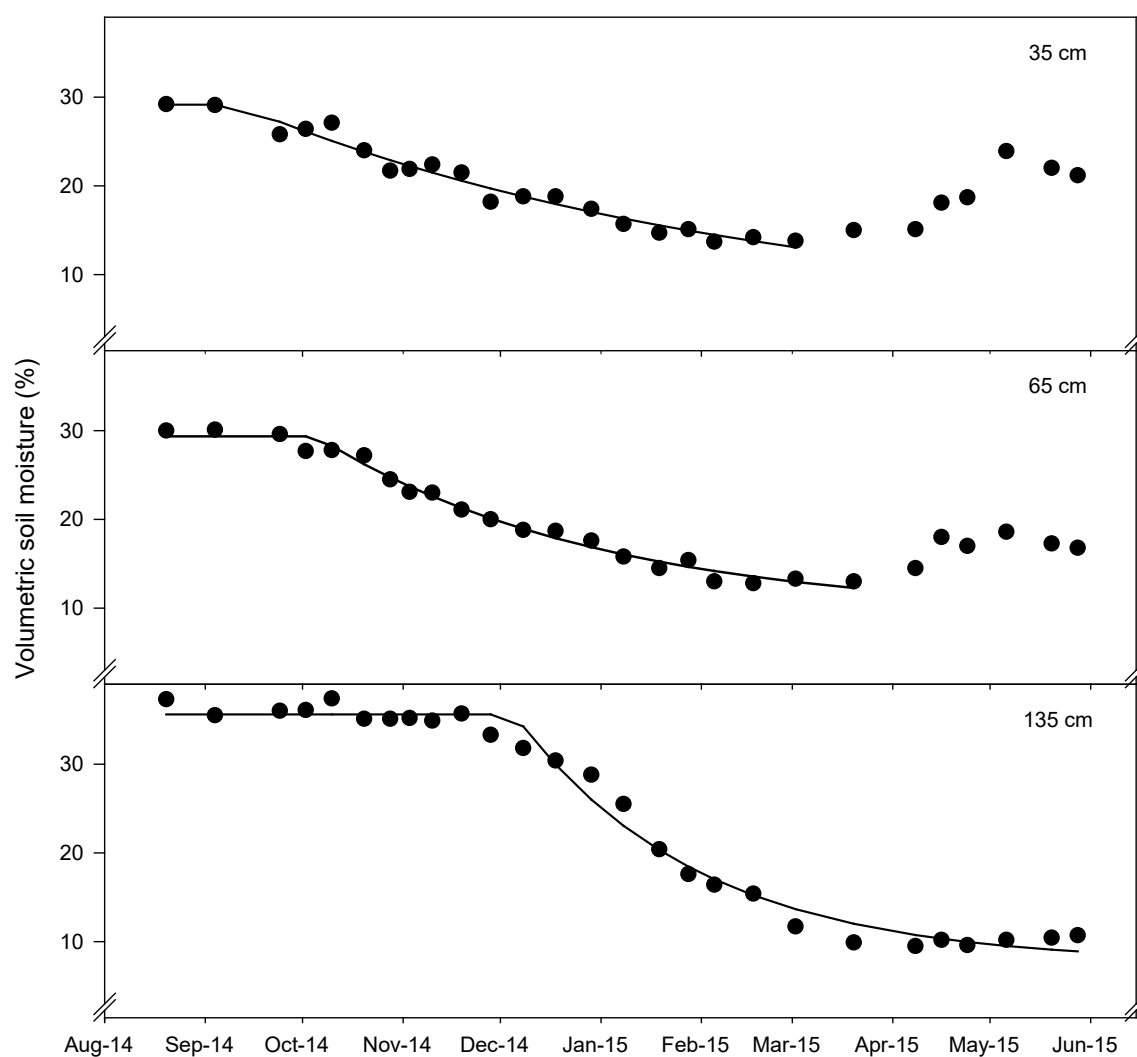
Figure 5.7 An example of seasonal water extraction patterns at different depths for a dryland lucerne pasture grown in paddock H12 at Lincoln University, Canterbury.

5.3.3.2 Plant available water capacity

The mean D_{UL} and LL of cf-lupin and lucerne pastures is given in Figure 5.8. Cf-lupin and lucerne pastures extracted water to a depth of about 165 cm during the 2014/15 growth season. Over this depth, cf-lupin pastures extracted 240 mm from the soil profile compared with 293 mm for lucerne. The mean D_{UL} was 29.9% for cf-lupin pastures, which was similar (P=0.077-0.957) to 28.8% for lucerne. Differences between the two pasture types became apparent in soil layers between 55 and 155 cm, where the mean PAWC for lucerne was 19.3 mm compared with 14.9 mm for cf-lupin pastures (P<0.001).
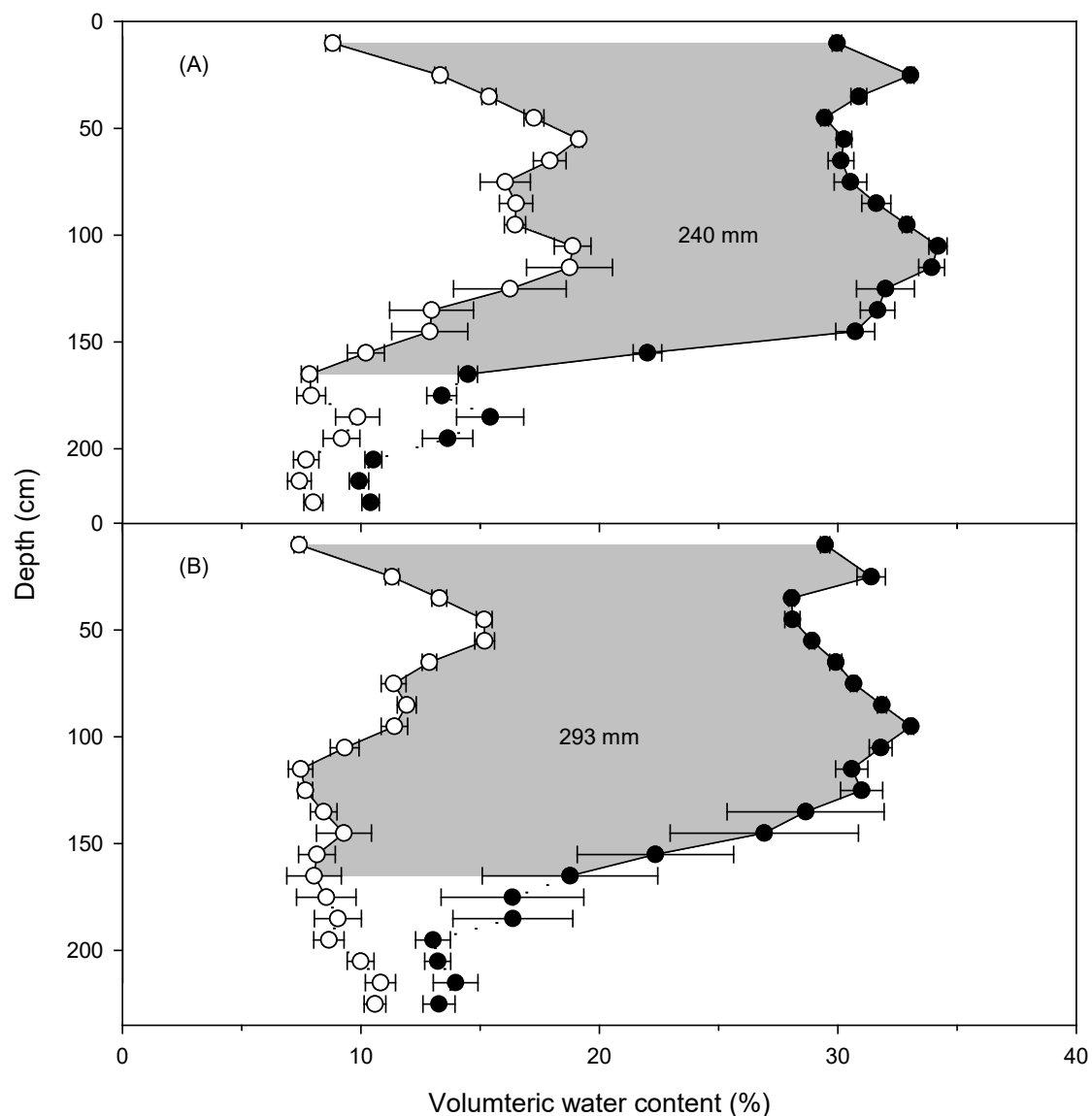
Figure 5.8 Drained upper (●) and lower (○) limits of water extraction for (a) cocksfoot-lupin and (b) lucerne pastures between 20 August 2014 and 28 May 2015 at Lincoln University, Canterbury. Shaded area and numbers represent the plant available water capacity. Error bars represent standard error of the mean.

5.3.3.3   Extraction front velocity

The EFV of cf-lupin and lucerne pastures is given in Figure 5.9. Both pasture types showed a linear EFV with a consistent progression down the profile. The EFV of cf-lupin pastures was 8.6 mm/day (R^2=0.77) which was similar (*P*=0.203) to 9.6 mm/day (R^2=0.90) for lucerne. The maximum extraction depth of 165 cm was reached for both pastures between the 15th and 25th of January 2015.
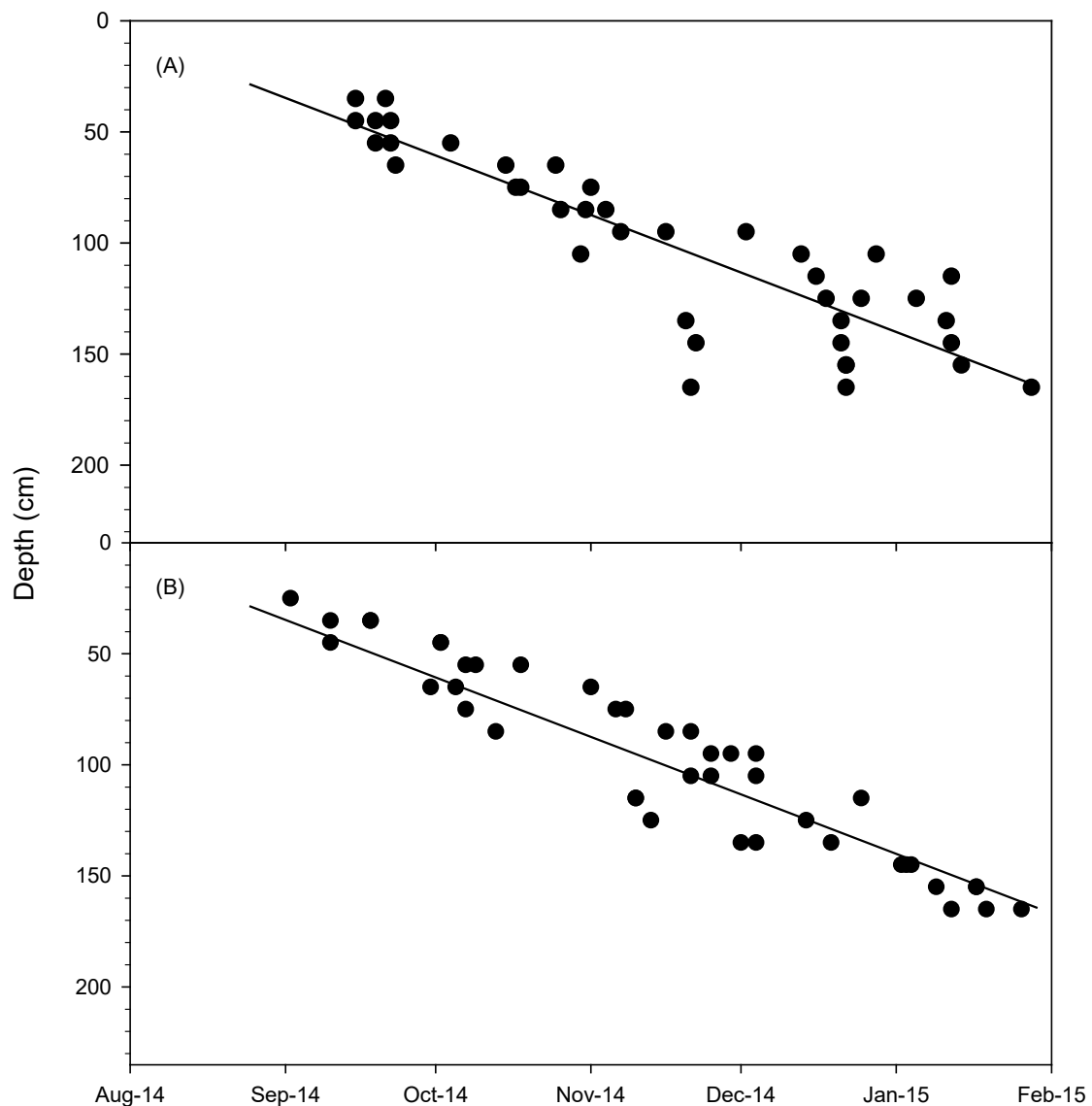
Figure 5.9   Extraction start time (t_c) at each depth interval of the soil profile for (A) cocksfoot-lupin and (B) lucerne pastures between 20 August 2014 and 1 February 2015 at Lincoln University, Canterbury.

Note: Linear regression; (A): (—) y = 0.8637x + 24.29 (R^2=0.77), (B): (—) y = 0.9583x + 13.11 (R^2=0.90).

5.3.3.4   Extraction decay constant

The extraction decay constant was variable over the depth of the soil profile for both pasture types (Figure 5.10). For cf-lupin pastures, the -kl ranged between 0.003 and 0.383/day and 0.005 to 0.099/day for lucerne. The mean –kl was 0.0190/day for lucerne, which was not different (*P*=0.128) from 0.015/day in cf-lupin pastures.
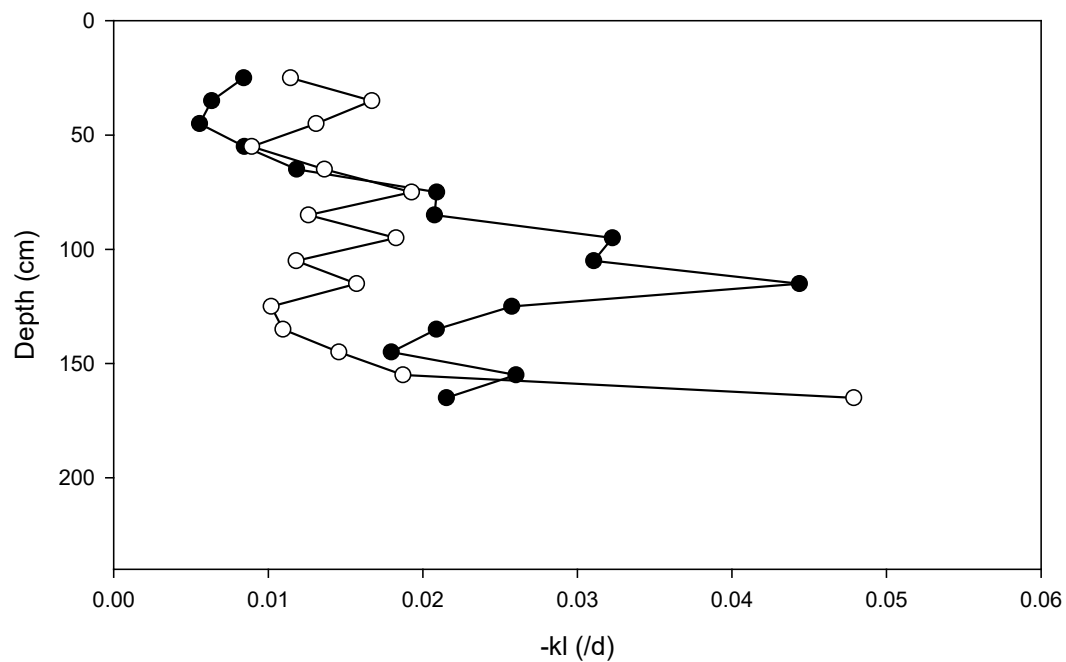
Figure 5.10 Extraction decay constant (-kl) of individual soil layers for dryland lucerne (●) and lupin-cf (○) pastures at Lincoln University, Canterbury.

5.4 Discussion

In Chapter 4, lucerne produced 3800 kg DM/ha/year more than cf-lupin pastures, which was converted to an additional 400 kg/ha of sheep liveweight gain. The additional productivity was attributed to a higher linear pasture growth rate for lucerne during the spring/summer period. The additional productivity resulted from the improved WUE and more efficient soil moisture extraction of lucerne. To quantify the pattern of soil moisture extraction, the 'Monteith framework' was successfully applied to both pasture types. This indicated that pastures were similar in their extraction depth but lucerne was more efficient at extracting moisture from soil layers between 55 and 155 cm.

In spring, both pasture types grew linearly in response to thermal time (Figure 5.2). Lucerne produced 3.8 kg DM/ha/^{o}Cd compared with 2.9 kg DM/ha/^{o}Cd for cf-lupin pastures. For lucerne, the rate of biomass accumulation in response to thermal time was lower than expected. For example, Mills *et al.* (2015b) found that lucerne produced herbage at a rate of 6.5 kg DM/ha/^{o}Cd regardless of the total spring yield it achieved. The two most probable reasons for differences between the two studies are either differences in yield accumulation or the parameters that were used in thermal time calculation. Differences in yield accumulation may have occurred as a result of the grazing management. Firstly, the lucerne was managed in unison with the cf-lupin pastures which meant grazing was not solely aimed at achieving maximal lucerne yields (Moot *et al.*, 2003). Secondly, is the method of estimating herbage mass that was used. In Chapter 4, herbage mass was calculated as the difference between post and pre-grazing pasture mass throughout a complete grazing rotation. It assumes that no growth is occurring while pastures are being grazed, which were periods of 3-10 days throughout the 2014/15 grazing season. This assumption will provide the lowest estimation of total biomass production when compared with methods such as cage cutting. When calculating thermal time, the choice of cardinal temperatures (e.g. T_b, T_{opt} and T_{max}) can considerably influence the rate of accumulation. In this study, T_b was set at 0°C for both pasture types, which gave the lowest coefficient of variation. The linear growth rate of cf-lupin pastures was similar to 3.2 kg DM/ha/^{o}Cd for irrigated (Mills *et al.*, 2006) and 3.1 kg DM/ha/^{o}Cd for dryland cocksfoot (Fasi *et al.*, 2008) monocultures that had not received N fertiliser. However, the values were considerably lower than 6.8 kg DM/ha/^{o}Cd for cocksfoot/sub clover pastures (Ates *et al.*, 2010) and 7.2 kg DM/ha/^{o}Cd for cocksfoot monocultures receiving N fertiliser (Mills *et al.*, 2006).

5.4.1 Water use efficiency

During the linear spring/summer growth phase, lucerne used water more efficiently than cf-lupin. Lucerne had produced 8440 kg DM/ha and used 404 mm of water compared with 6510 kg DM/ha and

350 mm for cf-lupin pastures when the C_{LD} was reached on 22 and 24 January 2014, respectively (Figure 5.2). This equated to a WUE of 20.9 kg DM/ha/mm for lucerne and 18.6 kg DM/ha/mm for cf-lupin pastures. Therefore, the additional 54 mm of water extraction for lucerne did not extend its period of non-limited growth but allowed it to produce more biomass while maintaining a higher WUE. The WUE of lucerne was similar to that reported by Dolling *et al.* (2005) in Western Australia but lower than 24 kg DM/ha/mm and 29 kg DM/ha/mm reported by Brown *et al.* (2005) and Sim (2014) at Lincoln University, respectively. In comparison, the WUE of 18.2 kg DM/ha/mm for cf-lupin pastures was lower than lucerne but was consistent with an annual WUE of 17 kg DM/ha/mm for dryland cocksfoot monocultures (Mills, 2007) and 19 kg DM/ha/mm for perennial ryegrass/white clover pastures (Black and Murdoch, 2013) both of which did not receive N fertilizer.

The WUE for both pasture types reduced considerably after they had reached C_{LD}. This occurred as a function of continued water use without substantial herbage growth. The WUE of cf-lupin pastures declined to 0.29 kg DM/ha/mm, whilst lucerne pastures had a WUE of 3.3 kg DM/ha/mm. In part, the significant reduction in WUE was probably caused by frequent small rainfall events that occured between February and May. During this period, 82% of the 143 mm of rainfall occurred in events that were less than 5 mm. Small rainfall events are often lost to soil evaporation, which causes an apparent increase in water used and thus a decline in WUE. Accounting for evaporative losses, and disentangling it from transpiration losses, is achievable when ground cover is quantified, however, this was not measured in this study. Despite this, both pasture types produced <600 kg DM/ha throughout this period, which was about 6% of their total annual production. Therefore, any advantage to lucerne would have only minor practical implications.

The importance of N nutrition in grass-based pastures for increased WUE is well documented (Black and Murdoch, 2013; Moot *et al.*, 2008; Peri *et al.*, 2002). Nitrogen deficient pastures will generally use the same amount of water as a pasture with sufficient N fertilisation (Black and Murdoch, 2013; Brown *et al.*, 2003), however, the lower photosynthetic capacity (Peri *et al.*, 2002) will limit biomass accumulation which reduces WUE. Given that *L. polyphyllus* was the only additional source of N for the cf-lupin pasture, its total productivity of about 1900 kg DM/ha between August 2014 and May 2015 would have meant that the potential for N fixation was low (~45 kg N/ha). Cocksfoot requires a minimum of about 28 kg N/t DM to maintain photosynthetic efficiency (Peri *et al.*, 2002). Thus, the amount of N fixed by lupin plants would have been inadequate to support the maximum WUE of a legume or N fertilized pasture.

5.4.2 Water extraction patterns

The 'Monteith framework', originally developed for annual crops (Monteith, 1986), has been validated for perennial crops (Brown, 2004; Mills, 2007; Sim 2014), and was successfully applied to lucerne (R^2=0.96; range = 0.81-0.99) and cf-lupin (R^2=0.95; range = 0.80-0.99) pastures in this study (Figure 5.6 and Figure 5.7). The model is used to understand patterns of water extraction and quantify the progression of roots down the soil profile. The model is frequently applied to monocultures, but in this study, the model was used to compare a monoculture with a mixed-pasture. The complexity of applying it to a mixed-pasture is evident. Differences between root morphology and potential differences in the seasonal demand of each species could limit its application. However, when applied to lucerne and cf-lupin pastures, the exponential fits for water extraction were within the range reported for lucerne (Brown, 2004), cocksfoot (Mills, 2007), and various annual crops (Dardanelli *et al.*, 1997; Meinke *et al.*, 1993).

Analysis of water extraction patterns indicated that maximum extraction depth and D_{UL} were not different between cf-lupin and lucerne pastures (Figure 5.8). The EFV was also not different between pasture types(Figure 5.9), which indicated that differences in water supply were caused by PAWC and the extraction decay constant (-kl) (Figure 5.10) of each pasture type. Both PAWC and -kl were similar for cf-lupin and lucerne at depths of 0-55 cm. However, between 55 and 155 cm, the mean PAWC of lucerne was 30% higher than cf-lupin pastures despite having a similar mean D_{UL} of about 30% v/v. Therefore, the additional PAWC was caused by greater extraction within each layer, which resulted in a mean LL of 7.8% v/v for lucerne and 12.3% v/v for cf-lupin pastures. This was also coupled with a mean -kl of 0.028/d (±0.004) for lucerne, which was twice that of the cf-lupin pastures. The PAWC is predominantly influenced by soil texture and root density, whilst the -kl is influenced by crop water demand and root length density (Passioura, 1983). Because the D_{UL} was not different between the pasture types, the higher mean PAWC and -kl of lucerne suggests that it had a greater root density between 55 and 155 cm rather than differences in soil texture.

There was some evidence of soil textural variation within both pasture types. For example, the D_{UL} of lucerne had a median SEM of 1.0% v/v (range=0.11-1.9% v/v) at soil depths of 25-125 cm compared with a median SEM of 6.3% v/v (range=1.4-7.8% v/v) at depths of 135 to 185 cm. However, below 185 cm, the soil texture appeared to be more consistent with mean D_{UL} values of 12.8% v/v (±0.66% v/v) for lucerne and 11.6% v/v (±0.82% v/v) for cf-lupin pastures. These results indicate that there was a consistent decline in water holding capacity (WHC) below 185 cm, which is associated with an increase in coarser materials (e.g. gravel) that have a low WHC (Ratliff *et al.*, 1983). However, in some areas of the experimental site, the depth to coarser materials was shallower, which resulted in a lower WHC

and production potential. This is consistent with other studies, on similar soils, where extraction patterns were affected by variable soil texture (Brown, 2004; Mills, 2007; Sim *et al.*, 2017) and may help to explain some of the production variability that was found in Chapter 4.

5.4.3 Effects of soil moisture deficit on growth

The broken-stick thermal time model suggested that C_{LD} of both pasture types was reached after about 2400°Cd accumulated. This caused the linear growth rate to decrease from 2.9 to 0.19 kg DM/ha/$^{\circ}$Cd for cf-lupin pastures and 3.8 to 0.41 kg DM/ha/$^{\circ}$Cd for lucerne. At this point, the EFV indicated that both pasture types had reached their maximum extraction depth of around 165 cm (Figure 5.9). However, cf-lupin pastures had depleted 37% of PAWC whilst lucerne had depleted 51%. Results for lucerne were consistent with Mills *et al.* (2006), who suggested that pasture growth will be significantly limited once 50% of PAWC has been depleted. However, the growth limiting effects of moisture stress occurred sooner than expected for cf-lupin pastures. Because cocksfoot was the dominant yield component (about 65% of annual yield), it was the predominant driver of the thermal time model. Therefore, the yield-limiting effects of SWD were probably more dependent on cocksfoot's primary root zone (<80 cm; (Mills *et al.*, 2006)) rather than the SWD for the entire extraction depth of 165 cm that included both cocksfoot and *L. polyphyllus* roots. Thus, the amount of moisture that could be extracted from depths between 85 and 165 cm was probably dependent on the relative abundance of *L. polyphyllus* (about 20% of annual yield) in the pasture and the resultant number of taproots. Taproot density was not quantified in this study, but a reduction in PAWC and -kl at these depths was indicative of a lower root density (Monteith and Greenwood, 1986), which generally decreases with depth (McKenzie *et al.*, 2006).

5.5   Summary and conclusions

The combined results of Chapters 4 and 5 indicated that the improved productive performance of lucerne compared with lupin-cocksfoot was mainly attributed to increased water extraction from the soil profile and improved WUE during spring/summer. Continued measurements of these pastures gave similar results and are presented in Black and Ryan-Salter (2016).

- Productivity of lucerne during non-limited growth was higher than cf-lupin pastures with growth rates of 3.8 and 2.9 kg DM/ha/^{o}Cd (T_b=0°C), respectively. This implies that, under the same conditions, lucerne will produce more herbage than cf-lupin pastures that are dominated by cocksfoot.

- Lucerne extracted an additional 60 mm of water from the soil profile and had a WUE of 20.9 kg DM/ha/mm compared with 18.6 kg DM/ha/mm for cf-lupin pastures. This allowed lucerne to produce an additional ~1700 kg DM/ha before SWD became limiting. Thus, lucerne was able to maintain a higher stocking rate for longer due to greater soil moisture access.

- The 'Monteith framework' was successfully fitted to both the lucerne and mixed cf-lupin pastures.

- The maximum extraction depth was the same for both pastures. However, differences in extraction were driven by an increased root density at depths of 65 to 155 cm where lucerne had a higher PAWC and -kl. This suggests that extraction was limited beyond 0.7 m for cf-lupin pastures and their yield potential may not have been met due to the low root density of *L. polyphyllus*.

- The depth to gravel was variable across the experiment at depths below ~135 cm, which was probably responsible for some of the yield variability that was found in Chapter 4.

6   Characterisation of rhizobial symbionts, use of inoculant and utilisation of soil inorganic N in *Lupinus polyphyllus*

6.1   Introduction

Lupinus polyphyllus was shown to have potential as a forage plant in Chapter 3 which confirms findings in other studies (Black *et al.*, 2014b; Scott, 1989, 2008). As for the majority of legumes, *L. polyphyllus* is capable of fixing atmospheric nitrogen (N$_2$) via symbiotic bacteria ("rhizobia") in root nodules. This ability gives legumes an advantage over non-fixing plants under low soil N conditions if other factors are favourable for growth (Andrews *et al.*, 2009; Andrews *et al.*, 2013).

Rhizobia belonging to the slow-growing, acid-tolerant, *Bradyrhizobium* genus have been recognised as the predominant symbiont partners for annual (e.g. *L. angustifolius*) and perennial lupin species (Andrews and Andrews, 2017; Jarabo-Lorenzo *et al.*, 2003; Stępkowski *et al.*, 2018; Stepkowski *et al.*, 2007; Stepkowski *et al.*, 2005; Weir, 2006; Weir *et al.*, 2004). Commercial inoculants that contain *Bradyrhizobium* sp. have been developed for annual lupins, and have also been recommended for use on *L. polyphyllus* in New Zealand (Scott, 1989). However, no attempt has been made to select rhizobial strains for use as an inoculum on *L. polyphyllus* or quantify the effectiveness of particular strains at improving productivity. Where tested, legumes were found to be able to utilise inorganic soil N in the form of both nitrate (NO$_3^-$) and ammonium (NH$_4^+$) (Andrews *et al.*, 2018; Liu *et al.*, 2016; Streeter, 1985b). Also, increased availability of soil inorganic N generally resulted in increased uptake of soil inorganic N and reduced N$_2$ fixation (Carlsson and Huss-Danell, 2003; Streeter, 1985a; Vance and Heichel, 1981). The ability of *L. polyphyllus* to utilise soil inorganic N has not been tested.

The first objective of this chapter was to evaluate nodulation of *L. polyphyllus* over a wide range of sites throughout the South Island of NZ. This was achieved by visiting a range of geographically separate sites and examining roots for nodulation. All plants at all sites were nodulated, which led to the second objective which was to isolate rhizobia from these nodules and broadly characterise them genetically to assess variability between the strains and possibly gain some understanding of their origin(s). The third objective was to assess whether there was variability in the effectiveness of these isolates on the growth of *L. polyphyllus* in high-country soils under glasshouse conditions. This would allow the effectiveness of isolates to be measured under controlled conditions and evaluate any opportunity for selection of 'elite' strains. Following this, the fourth objective was to test whether an inoculant would improve growth of seedling *L. polyphyllus* in a field sowing under high-country conditions. The fifth objective was to determine if *L. polyphyllus* and lucerne could utilise soil NO$_3^-$

(main form of inorganic N in cultivated agricultural soils) and, if so, did this depress N_2 fixation. Lucerne was included to maintain a consistent thread throughout the thesis.

6.2 Materials and methods

6.2.1 Plant sampling and rhizobial isolation

Ten sites containing *Lupinus polyphyllus* were visited in the South Island of NZ between 12 and 14 March 2013 (Table 6.1). At each site, three lupin plants were selected at random and dug from the soil so that the majority of the upper root system (ca. 40 cm) remained intact. Roots were rinsed to remove adhered soil and assessed for nodulation. Nodules were cut from the plant within 96 hours of collection. Because all plants were nodulated, two nodules were cut from the roots of each plant by severing the root on either side of the nodule with a scalpel.

Table 6.1 **Location of *Lupinus polyphyllus* populations used to investigate the presence of nodules and associated rhizobial strains in the South Island of New Zealand.**

Site	No. of strains isolated	Collection Date	Locality	Co-ordinates
1	4	12/3/2013	Arthurs Pass	S42°57.720′, E171°34.521′
2	4	12/3/2013	Arthurs Pass	S43°01.049′, E171°35.624′
3	1	12/3/2013	Arthurs Pass	S43°01.268′, E171°35.844′
4	1	12/3/2013	Arthurs Pass	S43°16.613′, E171°42.453′
5	2	14/3/2013	Mackenzie Basin	S44°04.838′, E170°31.945′
6	2	14/3/2013	Mackenzie Basin	S44°03.901′, E170°21.801′
7	2	14/3/2013	Mackenzie Basin	S44°03.925′, E170°29.357′
8	2	14/3/2013	Central Otago	S44°30.872′, E169°49.712′
9	1	14/3/2013	Central Otago	S44°53.561′, E168°59.600′
10	2	14/3/2013	Te Anau	S45°08.185′, E167°55.802′

Nodules were then transferred to a sterile laminar flow cabinet where they were processed according to the methods of Vincent (1970). Nodules were surface sterilised by immersion in 96% ethanol for approximately 10 seconds followed by 2-4 minutes in a 0.5% solution of sodium hypochlorite. They were then washed in five changes of sterile water in separate sterile petri dishes and transferred to a sterile petri dish for dissection. Nodules were then crushed using a sterile scalpel and forceps. A sterile loop (10 μl) was used to transfer fluid from the crushed nodule onto a yeast mannitol agar plate (YMA) (Table 6.2). The YMA plates were then sealed, inverted and incubated at 25°C in a dark incubator for 3-5 days. The YMA plates were inspected daily for colony growth and were removed from the incubator when colonies had grown adequately. Individual colonies were repetitively sub-cultured to obtain purified strains.

Table 6.2   Contents of YMB and YMA used for culturing rhizobial strains.

Medium	Contents	Weight (g/L)
YMB	Yeast extract	1
	Mannitol	10
	Dipotassium phosphate (K_2HPO_4)	0.5
	Magnesium sulphate ($MgSO_4$)	0.2
	Sodium chloride (NaCl)	0.1
YMA	YMB	as above
	Calcium carbonate ($CaCO_3$)	1
	Agar	15

Twenty-one rhizobial isolates were obtained from nodules of different *L. polyphyllus* plants sampled from four sites at Arthur's Pass (AP; 10 isolates), three sites in the Mackenzie Basin (MB; six isolates), two sites in Central Otago (CO; three isolates) and one site near Te Anau (TA; two isolates), South Island, New Zealand in March 2013 (Table 6.1). All isolates were obtained from wild populations of *L. polyphyllus* except for two isolates that were obtained from an agricultural stand at Sawdon Station, near Tekapo, in the Mackenzie Basin (MB5 and MB6).

A 'control' isolate was obtained from *L. polyphyllus* plants supplied with Group G inoculant (Becker Underwood), which is currently recommended for use on annual lupins. Plants were grown under controlled conditions in an incubation room supplied with filtered air (described in Section 6.2.4). The nodules were then cut from the plants and underwent the same isolation technique as described previously for the other isolates.

6.2.2   DNA extraction, PCR amplification and gel electrophoresis

A single colony of bacterial sub-cultures for each nodule was inoculated into 1.0 ml of yeast-mannitol broth (YMB) (Table 6.2) and incubated for 24 hours at 28°C on a shaking incubator. Following incubation, 500 µL of YMB culture was mixed with 500 µL of 20% glycerol and placed in a -80°C freezer for long-term storage. The Gentra PUREGENE™ Purification kit (Gentra Systems, USA) was used to extract Deoxyribosenucleic acid (DNA) from the remaining 500 µL of YMB culture; using the supplied protocol for gram-negative bacteria. The purity of extracted DNA was then assessed using a spectrophotometer (NanoDrop™) at wavelengths of 260 and 280 nm. An $OD_{260/280nm}$ ratio above 1.8 indicated that the extracted DNA was of high purity and could be used for further analysis. Samples were brought to a uniform DNA concentration of 50 ng/µL using sterile water.

The Polymerase Chain Reaction (PCR) was used to amplify gene fragments for sequencing using a customised master mix (Table 6.3). Three genes were amplified in this study. These genes were the

small subunit RNA (16s rRNA), nitrogenase iron protein (*nifH*), and the N-acyltransferase nodulation protein A (*nodA*). Primers for the 16s rRNA, *nifH* and *nodA* genes were selected from previous work and their respective sequences are listed in Table 6.4. All primers were manufactured by Integrated DNA Technologies, Auckland. The FastStart™ Taq DNA Polymerase kit (Roche Applied Science, Auckland) was used for all PCR reactions, with optimisations of annealing temperature and primer concentrations. The conditions of PCR for respective genes are listed in Table 6.5.

Table 6.3 **Contents of PCR mastermix used to amplify the 16s rRNA, *nifH* and *nodA* gene fragments of rhizobial isolates.**

Master Mix (per PCR tube)	Amount (µL)
PCR Buffer 10x + MgCl$_2$	2.50
2.5 mM dNTPs	2.00
Forward Primer	1.00
Reverse Primer	1.00
FastStart Taq Polymerase (5U/µL)	0.25
50ng genomic DNA	1.00
Distilled water	17.25
Total	25.00

Table 6.4 **Target genes and their PCR primers used in this study.**

Target Gene	Primer	Sequence (5' -3')	Reference
16srDNA	F27	AGA-GTT-TGA-TCM-TGG-CTC-AG	Weisburg *et al*, 1991
	R1494	CTA-CGG-YTA-CCT-TGT-TAC-GAC	Weisburg *et al*, 1991
nifH	PolF	TGC-GAY-CCS-AAR-GCB-GAC-TC	Poly *et al*, 2001a
	PolR	ATS-GCC-ATC-ATY-TCR-CCG-GA	Poly *et al*, 2001a
nodA	nodA1	TGC-RGT-GGA-ARN-TRN-NCT-GGG-AAA	Haukka *et al*, 1998
	nodA2	GGN-CCG-TCR-TCR-AAW-GTC-ARG-TA	Haukka *et al*, 1998
	nodAf.brad	GTY-CAG-TGG-AGS-STK-CGC-TGG-G	Chaintreuil *et al*, 2001
	nodAr.brad	TCA-CAR-CTC-KGG-CCC-GTT-CCG	Chaintreuil *et al*, 2001

[1]Symbols: A, C, G, T = standard nucleotides; Y= C or T; R= A or G; W= A or T; S= G or C; K= T or G; M= C or A; D= A or T or G; V= A or C or G; B= T or C or G; N= A or G or C or T

Table 6.5   PCR conditions for amplification of 16s rRNA, *nifH* and *nodA* gene fragments.

PCR	Temperature (°C)	Time	Cycle
	95	3 min	1 x
	95	30 sec	
16s rRNA	65	30 sec	35 x
	72	30 sec	
	72	7 min	1 x
	4	∞	-
	95	3 min	1 x
	94	30 sec	
nifH	55	30 sec	35 x
	72	45 sec	
	72	7 min	1 x
	4	∞	-
	95	3 min	1 x
	94	40 sec	
nodA	49	40 sec	35 x
	72	90 sec	
	72	7 min	1 x
	4	∞	-

PCR products were resolved with electrophoresis in a 1% agarose gel (1 g agarose in 100 ml of 1 x TAE buffer) stained with 10 µL of SYBR Safe (Life Technologies, Waltham). A 1 kb+ ladder (Life Technologies, Waltham) was used to ensure the correct target sequence had been isolated. Gels were immersed in a 1 x TAE buffer solution and run at 100 volts for 35 minutes, followed by viewing under UV light.

6.2.3   Nucleotide sequencing and phylogenetic analysis

Sequences were obtained for both directions using an ABI PRISM® 310 Genetic Analyser (Applied Biosystems, California, USA) and viewed with Sequence Scanner v 1.0 (Applied Biosystems, California, USA). Sequences were then edited and assembled using DNAMAN v6 (Lynnon Corporation). Sequences were compared with those of known origin using the Basic Local Alignment Search Tool (BLAST®, NCBI; Bethesta, USA) and GenBank.

DNA sequences were aligned and Maximum Likelihood trees were constructed with 1000 bootstrap replications with partial deletion and 80% coverage cut off using a Tamura 3-parameter model in MEGA6 software (Tamura *et al.*, 2007). Only bootstrap values ≥50% were shown for each tree. The type strain for *Ensifer/Sinorhizobium meliloti* was used as an out-group for all genes. Sequences for this type strain were obtained from the GenBank database. Sequences that were not submitted to the GenBank database are given in (Appendix 7, Appendix 8 and Appendix 9).

6.2.4 Confirmation of nodulation

The ability of the isolates to form nodules on *L. polyphyllus* seedlings upon re-inoculation was investigated under sterile conditions. Lupin seeds were obtained from Rosevear & Co. Ltd, Ashburton. The seeds were spun against a sandpaper cone using compressed air for a period of 10 seconds for scarification. Scarified seeds were then transferred to a sterile laminar flow cabinet for sterilisation. They were surface sterilised by immersion in ethanol (100%) for 5-10 seconds followed by three minutes in a 0.5% solution of sodium hypochlorite. Seeds were then rinsed in five changes of sterile water. Sterilised seeds were then placed on moist blotting paper within a sterile container which was incubated at 20°C for 2-4 days in the dark.

Inoculants were produced for each of the bacterial isolates. A sterile loop was used to transfer bacterial colonies for each of the isolates from YMA plates to 30 ml of sterile YMB. The YMB cultures were grown to log phase (~1×10^8 cfu/ml) on a shaking incubator at 27°C.

Germinated seeds were transferred to a laminar flow cabinet and single seedlings were sown into autoclaved vermiculite within individual 500 ml polyethylene terephthalate (PET) jars. All seedlings were supplied with 30 ml of a complete nutrient solution (pH 6.0) at establishment (Table 6.6). Seedlings were then inoculated with five ml of appropriate bacterial broth and watered with ~100 ml of sterile water. Control plants were supplied with sterile YMB. The lid of each PET jar was penetrated to allow air circulation, and all jars were placed in a plant growth chamber at 25°C with a photoperiod of 16 hours. There were three replicates per treatment.

Table 6.6   Concentrations of micronutrients and macronutrients applied to the plants. The nutrient solution had a pH of 5.8.

Nutrient	Molarity
Micronutrients (µM)	
Iron chloride (FeCl$_2$ 4H$_2$O)	5.00
Cobalt chloride (CoCl$_2$ 6H$_2$O)	0.02
Copper sulphate (CuSO$_4$ 5H$_2$O)	0.10
Boric acid (H$_3$BO$_3$)	5.00
Sodium molybdate (Na$_2$MoO$_4$ 2H$_2$O	0.50
Manganese chloride (MnCl$_2$ 2H$_2$O)	1.00
Zinc sulphate (ZnSO$_4$ 7H$_2$0)	0.10
Macronutrients (mM)	
Ammonium nitrate (NH$_4$NO$_3$)	0.1
Calcium chloride (CaCl$_2$)	1.0
Potassium chloride (KCl)	1.0
Magnesium sulphate (MgSO$_4$ 7H$_2$O)	1.0
Monosodium phosphate (NaH$_2$PO$_4$)	1.0
Sodium phosphate (Na$_2$HPO$_4$)	0.1

After six weeks, plants were removed from the growth chamber and examined for nodulation. Individual plants were carefully withdrawn from the vermiculite and washed to remove any adhering contaminants. Roots were cut from the shoot, assessed for the presence of nodules, and transferred to a 20 ml sealed glass vial. In this form, the roots could then be assessed for nitrogenase activity using the acetylene reduction assay (ARA) (Cummings *et al.*, 2009). AcetPure acetylene (1.2 ml) was introduced to the vial and allowed to sit for a period of 30 minutes. After 30 minutes, a 10 ml sample was taken from each vial and transferred to a separate sealed glass vial. Samples were taken to the National Centre for Nitrous Oxide Measurement at Lincoln University, and analysed for ethylene content using gas chromatography.

6.2.5   Glasshouse Experiments 1 and 2 – inoculant effects on growth

Selected isolates were evaluated for their effect on plant growth on two separate occasions. Isolates were selected on the basis of their distinct phylogenetic groupings for the 16s rRNA and *nodA* sequences. The first experiment was conducted between 25 February 2014 and 14 May 2014, whilst the second experiment was conducted between 16 June 2014 and 9 October 2014. Both experiments were located in the Alluminex glasshouse at Lincoln University, Canterbury. The glasshouse was under natural lighting conditions for the duration of the experiments.

6.2.5.1 Preparation and design

Soil was collected from Glenmore Station (43°55'43.58" S, 170°29'15.72"E and 723 meters above sea level), Tekapo on 30 January 2014. The soil had an upper layer of decaying thatch (about 5 cm) that was removed prior to collection. Once removed, top soil was taken from a depth of 5-30 cm and transported back to Lincoln University. Soil was mixed with a spade and shaken through a 4 mm sieve to give a consistent particle size. The mixed soil was then tested for nutrient status (Table 6.7).

Table 6.7 **Test results of soil collected from Glenmore station on 30 January 2014. Soil was collected from a depth of 5-30 cm.**

pH	Olsen P	K	Ca	Mg	Na	Exch Al
H_2O	Mg/L		me/100 g			$CaCl_2$ mg/kg
5.5	14	0.69	4.8	0.83	0.07	2.5

In both experiments, 60 two litre pots were filled with soil and placed on clean saucers. Pots were watered and allowed to consolidate for two weeks before treatments were applied. Pots were arranged in a randomized complete block design with 12 inoculant treatments and five replicates.

6.2.5.2 Inoculant and seed preparation

Sterile Falcon® tubes were filled with 50 ml of YMB and inoculated with a single colony of each strain (one strain per tube). Tubes were sealed, agitated and grown to log phase, ca. $1x10^8$ cfu/ml, at a temperature of 27°C.

Lupinus polyphyllus seeds were obtained from Rosevear & Co. Ltd, Ashburton. The seeds were scarified, sterilised and germinated using the technique given in Section 6.2.4.

6.2.5.3 Establishment, management and measurements

At the commencement of both experiments, two seedlings were sown to a depth of 10 mm in each pot. After sowing, five ml of inoculant was applied to each seedling using a sterile pipette. After inoculation, about 5 mm of inert perlite was applied to the surface of each pot to reduce the chances of cross-contamination between isolates. In both experiments, pots were checked daily and watered when required. In the first experiment, pots were watered with tap water. In the second experiment, pots were watered with sterile water to reduce the chances of contamination.

At the completion of each experiment, the pots were removed from the glasshouse for assessment. Each plant was withdrawn from the soil and the roots were washed. The number of fully expanded leaves was counted and shoots were cut from the roots at the crown. Samples were then placed in a

forced-air oven at 68°C for 48 hours. Dried samples were then weighed individually to determine root and shoot dry weights.

6.2.6 Glasshouse Experiments 3 and 4 – Effect of nitrate on N$_2$ fixation

Two separate glasshouse experiments were undertaken to quantify the effect of increased soil inorganic N on N utilisation and N$_2$ fixation in *L. polyphyllus*. Lupin was compared with lucerne in its ability to use soil NO$_3^-$. The first experiment was established on 11 April 2014 and the second on 2 September 2015. Both experiments were located in the Alluminex glasshouse at Lincoln University. The Alluminex glasshouse was under natural lighting conditions for the duration of the experiments. Unless otherwise stated, establishment and analytical techniques were consistent between the two experiments.

6.2.6.1 Preparation and design

Lupinus polyphyllus seeds were obtained from Rosevear & Co. Ltd, Ashburton, NZ. Coated lucerne ('Force-4') seed was obtained from Seed Force, NZ. Lupin seeds were spun against a sandpaper cone using compressed air for a period of 10 seconds for scarification. Both experiments were undertaken using a non-nitrogenous potting mix that was specifically formulated for this experiment (Table 6.8).

Table 6.8 **Composition of non-nitrogenous fertiliser used for the assessment of N uptake at Lincoln University, Canterbury. Superphosphate was sourced from Ravensdown, New Zealand. All other nutrient fertilisers and wetting agents were obtained from Everris International; Geldermalsen, Netherlands.**

Composition	Amount
Growing medium	
Composted bark	60%
Fine pumice	40%
Nutrients (pH = 5.8)	
Agricultural lime	1 g l^{-1}
Superphoshpate (0-9-0-12)	0.3 g l^{-1}
Osmocote (0-0-37-0) - 6 month	0.3 g l^{-1}
Micromax trace elements	0.3 g l^{-1}
Hydraflo wetting agent	1 g l^{-1}

Experiment 3 was a randomised complete block design with two legume species (*L. polyphyllus* and lucerne) and five rates of nitrogen that was replicated six times giving 60 pots in total. Nitrogen treatments were 0, 25, 50, 100 and 200 kg N/ha.

Experiment 4 was a randomised complete block design with six rates of N and two species that was replicated four times giving 48 pots in total. The two species were *L. polyphyllus* and lucerne. Nitrogen treatments were 0, 50, 100, 200, 400 and 600 kg N/ha.

For both experiments, four litre pots were filled with soil and sown with about 20 seeds of lupin and lucerne. Following sowing, a 1M solution of liquid urea (46% N) was diluted with water and applied to the surface of each pot in 100 ml volumes so that they were receiving 25 kg N/ha. Lupin and lucerne pots received a 5 ml solution of Group G and Group AL commercial inoculants (Becker Underwood, Australia), respectively. The pots were then moved to the glasshouse where they were arranged into blocks and placed on clean saucers. Plants were checked and watered daily with tap water to a constant weight.

Plants were cut to a height of 3 cm and thinned to a population of 10 plants/pot at even spacings, which occurred on 28 May 2014 for Experiment 3 and 8 October 2015 for the Experiment 4. Labelled $^{15}KNO_3$ (10% ^{15}N atom%) fertiliser (Sigma-Aldrich; Missouri, USA) was dissolved in tap water to form a 1M stock solution. Nitrogen rates were then applied to individual pots in 100 ml volumes by combining the respective volume of stock solution with reverse osmosis water.

6.2.6.2 Measurements

Pots were removed from the glasshouse for destructive harvest on 23 July 2014 (57 days after N application) for Experiment 3 and 23 November 2015 (47 days after N application) for Experiment 4. Whole plants were carefully withdrawn from the potting mix and the roots were washed. Plants were counted and placed in a forced-air oven at 65°C for 48 hours for drying. Shoot material was cut from the root at the crown before both components were weighed. Following weighing, roots and shoots were combined for chemical analysis. In Experiment 3, replicates were combined (1 & 2, 3 & 4, 5 & 6) to reduce the number of samples for analysis.

Samples were then ground, using a centrifugal rotary mill (Retsch, GmbH, Haan, Germany), to pass through a 2 mm sieve which was followed by a second grinding that passed particles through a 0.5 mm sieve. Ground samples were then analysed for total N and isotopic ^{15}N concentration using mass spectrometry at the Lincoln University Centre for N_2O emissions. Plant ^{15}N concentration was calculated (Equation 6.1).

Equation 6.1 $\qquad$ **%Ndfs = (^{15}N atom% - $\delta^{15}N_{AIR}$%) × 10**

Where %Ndfs is the amount of N derived from soil, ^{15}N atom% is the percentage of N present as 15N in the plant material, and $\delta^{15}N_{AIR}$% is the proportion of atmospheric N_2 present as ^{15}N (generally constant at 0.3663% ^{15}N).

6.2.7   Field experiment

6.2.7.1   Establishment and measurements

Lupin seeds were obtained from Rosevear & Co. Ltd, Ashburton, NZ. On 29 September 2015, seeds were spun against a sandpaper cone using compressed air for a period of 10 seconds for scarification. Scarified seeds were then transferred to a sterile laminar flow cabinet for sterilisation. They were surface sterilised by immersion in ethanol (100%) for 5-10 seconds followed by three minutes in a 0.5% solution of sodium hypochlorite. Seeds were then rinsed in five changes of sterile water. Seeds were then placed into a sterile container to imbibe overnight.

On 30 September 2015, the experiment was established across six high-country farms within the Mackenzie and Otago districts (Table 6.9).

Table 6.9   Localitions of six experimental sites where lupin plants were established on 5 October 2015.

Site #	Farm name	Locality	Latitude/longitude	Elevation (m a.s.l.)
S1	Sawdon	Tekapo	44° 3'5.23"S, 170°31'19.84"E	717
S2	Glenmore	Tekapo	43°54'12.05"S, 170°28'13.58"E	727
S3	Simon's Hill	Pukaki	44°11'38.35"S, 170°18'35.91"E	518
S4	Omarama Station	Omarama	44°30'3.55"S, 169°56'51.83"E	442
S5	Bog Roy	Omarama	44°33'33.47"S, 170° 5'58.25"E	398
S6	Mt Grand	Lake Hawea	44°37'49.22"S, 169°19'12.68"E	473

The sites were randomly selected on flat (<5° slope) areas of each property. At each site, two adjacent 1 × 1 m^2 plots were marked with a separation distance of >5 m. Live plant material was removed from the surface of each site with a spade. A powered rotary hoe was used to cultivate at each site. Following cultivation, plots were compacted by foot.

Weed mat was cut into 1 × 0.5 m rectangles and a hole punch was used to put 36, two cm^2 holes at a five cm spacing into each mat (Figure 6.1). Mats were then pegged over the cultivated surface prior

to sowing. A single imbided lupin seed was placed into each of the 36 holes of the weed mat and sprinkled with 1 cm of soil.

Group G commercial inoculant (Nodulaid®; BASF, New Zealand) was suspended in sterile water (2 gm inoculant/50 ml water) at each site. One plot was randomly selected for inoculation and individual seeds were supplied with 1 ml of inoculant using a sterile pipette. A watering can was used to apply 80 mm of sterile water to each plot immediately after sowing. Plots were then covered with a cage to prevent animal disturbance. Sites were visited on 6 November to check for establishment and again on 17 December 2015. At both visits, plots received 70 mm of sterile water.
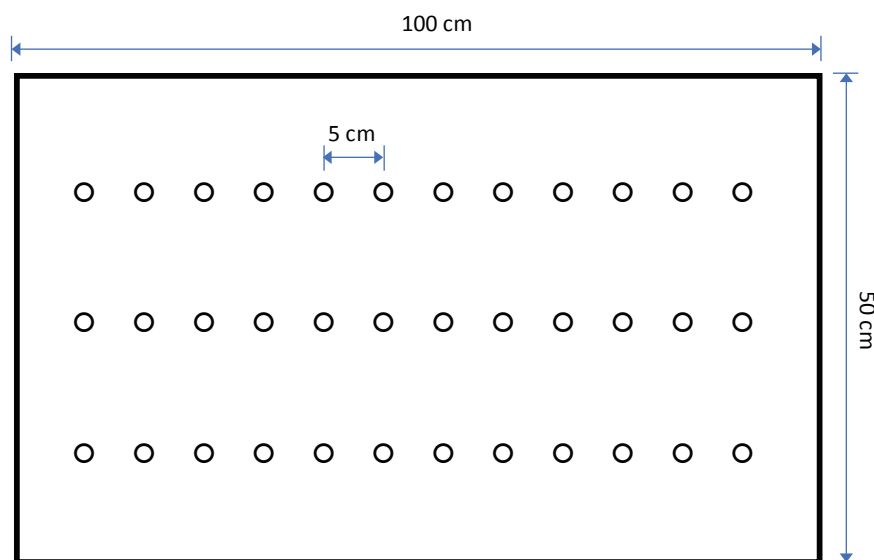
Figure 6.1 Example of weed mat used at each high-country site to prevent weed ingress.

On 8 February 2016, plants were dug from each plot with a spade and assessed for nodulation. Plant shoots were cut from the root at the crown and placed in a cooler bin at 4°C. On the following day, the shoots were placed in a forced-air oven at 68°C for 48 hours before being weighed.

6.2.8 Data analysis

All analyses were conducted with Genstat 16 Ed. (VSN International, 2014). A general analysis of variance was used to determine significant (P=0.05) effects in all experiments. In Glasshouse experiments 1 and 2, inoculant type was used as a fixed factor and leaf number was tested as a covariate. In Glasshouse experiments 3 and 4, N rate and plant species were used as fixed factors and plant number was tested as a covariate (Appendix 5 and Appendix 6). Regression curves were also fitted to the data for Glasshouse experiments 3 and 4. Linear regressions were applied to growth and

herbage N data, whilst polynomial regressions were fitted to %Ndfs data for both species. In the field experiment, inoculant treatment was used as a fixed factor and sites were used as replicates.

6.3 Results

6.3.1 Field evaluation and genetic analysis

At all 10 field sites, all *L. polyphyllus* plants sampled were heavily nodulated. All nodules were pink inside and assumed to be functional. Twenty-one isolates were then obtained from these nodules under laboratory conditions (Figure 6.2). The 21 isolates were then shown to form nodules on *L. polyphyllus* plants when they were inoculated under sterile conditions. The acetylene reduction assay confirmed that nitrogenase activity was occurring due to the high concentration of ethylene (C_2H_4) produced by inoculated plants. The mean concentration of ethylene from uninoculated plants was 7.8 µl/L (2.64-16.92 µl/L) whilst the mean concentration for inoculated plants was 508.8 µl/L (105-1968.3 µl/L).
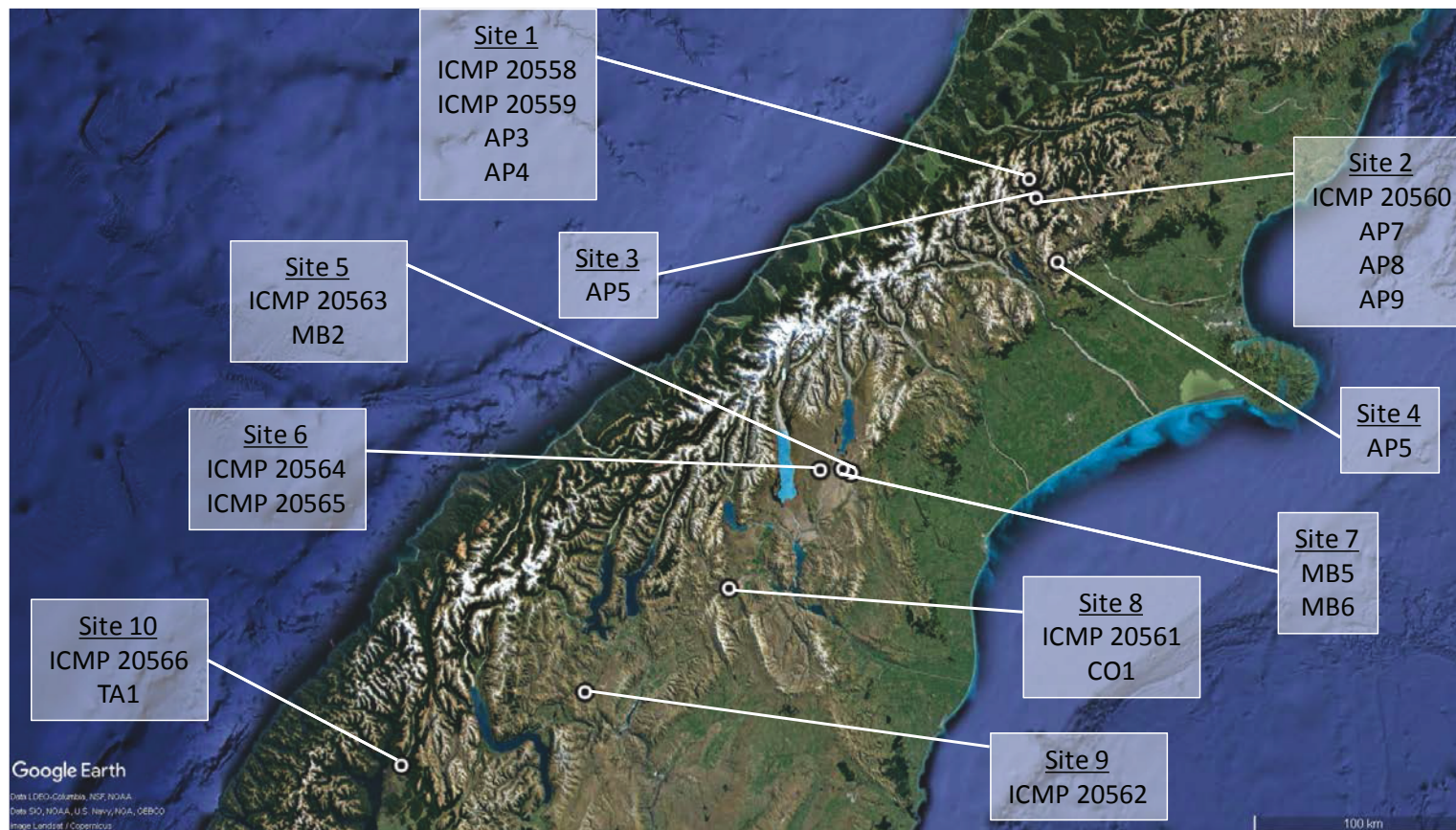
Figure 6.2 Location of *L. polyphyllus* populations that isolates were obtained from across the South Island of New Zealand in March 2013. The precise location of sites is provided in (Table 6.1).

6.3.1.1   16s rRNA

Amplification of the 16s rRNA gene sequences was successful for all isolates and identified them all as *Bradyrhizobium* sp. (Figure 6.3)*.* One isolate (AP6) showed a substantially different 16s rRNA sequence from the others. Its 16s rRNA sequence showed 99.77% (1298 bp) similarity to the *B. paxllaeri* type strain (LMTR 21) isolated from *Phaseolus lunatus* (Lima bean) in San Camilo, Peru.

The other 20 isolates from *L. polyphyllus* had similar 16s rRNA sequences that separated into four groups. Groups 1 and 2 (10 isolates) were most closely related to the Group G inoculant (Group 1: 99.20% similarity, 1257 bp; Group 2: 99.12% similarity, 1257 bp) and the *B. canariense* type strain (Group 1: 99.77% similarity, 1298 bp; Group 2: 99.69% similarity, 1282 bp) isolated from *Chamaecytisus palmensis* (tree lucerne) in La Laguna (Tenerife), Spain (Jarabo-Lorenzo *et al.*, 2003). Group 1 showed identical sequences to isolates of *Bradyrhizobium* sp*.* from *Lotus pedunculatus* (KM018177,KM018174; 1285 bp) and *C. palmensis* (KM018155, KM018151; 1259 bp) in New Zealand, and *Cytisus triflorius* (KF527973; 1299 bp) in Algeria (Ahnia *et al.*, 2014).

Group 3 was similar to, but clearly separate from, Group 4 (99.76% similarity, 1240 bp). Both groups were most closely related to the *B. ottawaense* type strain (Group 3: 99.60% similarity, 1240 bp; Group 4: 99.85% similarity, 1296 bp) isolated from *Glycine max* (soybean) in Ontario, Canada (Yu *et al.*, 2014). Isolates in Group 4 showed identical sequences to *Bradyrhizobium* sp*.* isolated from *Cytisus scoparius* (common broom) (AY491079; 1296 bp) (Weir *et al.*, 2004) and *Chamaecytisus palmensis* (KM018152; 1285 bp) in New Zealand.
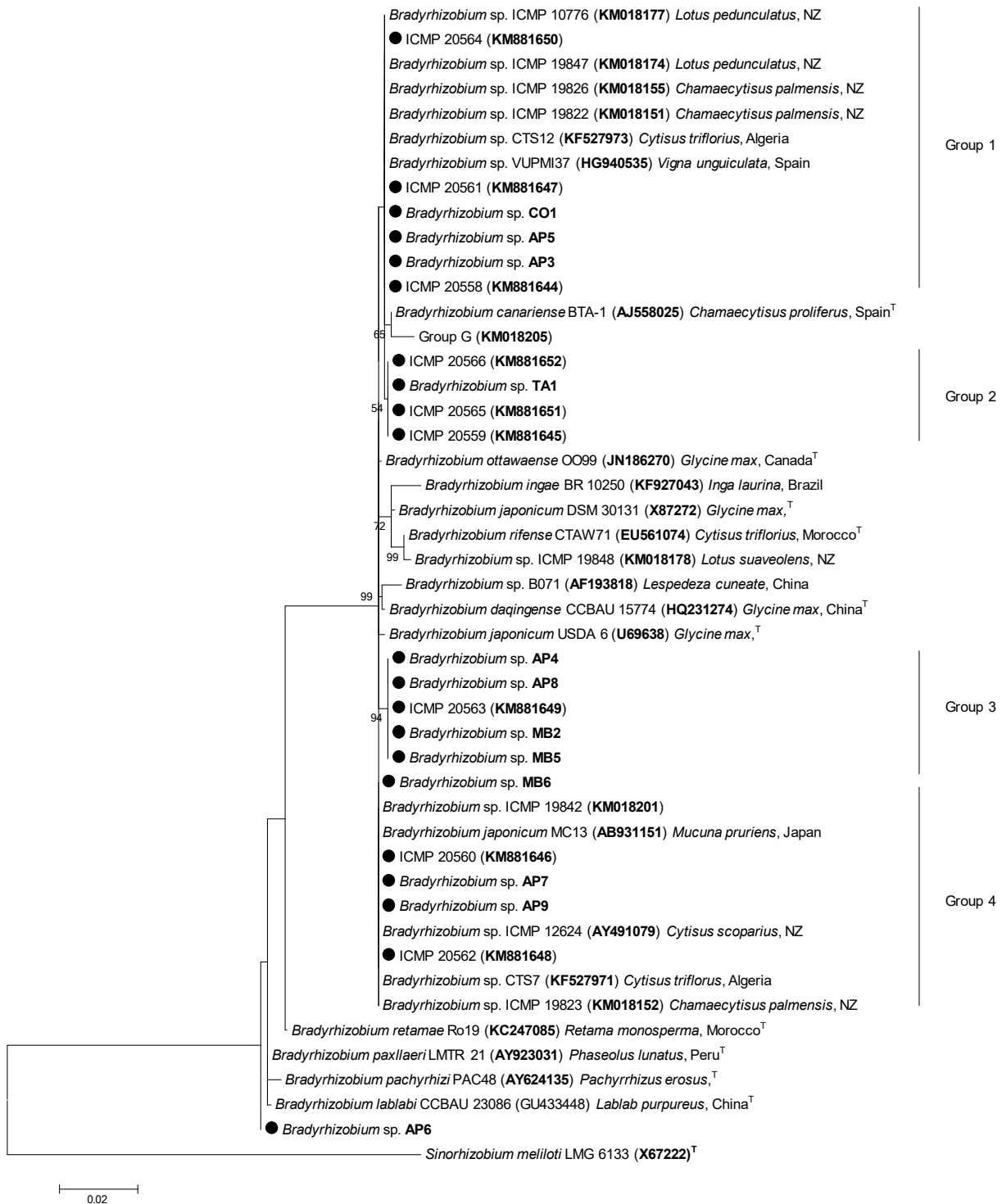
Figure 6.3 Phylogenetic tree of 16s rRNA gene sequences (ca. 1300 bp) of 21 bacterial isolates collected from the nodules of *Lupinus polyphyllus* within the South Island of New Zealand (●), closely related strains and type strains of *Bradyrhizobium* sp.. *Sinorhizobium meliloti* LMG 6133[T] was used as an outgroup. Scale bar = 2% sequence divergence (1 substitution per 50 nucleotides). Values in brackets represent the GenBank accession number for each strain. Superscript 'T' indicates type strain.

6.3.1.2   *nifH gene*

Amplification of the *nifH* gene was successful for the majority of isolates. However, isolates from the field sowing (MB5 and MB6) and one isolate from Arthurs Pass (AP6) were not successfully amplified.

The other 18 isolates divided into three separate groups (Figure 6.4). Group 1 contained 13 isolates which were most closely related to the *B. cytisi* type strain (97.83 – 98.90% similarity, 273 – 323 bp) isolated from *Cytisus triflorius* (GU001618; hairy broom) in the mountainous Moroccan Rif of northern Morocco (Chahboune *et al.*, 2011). Isolates from Group 1 were also similar (97.36 – 100% similarity, 227 – 273 bp) to isolates of *Bradyrhizobium* sp*.* taken from *L. pedunculatus* (KM018115) and *C. palmensis* (KM018093) in New Zealand. The pair of isolates from Group 2 were also closest to the *B. cytisi* type strain (GU001618; 96.59% similarity, 323 bp) whilst sharing identical sequences (287 bp) to isolates of *Bradyrhizobium* sp*.* from *C. palmensis* (KM018092) and *L. pedunculatus* (KM018118) in New Zealand. The Group G inoculant separated from the three groups and was closest to the *B. canariense* type strain (EU818926; 99.89% similarity, 297 bp) isolated from *Glycine max* in India (Chinnaswamy *et al.*, 2008).

Isolates in Group 3 showed significant deviation (41.07 – 45.28% similarity, 280 – 325 bp) from Groups 1 and 2. They were closest to, but clearly separate from, the *B. jicamae* type strain (HM047124; 45.45% similarity, 330 bp) from Southern China.
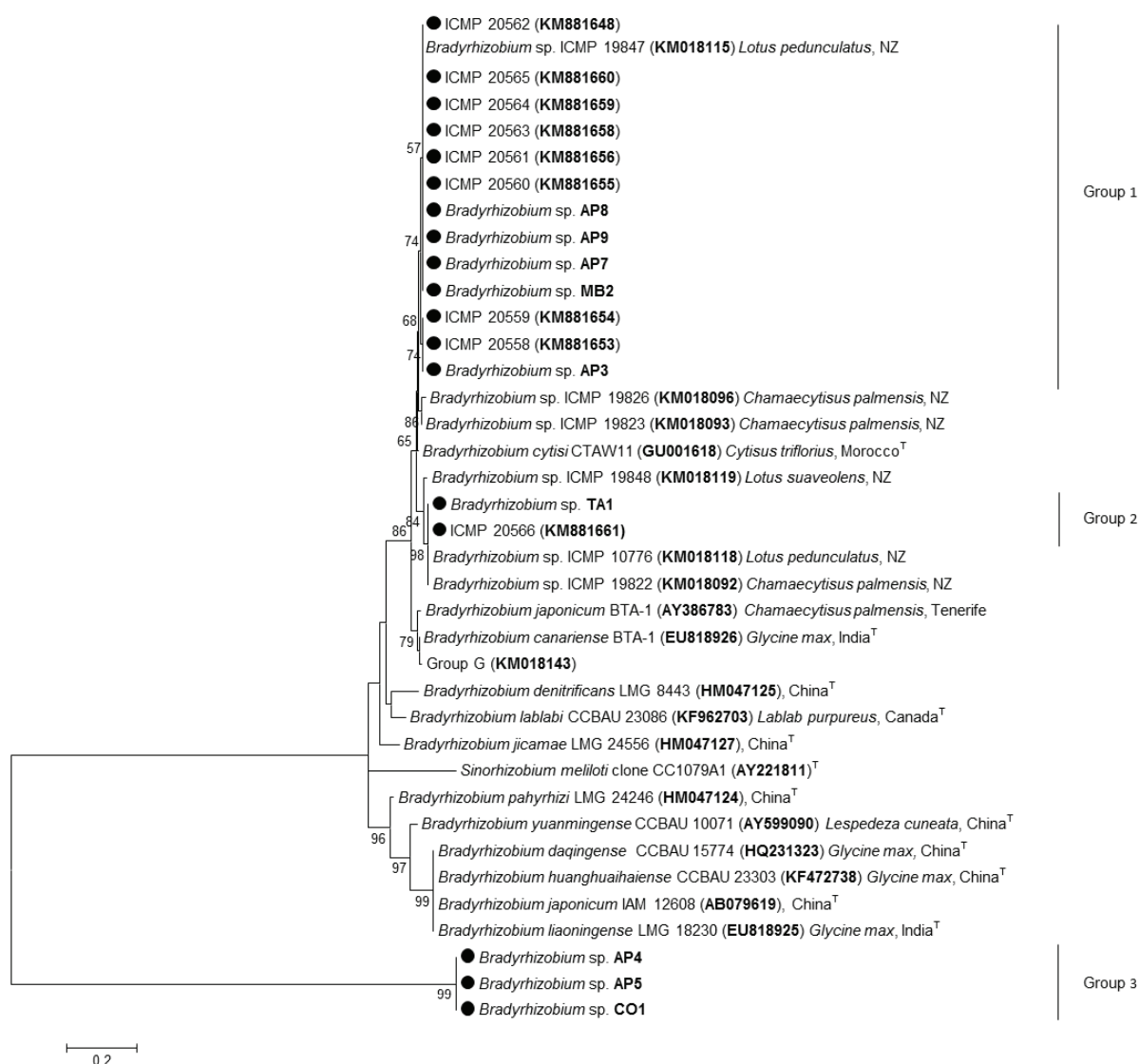
Figure 6.4 Phylogenetic tree of *nifH* gene sequences (ca. 300 bp) of 18 bacterial isolates from the nodules of *Lupinus polyphyllus* within the South Island of New Zealand (●), closely related strains and type strains of *Bradyrhizobium* sp. *Sinorhizobium meliloti* CC1079A1[T] was used as an outgroup. Scale bar = 20% sequence divergence (2 substitutions per 10 nucleotides). Values in brackets represent the GenBank accession number for each strain. Superscript 'T' indicates a type strain.

6.3.1.3   nodA gene

Amplification of the nodA gene was successful for all isolates. However, isolate AP6 showed substantial deviation from all other isolates and was closest to the type strain for *B. pachyrhizi* (KC509198; 84.02% similarity, 463 bp) isolated from *Pachyrhizus erosus* in Costa Rica (Figure 6.5).

The other isolates were divided into three groups. Group 1 contained 15 isolates that were either identical or very similar to each other (99.04 – 100% similarity, 522 bp). Isolates in Group 2 were identical to each other and were similar (98.55 – 98.74% similarity, 523 – 557 bp) but clearly separate from those in Group 1. Isolates in Group 3 were identical (523 bp) to *Bradyrhizobium sp.* isolates from plants of the *Lotus* genus in Australia, New Zealand and Portugal. Groups 1, 2, 3, and the Group G inoculant were closest to the type strain for *B. cytisi* (KC509202; Group 1: 96.13 - 96.56% similarity, 465 bp; Group 2: 96.77% similarity, 465 bp; Group 3: 92.90% similarity, 465 bp; Group G: 93.89% similarity, 360 bp) which was isolated from *C. villosus* in Morocco. Isolates from Group 2 were both from Te Anau and had identical sequences to each other (465 bp) and isolates from *Lotus* sp. from New Zealand and Portugal.
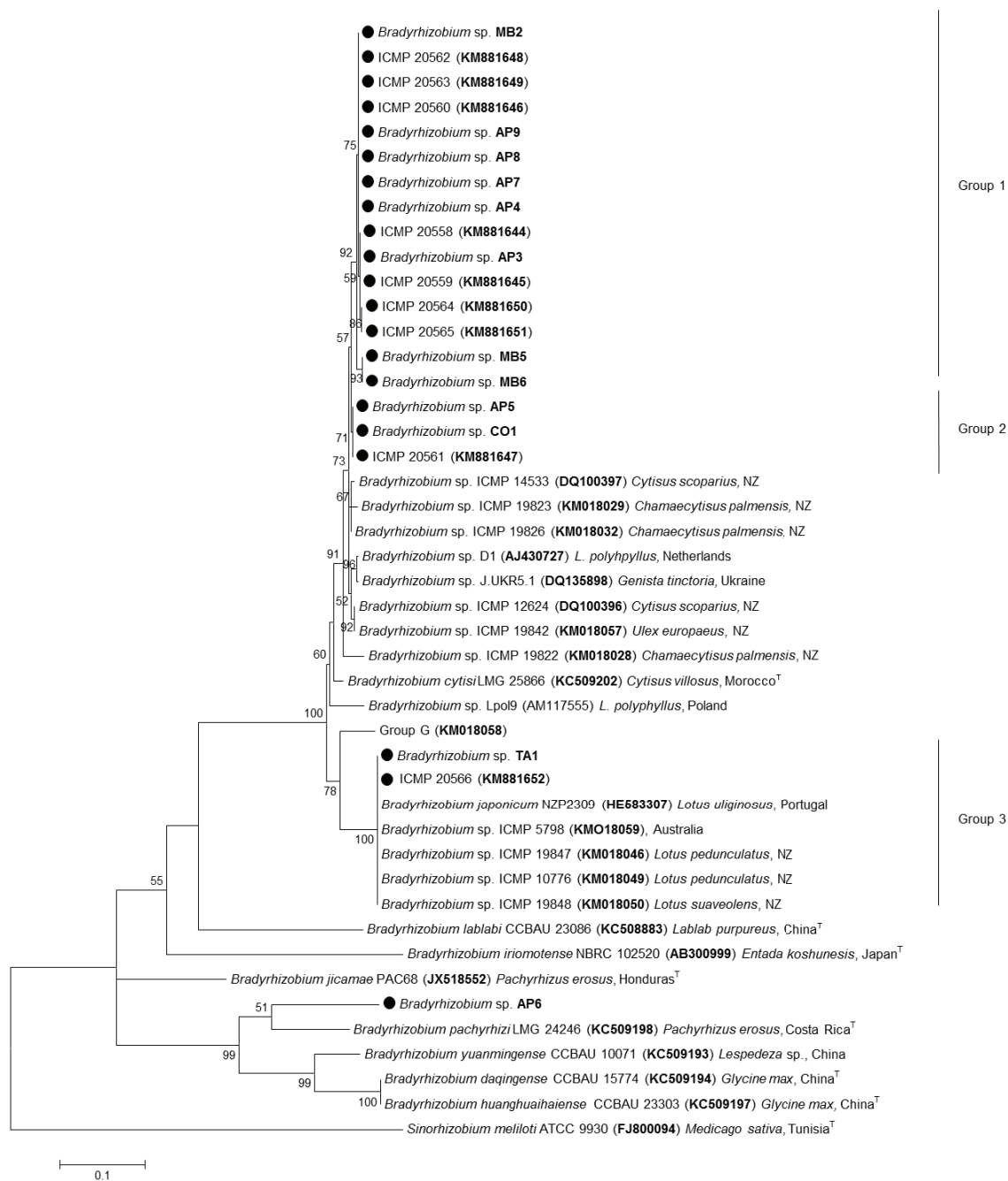
Figure 6.5 Phylogenetic tree of *nodA* gene sequences (ca. 350 bp) of 21 bacterial isolates from the nodules of *Lupinus polyphyllus* within the South Island of New Zealand (●), closely related strains and type strains of *Bradyrhizobium* sp.. *Sinorhizobium meliloti* ATC 9930^T was used as an outgroup. Scale bar = 10% sequence divergence (1 substitution per 10 nucleotides). Values in brackets represent the GenBank accession number for each strain. Superscript 'T' indicates a type strain.

Glasshouse Experiments 1 and 2 – inoculant effects on growth

In both glasshouse studies, all plants were nodulated at harvest regardless of inoculum treatment. The application of inoculant did not increase (P=0.439) plant weight in the first glasshouse experiment (Figure 6.6). Results of the second glasshouse experiment trended (P=0.066) toward increased plant weight with the application of inoculant (Figure 6.6). In both glasshouse studies, the Group G inoculant did not provide improved growth above the control or any other inoculant treatment.
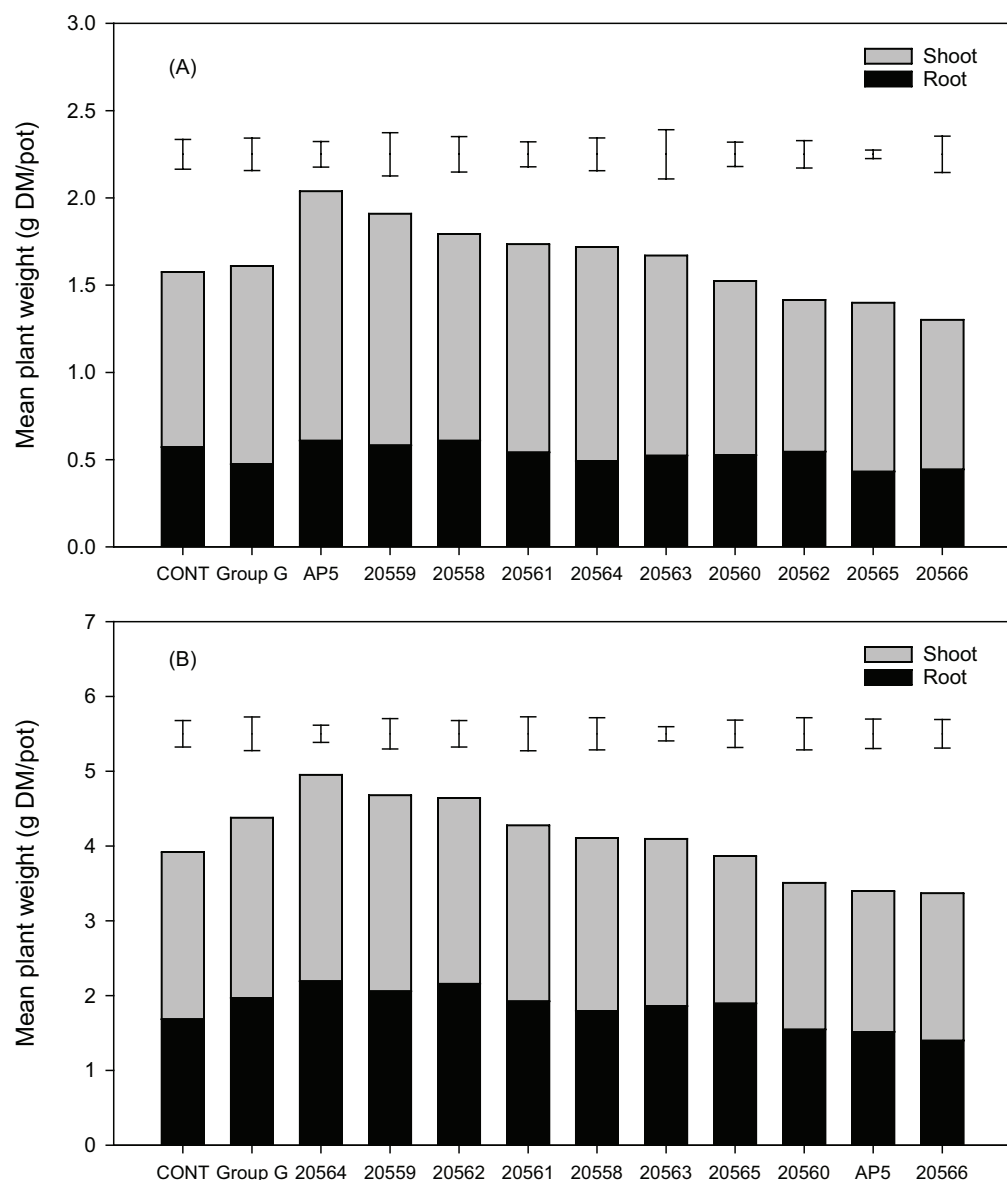
Figure 6.6 Mean dry weight of *L. polyphyllus* plants inoculated with rhizobial isolates in two separate glasshouse studies [(A): 25/2/14 – 14/5/14 and (B): 16/6/14 – 9/10/14] at Lincoln University, Canterbury. Plant weight is compared against an uninoculated control (CONT) and a commercial inoculant (Group G). The origin of the isolates is presented in Figure 6.2. Numerical values represent the ICMP number of some strains. Error bars represent one standard error of the mean for total plant weight.

6.3.3 Glasshouse Experiments 3 and 4 – effect of nitrate on N_2 fixation

The yield of *L. polyphyllus* was not (*P*=0.423-0.641) affected by the application of N fertilizer in either of the glasshouse experiments (Figure 6.7). Yield ranged between 3.1-4.2 g DM/pot in Experiment 3 and 3.4-6.2 g DM/pot in Experiment 4. Similarly, the yield of lucerne was not influenced (*P*=0.138) by N application in the first glasshouse experiment and ranged between 3.8-5.1 g DM/pot (Figure 6.7). In Experiment 4, lucerne yield was affected (*P*<0.001) by N fertilizer and followed a polynomial regression (R^2=0.41). This meant that yield was highest at an application rate of 250 kg/ha where yield was 12.5 g DM/pot and then declined to 9 g DM/pot at 600 kg N/ha. Fertiliser application had no (*P*=0.185-0.246) effect on the shoot:root of *L. polyphyllus* which was 2-2.6 in both experiments. In Experiment 3, the shoot:root of lucerne was 1.1-1.5 and did not (*P*=0.320) respond to N application. In Experiment 4, lucerne plants receiving 0 to 200 kg N/ha had a shoot:root of 1.22 which was lower (*P*=0.026) than 1.5 for plants grown at 400 kg N/ha.

The N concentration of *L. polyphyllus* was not (*P*=0.466-0.688) affected by N application in either experiment (Figure 6.8). In both experiments, the mean N concentration was 3.4% and ranged between 3.33-3.53% in Experiment 3 and 3.33-3.64% in Experiment 4. Mean plant N concentration was 2.90% (2.71-3.17%) for lucerne and was not (*P*=0.07) influenced by N rate in Experiment 3 (Figure 6.8). The N concentration of lucerne was affected (*P*<0.001) by N application in Experiment 4. However, changes in N concentration were not systematically related to the rate of N application (linear regression, R^2=0.09).

The application of N fertilizer had a significant (*P*<0.001) effect on the %Ndfs for *L. polyphyllus* and lucerne (Figure 6.9). In Experiment 3, *L. polyphyllus* was non-linear (R^2=0.98) in its response to N application. The %Ndfs increased from 5.1% to 43.1% as N application increased from 50 to 200 kg N/ha. Similarly, for lucerne, %Ndfs increased from 9.5% to 56.8% at rates of 50 and 200 kg N/ha, respectively (R^2=0.95). In Experiment 4, %Ndfs for *L. polyphyllus* increased (*P*<0.001) to 26.3% when 100 kg N/ha was applied. The %Ndfs increased (R^2=0.90) to a maximum of 62% for *L. polyphyllus* plants receiving 600 kg N/ha. For lucerne, the %Ndfs increased from 10.0% to 56.3% as fertilizer application increased from 50 to 200 kg N/ha, respectively (R^2=0.98). The %Ndfs peaked at 74% for lucerne plants receiving 600 kg N/ha.
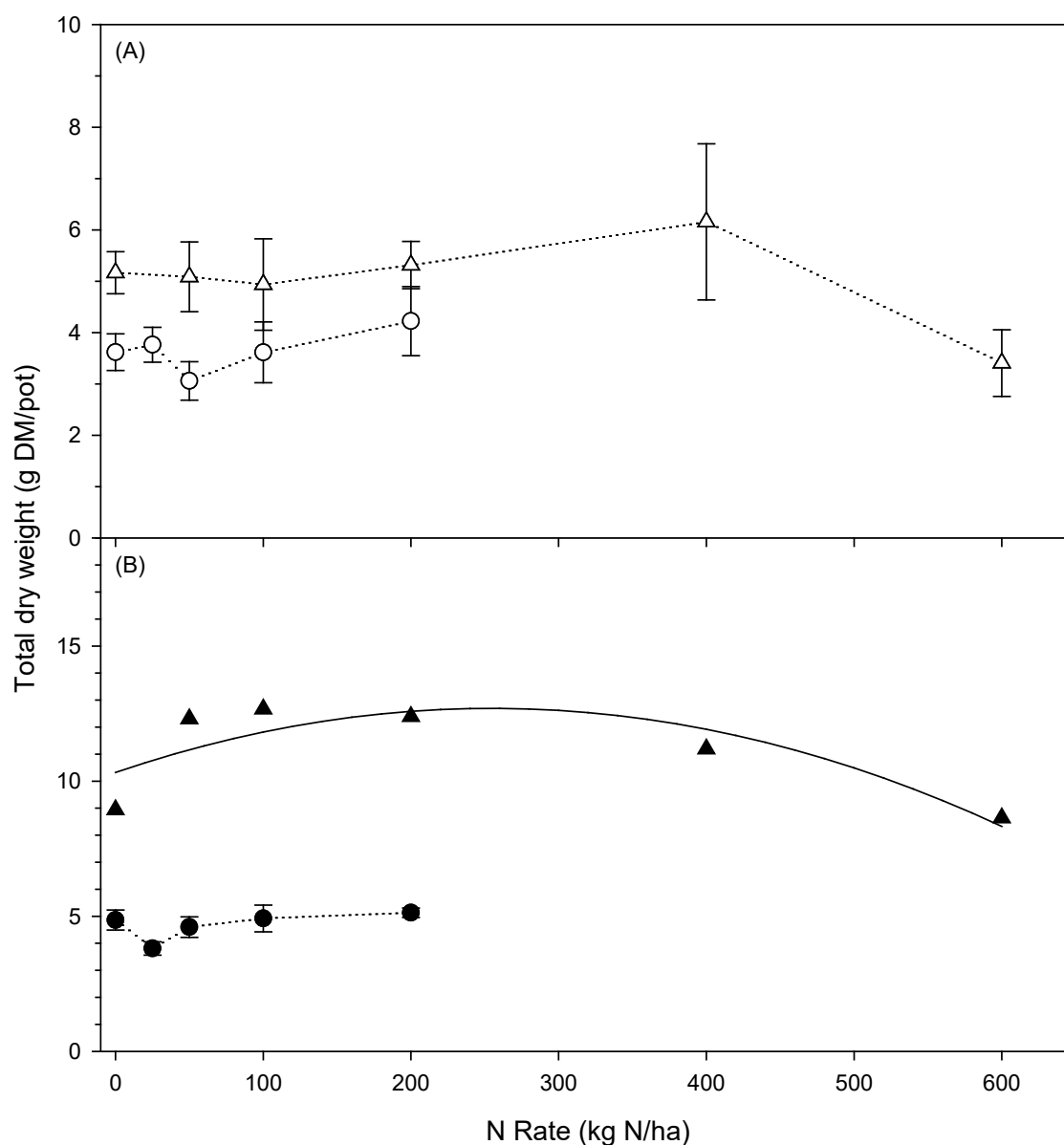
Figure 6.7 Yield (g DM/pot) of (A) *L. polyphyllus* and (B) lucerne in response to the application of labelled potassium nitrate ($^{15}KNO_3$ (10% ^{15}N atom%)) fertiliser in the Alluminex glasshouse at Lincoln University, Canterbury. Experiment 3 was conducted between 11 April 2014 and 23 July 2014 (○/●) and Experiment 4 was conducted between 2 September 2015 and 23 November 2015 (△/▲). Error bars represent the standard error of the mean.

Note: Polynomial regressions – (B) EXP 4: Dry Weight = 10.32 + 0.019 * N rate – 0.0000366 * N rate^2 (R^2 = 0.41).
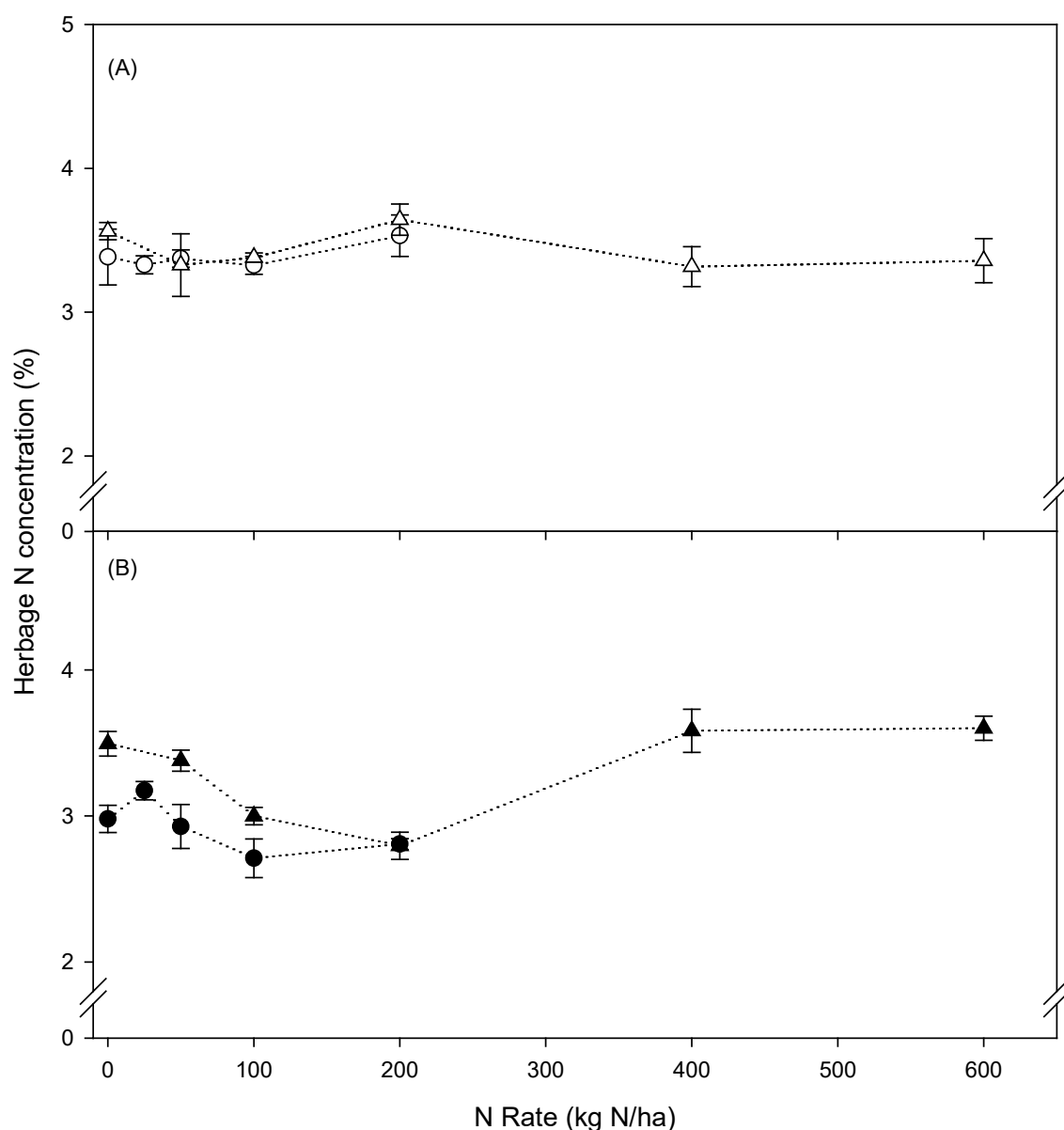
Figure 6.8 Herbage N concentration (%) of (A) *L. polyphyllus* **and (B) lucerne in response to the application of labelled potassium nitrate ($^{15}KNO_3$ (10% ^{15}N atom%)) fertiliser in the Alluminex glasshouse at Lincoln University, Canterbury. Experiment 3 was conducted between 11 April 2014 and 23 July 2014 (○/●) and Experiment 4 was conducted between 2 September 2015 and 23 November 2015 (△/▲). Error bars represent the standard error of the mean.**
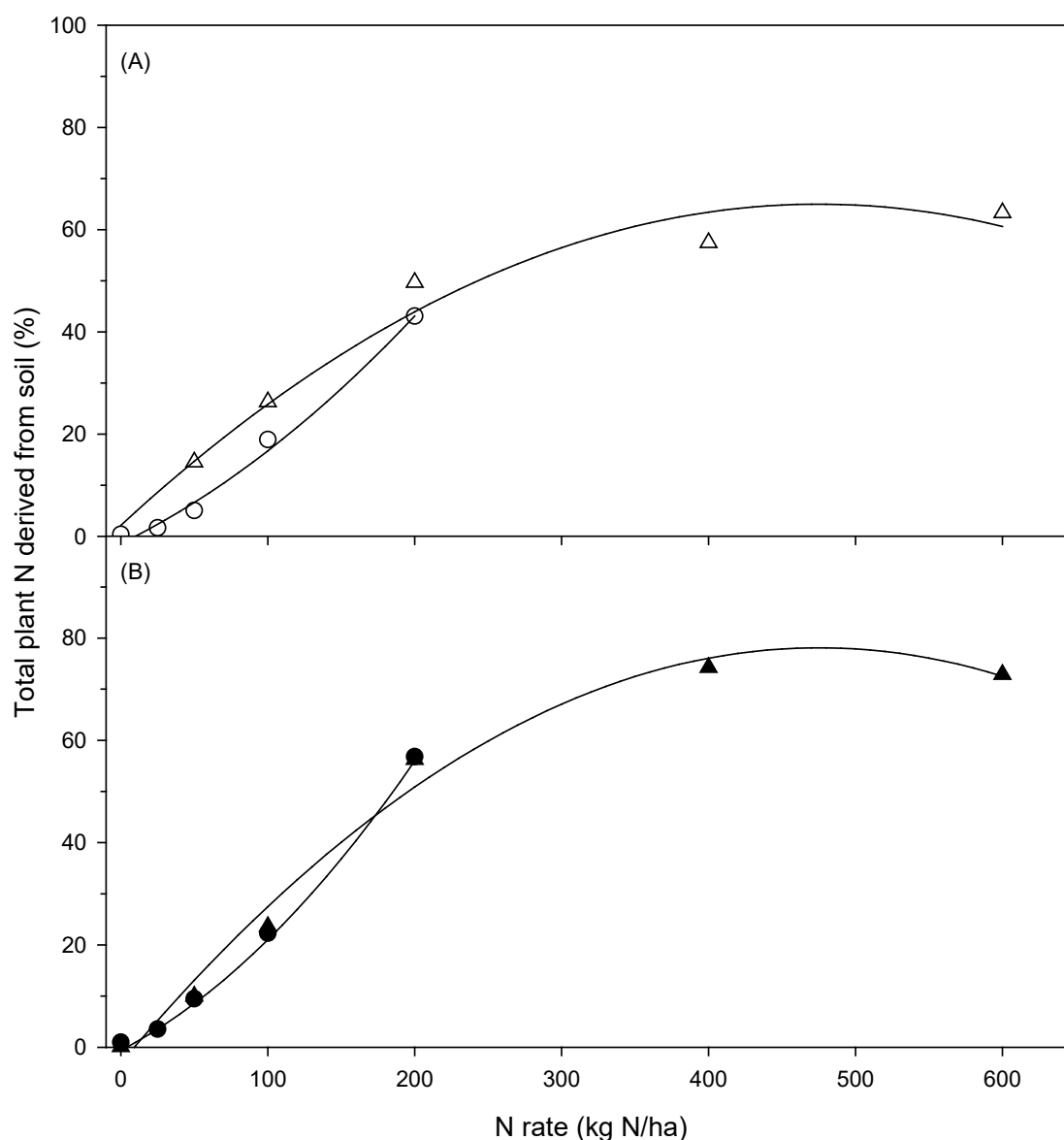
Figure 6.9 Total plant N derived from the soil (%Ndfs) for (A) *L. polyphyllus* **and (B) lucerne in response to the application of labelled potassium nitrate ($^{15}KNO_3$ (10% ^{15}N atom%)) fertiliser in the Alluminex glasshouse at Lincoln University, Canterbury. Experiment 3 was conducted between 11 April 2014 and 23 July 2014 (○/●) and Experiment 4 was conducted between 2 September 2015 and 23 November 2015 (△/▲).**

Note: Polynomial regressions – (A) EXP 3: %Ndfs = -1.38 + 0.14 * N rate + 0.000413 * N rate^2 (R^2 = 0.98), EXP 4: %Ndfs = 2.19 + 0.2646 * N rate - 0.00028 * N rate^2 (R^2 = 0.90), (B) EXP 3: %Ndfs = -0.6 + 0.1493 * N rate + 0.00067 * N rate^2 (R^2 = 0.95), EXP 4: %Ndfs = -3.08 + 0.3417 * N rate – 0.00036 * N rate^2 (R^2 = 0.98)

6.3.4 Field experiment

L. polyphyllus plants were nodulated regardless of inoculant application at all high-country sites. Inoculation significantly (*P*=0.007) improved the growth of seedling plants from 1.32 g (±0.15) to 1.89 g (±0.14) across the six sites (Figure 6.10). Visual assessment also indicated that plants receiving inoculant were healthier (Figure 6.11).
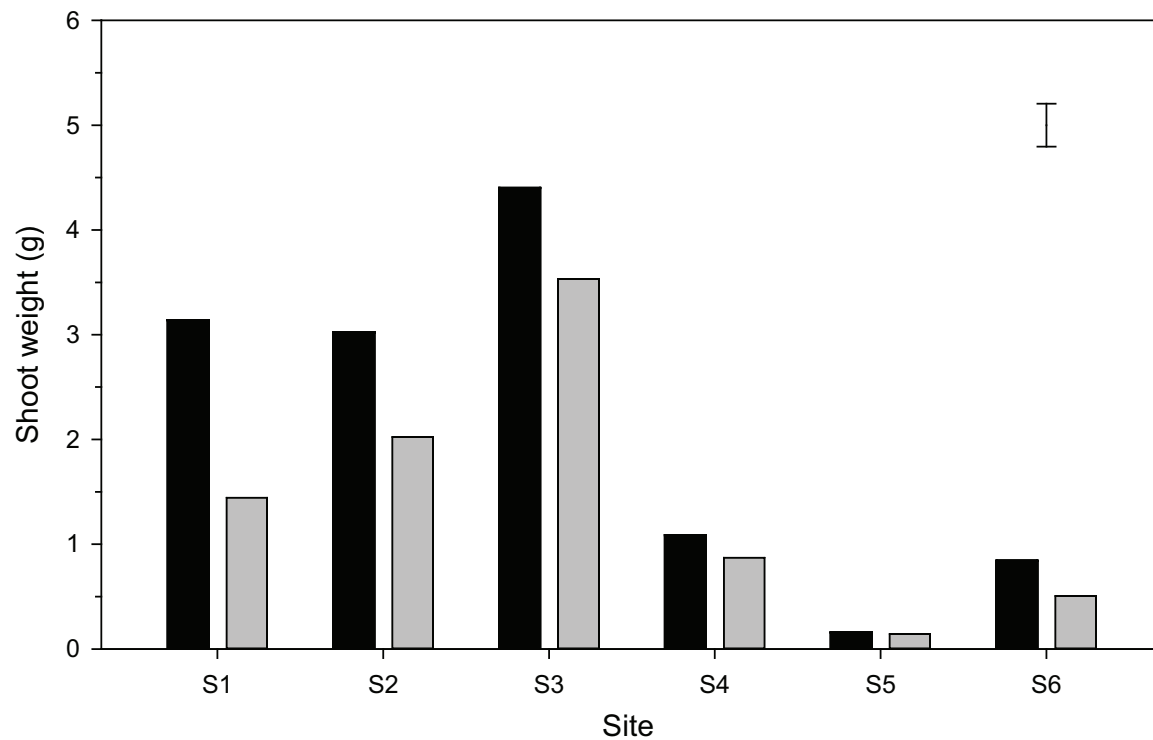
Figure 6.10 Shoot dry weight (g/plant) of *L. polyphyllus* plants sown with Group G inoculant (●) or without inoculant (●) at six high-country sites in the South Island of New Zealand. A full description and location of these sites can be found in Table 6.9. Error bar represents the LSD (*P*<0.05).
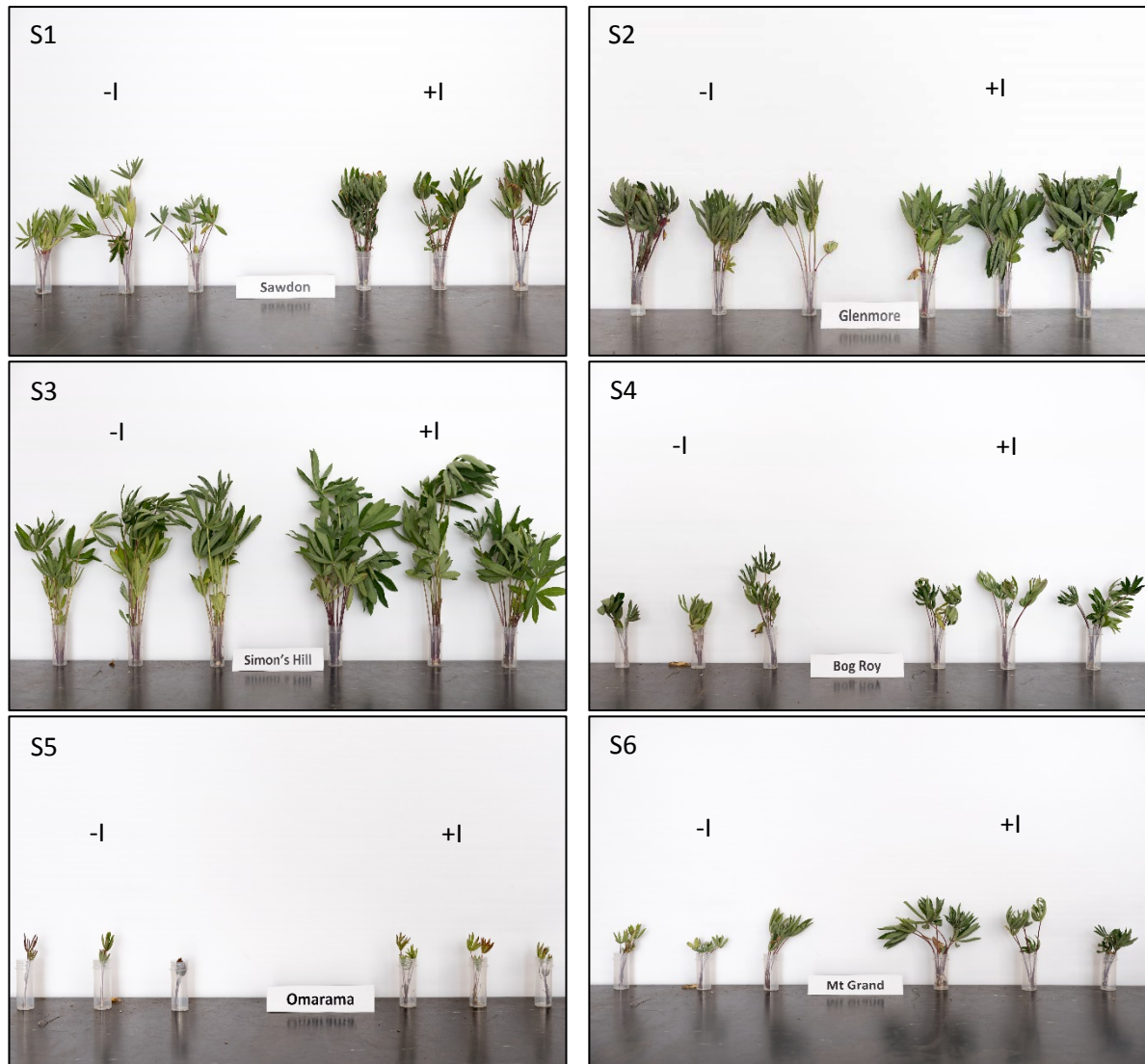
Figure 6.11 Representative examples of plants sown with (+I) and without (-I) at six high-country sites in the South Island of New Zealand. A full description and location of these sites can be found in Table 6.9.

6.4   Discussion

This chapter was a broad-ranging investigation into the relationship between *L. polyphyllus* and its N_2 fixation in the South Island of NZ.

L. polyphyllus plants were heavily nodulated at all field sites across the South Island (Table 6.1). These nodules were pink inside and assumed to be functional (Burton, 1972; Denison and Okano, 2003). Isolates from these nodules were then used to reinoculate *L. polyphyllus* plants under laboratory conditions and their effectiveness was evaluated with the acetylene reduction assay. Isolates gave a 60-fold increase in ethylene production over uninoculated control plants. This confirmed that nitrogenase activity was occurring and implies that rhizobial symbionts capable of forming functional nodules on *L. polyphyllus* are present throughout the South Island. In particular, isolates MB5 and MB6 were obtained from a successful agricultural stand of *L. polyphyllus* that was established using uninoculated seed near Lake Tekapo (Black *et al.*, 2014b).

The 16s rRNA housekeeping gene identified *Bradyrhizobium* spp*.* as the primary symbiont partner to *L. polyphyllus* in the current study (Figure 6.3). The isolates were highly similar, in their 16s rRNA, to *Bradyrhizobium* strains isolated from a range of species within NZ and overseas. Several isolates were identical to strains isolated from *C. scoparius*, *L. pedunculatus* and *C. palmensis* in NZ (Liu, 2014) and were also similar to the *Bradyrhizobium* type strain for the woody perennial *C. proliferus* in Spain (Jarabo-Lorenzo *et al.*, 2000) and the commonly grown annual *G. max*. Furthermore, a grouping of 6 isolates showed identical sequences to *Bradyrhizobium* isolates from *Cytisus triflorius* in Algeria and *Vigna unguiculata* in Spain. This implied that *L. polyphyllus* was exclusively nodulated by *Bradyrhizobium* but showed some degree of 'promiscuity' in its selection of symbiont partners. This is consistent with other studies that have shown Genistoid legumes (*C. palmensis*, *C. scoparius*, *Lupinus spp.*) to be 'promiscous' in their selection of symbiont partners (Kalita *et al.*, 2006; Liu, 2014; Sajnaga *et al.*, 2001). There is evidence that *Lupinus* spp*.* are capable of nodulating with rhizobia from other genera (Miller and Pepper, 1988; Trujillo *et al.*, 2005). However, this is uncommon (Stepkowski *et al.*, 2011) and this was not apparent in the current study.

The phylogenetic analysis of 16s rRNA sequences divided 20 of the isolates into four separate clusters, however, one isolate from Arthur's Pass was distinctively different than all other isolates. Differences between the four groupings were small compared with isolate AP6 from Arthur's pass. A comparative analysis of the isolates that fell within the four groups showed that their 16s rRNA sequences had a similarity of 99.84%. However, when those isolates were compared with AP6, similarity ranged

between 97.29 and 97.61%. This indicated that the 16s rRNA sequence of AP6 was more similar to a *Bradyrhizobium* isolate from *Phaseolus lunatus* in Peru (Duran *et al.*, 2014) than it was to isolates from New Zealand. The association with *P. lunatus*, a commonly grown crop within NZ, may have occurred from past use of inoculant with *Phaseolus* sp. in NZ. However, further analysis of its origin was limited by the unsuccessful amplification of the *nodA* gene in this study.

Phylogenetic analysis of the *nifH* and *nodA* genes showed general congruency between the symbiosis genes. In contrast to the broad groupings of the 16s rRNA sequences, 85% of the *nodA* sequences formed a single group that were clearly separate from European isolates of *L. polyphyllus, Bradyrhizobium* type strains and the commercial Group G inoculant (Figure 6.5). Similarly, for *nifH*, >70% of isolates resided in a single group that contained only one isolate from outside this study (isolated from *Lotus pedunculatus* in Taupo, NZ) (Figure 6.4). Therefore, despite being sampled across several geographic localities, the symbiosis genes remained distinctly related to the host plant, *L. polyphyllus*. The widespread occurrence of these bradyrhizobia may have resulted from 1) an inoculant used in New Zealand in the past, 2) a strain from outside New Zealand that has become established with *L. polyphyllus* throughout the South Island, and 3) naturally occurring bradyrhizobia that nodulate *L. polyphyllus* in NZ. Further work, including sequencing other housekeeping (e.g. *glnII, dnaK*) and symbiosis (e.g. *nodC*) genes, is required to clarify this point.

Inoculation of glasshouse plants with a selection of *Bradyrhizobium* isolates and the commercial inoculant did not improve plant growth above the uninoculated control (Figure 6.6). Results of the second glasshouse study were approaching significance. However, this was related to differences in performance between the highest and lowest performing treatments rather than inoculated versus uninoculated treatments. All uninoculated plants nodulated in both glasshouse studies. Given the widespread occurrence of effective *Bradyrhizobium* in NZ (Liu, 2014; Ryan-Salter *et al.*, 2014; Weir *et al.*, 2004), and efforts made to reduce cross-contamination in this experiment, it appears that a rhizobial symbiont was present in the agricultural soil that had no known history of *L. polyphyllus*. The results indicated that there may be performance differences between isolates. However, any advantage in seedling growth as a result of inoculation was not confirmed.

The use of a rhizobial inoculant led to improved seedling growth of *L. polyphyllus* at high-country sites (Figure 6.10). *Lupinus polyphyllus* was nodulated regardless of inoculant treatment, however, the use of commercial Group G inoculant increased mean shoot weight by 40% despite considerable variation in shoot weight between sites. These results were a contrast to Glasshouse Experiments 3 and 4 where

the Group G inoculant did not offer growth improvements over control plants. However, they agree with Scott (1989), whose field observations suggested that inoculants are not essential but can lead to improvements in early growth of *L. polyphyllus*. In some cases, plant growth benefits from inoculation are attributed in improved symbiotic effectiveness of the rhizobia that were introduced through the inoculant (Ballard *et al.*, 2002; Howieson *et al.*, 2011). However, Ballard *et al.* (2003) found that inoculation can lead to improved legume growth, even where suitable resident rhizobia exist, due to the significant increase in rhizobia at close proximity to the seed. This appears to be the more logical explanation for the results given the lack of an effect from Group G inoculant under controlled conditions.

The application of N fertiliser led to the uptake of inorganic soil N and downregulation of N_2 fixation in *L. polyphyllus* and lucerne. (Figure 6.9). In both glasshouse experiments, the dry weight of *L. polyphyllus* plants and the herbage N concentration were not affected by N application. However, the %Ndfs of *L. polyphyllus* consistently increased with N application up to a maximum of 62% at 600 kg N/ha. This confirmed that *L. polyphyllus* was downregulating N_2 fixation and utilising inorganic soil N. This was similar for lucerne, where changes in yield and N concentration were minor compared to the considerable increase in %Ndfs, which peaked at 74% when 400 kg N/ha was applied. These results agree with several other studies that associate increased %Ndfs with the downregulation of N_2 fixation in legumes under glasshouse and field conditions (Carlsson and Huss-Danell, 2003; Dayoub *et al.*, 2017; Ledgard *et al.*, 2001; Liu *et al.*, 2016). The ability of *L. polyphyllus* to downregulate N_2 fixation in response to elevated levels of inorganic soil N has important environmental implications that will be discussed at greater length in Section 7.3.

6.5   Summary and conclusions

- The widespread presence of rhizobia capable of nodulating *L. polyphyllus* was confirmed by the presence of healthy nodules on *L. polyphyllus* plants throughout a range of geographically separate sites. Plants were nodulated with *Bradyrhizobium* sp. at all high-country sites and have similar 16s rRNA sequences to *Bradyrhizobium* that nodulate several Genistoid legumes in NZ.

- The symbiosis genes (*nifH* and *nodA*) were generally congruent, which indicated there was a degree of specificity between *L. polyphyllus* and its nodulation partners. However, further genetic analysis is required to clarify this point.

- The exact source of *Bradyrhizobia* that nodulate *L. polyphyllus* in NZ is unknown and further work would be required to clarify this.

- Lupins showed similar growth in pot trials regardless of inoculant treatment. Control plants were nodulated which indicated the presence of rhizobia within the high-country soil. Inoculated plants did show some differences in growth, which indicated there was a possibility of selecting eite rhizobia strains. However, this was beyond the scope of this experiment and further work would be required.

- Use of Group G inoculant improved seedling growth of *L. polyphyllus* in high-country field experiments. Given the relatively low cost of inoculant, compared to other aspects of pasture renovation, its use is advised.

- Elevated levels of inorganic soil N led to the downregulation of N_2 fixation and soil N assimilation in *L. polyphyllus* and lucerne. This means that both species were capable of utilising soil nitrogen.

7   General discussion

The aim of this thesis was to understand the potential of *L. polyphyllus* to improve productivity of high-country grazing systems. Information was gathered from on-farm studies to glasshouse experiments. The results will aid farmers to understand the role that *L. polyphyllus* can play within their grazing system and give them greater confidence in its use as a forage legume.

7.1   Productivity of lupin-based pastures

In Chapter 3, the on-farm study at Sawdon Station provided insight into the productivity of lupin-based pastures. Previous studies have quantified the productivity of lupin-based pastures through agronomic measurements or grazing with dry livestock. However, to our knowledge, this was the first study to evaluate lupin-based pastures as a feed platform for reproductive ewe flocks.

The reproductive potential of ewes was maintained when grazing lupin-based pastures. Ewes were grazed on the lupin-based pasture for flushing, mating, lambing and lactation, which are considered the most important periods for the reproductive performance of ewes. The mean lambing rate was 112% across both measurement seasons. In comparison, the control mob had a mean lambing rate of 105% and the Merino NZ benchmark group was 103%. Liveweight data showed that ewes were of similar liveweight between lupin and control mobs. A lack of agronomic measurements on control pastures made it difficult to evaluate the exact mechanism for these results. However, the comparatively smaller paddocks and abundance of feed may have reduced the potential for mis-mothering on the lupin block. Additionally, survival of young lambs may have been improved through the shelter provided by *L. polyphyllus* plants from birth to weaning (Pers. Comm. Will Murray, Glenmore Station, Lake Tekapo).

There is limited opportunity to produce feed when moisture is not limiting in dryland farming. Thus, the period of greatest importance is the spring, when conditions are generally optimum for pasture growth. Specifically, soil moisture recharge is expected to occur during winter which then provides opportunity for rapid spring growth when temperatures warmup. In both seasons, the pasture cover of lupin-based pastures at Sawdon station increased by about 4500 kg DM/ha between October and mid-December whilst being grazed at 14-16 SU/ha. This period of growth primarily occurred between October and November when the ungrazed paddock accumulated about 9000 kg DM/ha before rotational grazing occurred. This equated to a growth rate of 151 kg DM/ha/day in 2012 and 115 kg DM/ha/day in 2013. The rate of accumulation was similar to the lupin-based pastures at Glenmore station (Moot and Pollock, 2014). The rapid growth rate meant that there was an over abundant feed

supply in spring that could be carried forward into summer where palatable components were likely to maintain most of their quality (Section 3.3.4). Therefore, *L. polyphyllus* was suitable for the 'boom then bust' pattern of pasture growth that is commonly associated with high country pastures.

The results of Chapter 3 were a contrast to the relatively low performance of *L. polyphyllus* in the intensive grazing study of Chapter 4. *L. polyphyllus* had a contribution of 30% when averaged over the establishment and second season of grazing in Chapter 4, which declined to a mean contribution of 25% over a three year period in Black and Ryan-Salter (2016). However, because *L. polyphyllus* represented up to 70% of herbage during the establishment phase, it appears that poor establishment was not the cause for the low contribution. The primary difference between the two experiments was the grazing intensity, particularly during the establishment phase. For example, subplots of Experiment 4 were stocked at a density of 85 sheep/ha during the first two full grazing cycles of cf-lupin pastures. This compares with a spring set-stocking density of 17 sheep/ha on farm paddocks with established plants in Chapter 3. The early spring grazing intensity in Chapter 4 suppressed reproductive stem growth, which peaked at about 10% during summer. In contrast, stem formed a much larger component of pasture yield in Chapter 3, where the plants became strongly apically dominant. Given the rapid productivity of *L. polyphyllus* during spring, it may be necessary for some stem to be allowed to form before heavy grazing is imposed, particularly during the vulnerable establishment phase (Scott, 1989). It seems likely that small manipulations to the establishment and grazing management used in Chapter 4 could have improved the contribution of *L. polyphyllus* to herbage yield and potential for nitrogen fixation. Specifically, the cf-lupin pastures could have been set-stocked during early spring (August to September) or grazed at a lower intensity to allow some apical dominance to occur. In mixed pastures, sheep would likely graze other species before lupin leaf material (Figure 4.14 and Figure 4.15). Therefore, less intensive grazing would probably use a combination of a lower stocking rate (~60 head/ha) and faster rotation, which would reduce the grazing pressure on *L. polyphyllus* in early spring. Another factor that possibly affected longevity was infection of the crown and root by *Fusarium heterosporum* Nees. This has been recognised as a probable cause of plant death in other studies at Lincoln University (Kitessa, 1992) and may have contributed to the plant population decline but no disease assessment was undertaken.

Livestock acceptability is a commonly debated aspect of *L. polyphyllus* grazing. The presence of quinolizidine alkaloids in the plant tissue of *L. polyphyllus* causes a bitterness that can deter grazing (Section 2.3.9.5). However, the experiments of Chapters 3 and 4 showed that sheep quickly adapted to *L. polyphyllus*. In Chapter 4, *L. polyphyllus* contributed to 31% of total yield and had a mean

utilisation of 67%, with the palatable leaf lamina components having a utilisation of 73%. This was more challenging to quantify in Chapter 3 due to a combination of stocking methods (set-stocking and rotational grazing) and the relatively low stocking rates. Observations during the on-farm trial indicated that sheep preferred companion species and small amounts of lamina material, with an increasing preference for the latter as the abundance of companion species declined. This did not appear to occur in the disappearance measurements of Chapter 4, where *L. polyphyllus* lamina utilisation was between 70 and 90%. However, being a relatively small component of the diet meant that the novelty factor was probably still high. *Lupinus polyphyllus* inflorescences were a highly preferred component for grazing sheep (Chapters 3 and 4), where utilisation was 90-100%. The presence of alkaloids is therefore a paradox. Voluntary feed intake is undoubtedly limited to some degree by the bitterness of material when animals are given a choice of feeds. However, its presence reduces the potential for overgrazing, which can considerably reduce persistence and potential for nitrogen fixation in pastures. Thus, the alkaloid may be advantageous in an extensive grazing situation where animals often exhibit selection.

The results of Chapters 3 and 4, based on high fertility sites, should be applicable to sites of lower fertility that receive small inputs of fertiliser. Despite the large geographical distance between the sites in Chapters 3 and 4, the soil conditions were similar for both. The pH at both sites was 6.0, which is generally considered optimum for pasture growth and this resulted in a low level of exchangeable aluminium. Additionally, soil test results for Olsen P were 17-24 mg/kg which would not be limiting plant growth. Therefore, neither of the studies were conducted on the target niche for this species, which is acidic soils (pH 5.0-5.5) with high levels of exchangeable aluminium (> 3 mg/kg $CaCl_2$ Al). However, the productivity of lupin-based pastures in Chapter 3 were similar to those reported by Moot and Pollock (2014) in the same district. In their study, lupins were grown on a soil with a pH of 5 and exchangeable Al of 5 mg/kg. Additionally, those authors also found that the addition of 3 t lime/ha, 6 months before establishment, had no effect on yield of *L. polyphyllus*. Similarly, at the Mt John trial site, *L. polyphyllus* became the dominant pasture component on soils with a pH of 5.2 and exchangeable aluminium of 3.0 mg/kg (Scott, 2014). However, both experiments did use inputs of fertiliser (Scott (1989); 10 kg P & 20 kg S/ha/year, and Moot and Pollock (2014); 100 kg Cropmaster 20 (19% N, 10% P and 12.5% S) at establishment. This indicates that *L. polyphyllus* yield may not be heavily influenced by pH and aluminium provided that its P and S requirements are being met.

Chapter 4 drew the important correlation between liveweight yield and herbage intake. The strong linear relationship showed that, regardless of pasture type, seasonal productivity and botanical

composition, herbage intake remained the primary driver of liveweight gain. This was a function of the amount of palatable feed on offer rather than the total amount of herbage on offer. Therefore, despite the increasing abundance of stem material during reproductive phases, sheep still selected their preferred diet, which was mostly comprised of leaf material. Leaf material remained palatable throughout the season for both *L. polyphyllus* and lucerne, which was reflected in its consistent nutritive value and grazing preference (Table 4.3). This contrasts with grasses under dryland conditions, which commonly experience a reduction in palatable feed production over summer due to an earlier reduction in biomass production (McGowan *et al.*, 2003; Mills *et al.*, 2015b) and a reduced nutritive value that is associated with morphological development (Buxton and Marten, 1989; Buxton, 1996). Based on this, farmers are encouraged to consider the proportion of palatable feed that is produced by a given pasture species rather than focusing on total production. They need to use a combination of pasture species are suitably matched to the environmental 'niches' that exist across their property.

Chapters 4 and 5 highlighted the importance of maximising productivity in spring when soil moisture is non-limiting. Cocksfoot-lupin pastures produced 62% of their total liveweight gain during the spring (1 September – 30 November) which was similar to 55% for lucerne. This agrees with the long-term averages found by Mills *et al.* (2015a) where cocksfoot-dominant pastures produced 63% of their total during spring and lucerne 47%. This is primarily driven by high animal and plant growth rates during spring, and also by the large proportion of palatable herbage that is produced. The growth rates were maintained until mid-summer. However, the transition to reproductive development in both pasture types meant that liveweight gain was limited by the abundance of palatable feed. For most pastures, palatability acts as a function of grazing management and temperature-driven reproductive development, whilst annual biomass productivity is limited by plant nutrition and soil moisture. The impact of environmental factors was largely responsible for the animal and pasture yield differences between cf-lupin and lucerne described in Chapters 4 and 5.

7.2   Influence of environmental variables

The productivity and palatability of cocksfoot in Chapter 4 was limited by inadequate N nutrition from N_2 fixation and soil N mineralisation. This became evident in Chapter 5, where the linear spring growth rate of cf-lupin pastures was 2.9 kg DM/ha/^{o}Cd compared with 6-9 kg DM/ha/^{o}Cd that can be expected from cocksfoot pastures receiving adequate N inputs (Fasi *et al.*, 2008; Mills *et al.*, 2009). The N limited growth was reflected by an N concentration of 2.35% for cocksfoot leaves between November and February and an annual mean of 2.7%. This meant that cocksfoot spent a considerable

proportion of the growth season at an N concentration close to the critical limiting deficit of 2.6%, which would have strongly limited its growth through reductions in photosynthetic efficiency (Peri *et al.*, 2002).

In Chapter 5, cf-lupin and lucerne pastures had a similar soil moisture extraction depth but lucerne produced about 1700 kg DM/ha during the linear spring growth phase. The yield advantage was driven by a higher linear growth rate, improved WUE and the extraction of an additional 50 mm of water from soil depths between 65 and 155 cm. This meant that productivity was increased within the spring period but did not extend the period of linear growth (Figure 5.2). For cf-lupin pastures, extraction to a depth of 1.65 m implied that the taproot of *L. polyphyllus* had increased moisture extraction beyond 0.8 m; which is the extraction depth expected for cocksfoot (Mills, 2007; Mills *et al.*, 2006). However, the opportunity for additional moisture extraction was limited by the relatively small contribution of *L. polyphyllus* to herbage production over summer (Section 4.3.6). This meant that the productivity of cf-lupin pastures was mostly dependent on the dominant cocksfoot component.

The productivity of both pasture types was variable in Chapter 4. For example, annual herbage yield ranged from 5141 to 8972 kg DM/ha for cf-lupin pastures and 7316 to 10,991 kg DM/ha for lucerne. This variability was partially described by the results of Chapter 5 where the PAWC of lucerne pastures in Paddock 2 ranged from 275 mm to 366 mm. Defining this pattern was confined to Paddock 2 due to the limited number of neutron probe access tubes that were installed. However, the effects of soil textural variability were evident.

7.3   Nitrogen fixation and N assimilation

The common occurrence of the symbiotic partner, *Bradyrhizobium* spp*.*, for *L. polyphyllus* throughout the South Island would suggest that an inoculant is not required for successful nodulation. Chapter 6 confirmed that wild populations of *L. polyphyllus* throughout the South Island have formed effective relationships with rhizobia of the *Bradyrhizobium* genus. The widespread occurrence of these rhizobia may have resulted from 1) widespread use of an inoculant in the past, 2) a strain from outside NZ that has become associated with *L. polyphyllus* or 3) naturally occurring *Bradyrhizobia* in NZ. Of all the sites examined, isolates from Te Anau were the only strains that gave identical sequences to a commercial inoculant. Aside from this, defining the exact source of the rhizobia is limited by the number of genes that were examined in this study (16s rRNA, *nifH* and *nodA*). Whilst untested, Scott (1989) suggested that uninoculated *L. polyphyllus* plants would eventually nodulate with resident soil rhizobia, however, the use of inoculant may lead to improved early growth. Also, Berenji (2015) found that inoculation of

perennial lupin had no effect on its growth when grown in mixed-pastures at a lowland field site. This then formed the second research question of Chapter 6, which was to address the requirement of an inoculant and also evaluate whether there was value in selecting 'elite' rhizobia for a more efficient symbiotic relationship. Glasshouse experiments in Chapter 6 were inconclusive and showed considerable variability in the growth of plants that were either inoculated with a) isolates from the study, b) a commercial inoculant or c) left uninoculated. In the field experiment, *L. polyphyllus* plants nodulated regardless of inoculant treatment. However, the use of a commercial inoculant led to improved growth of *L. polyphyllus* seedlings throughout the high-country sites. This suggested that, despite the widespread presence of *Bradyrhizobia* in the South Island of NZ, the use of a commercial inoculant would improve growth of *L. polyphyllus* seedlings. Therefore, given the cost of pasture development, it seems logical to inoculate *L. polyphyllus* for improved seedling growth and survival.

In Chapter 6, *L. polyphyllus* and lucerne downregulated N_2 fixation in response to elevated levels of inorganic soil N. Both species remained relatively consistent in their herbage productivity and N concentration as the application rate of N fertilizer increased. However, the %Ndfs consistently increased for both species, where 60-75% of plant N was derived from the soil at fertilizer application rates of 400 kg N/ha. This has important implications for pasture development where increased productivity is often met with greater grazing intensity (Scott, 1994) and potential for N leaching.

In grazed systems, N rates as high as 400 kg N/ha are only likely to occur under urine patches (Cameron *et al.*, 2013; Haynes and Williams, 1993; McLaren and Cameron, 1996a; Selbie *et al.*, 2015; Silva *et al.*, 1999; Woods *et al.*, 2016). However, the frequency of these will increase as pasture management intensifies with the addition of more productive species (Scott, 1999a, 2000b) and can have negative environmental impacts caused by N loss from the root zone (McLaren and Cameron, 1996b; Selbie *et al.*, 2015). The latter has been drawn into focus by the results of recent research that have shown N loss under legume monocultures to be double that of conventional pastures over a 17 month period (Woods *et al.*, 2016, 2017). However, in these experiments, the root zone was limited to 0.7 m (lysimeter depth), which is considerably less than the 1.65 m found for cf-lupin pasture in Chapter 5 and 2.25 m found for lucerne in other studies (Brown *et al.*, 2009; Sim *et al.*, 2017). Furthermore, the soil was maintained at close to field capacity throughout the duration of that experiment to simulate an irrigated dairy pasture. This meant that plots received a total of 1965 mm of water over a 17-month period, which is about 3 times more than normally expected in dryland properties of Canterbury (Moot *et al.*, 2010) and inland high-country areas (Maxwell *et al.*, 2010; Scott, 1999a). Under dryland conditions, the extraction front progresses down the soil profile, which leads to a systematic depletion

of PAWC. This led to a SWD of 330 mm for lucerne and 275 mm for cf-lupin pastures in Chapter 5. The high SWD created by these pasture types, means that complete soil moisture recharge would have to occur before there was a risk of nutrient loss from the root zone, which did not occur in the third year of this experiment (Black and Ryan-Salter, 2016).

7.4 Conclusions

The research presented in this thesis answered several research questions around the suitability of perennial lupin for livestock grazing. In general, results of field experiments gave a comprehensive overview of the agronomic characteristics under contrasting grazing regimes. The practical application of glasshouse results were limited due to time and resource constraints. However, from the work covered, the specific conclusions were:

- Perennial lupin generates herbage rapidly during the spring period, where pastures produce about 80% of the annual total between September and December.

- Lupin-based pastures are a suitable feed for grazing maternal livestock. There were no indications of adverse effects on the reproductive cycle of Merino ewes.

- Liveweight production is primarily determined by the amount of DM consumed. Seasonal influences on pasture composition affected the overall quality of herbage but palatable components were still selected by sheep.

- Maintaining lupin-dominant pastures requires careful management during establishment. Lax grazing via set-stocking is recommended for early spring growth, however, rotational grazing may be used once plants have produced reproductive stem.

- Dryland pasture yield remains closely related to the amount of water available and how efficiently that water is used. Maximising pasture productivity will come through an abundance of legume.

- Lupin rhizobia are present throughout the South Island of New Zealand. This suggests that plants will freely nodulate without the use of inoculum. However, given the cost of pasture renovation, inoculation is still recommended due to indications of improved growth during establishment.

- Lupin and lucerne respond to increasing levels of soil nitrate by downregulating nitrogen fixation. Consistent plant N concentration indicated that N uptake was closely related to biomass production.

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Appendices
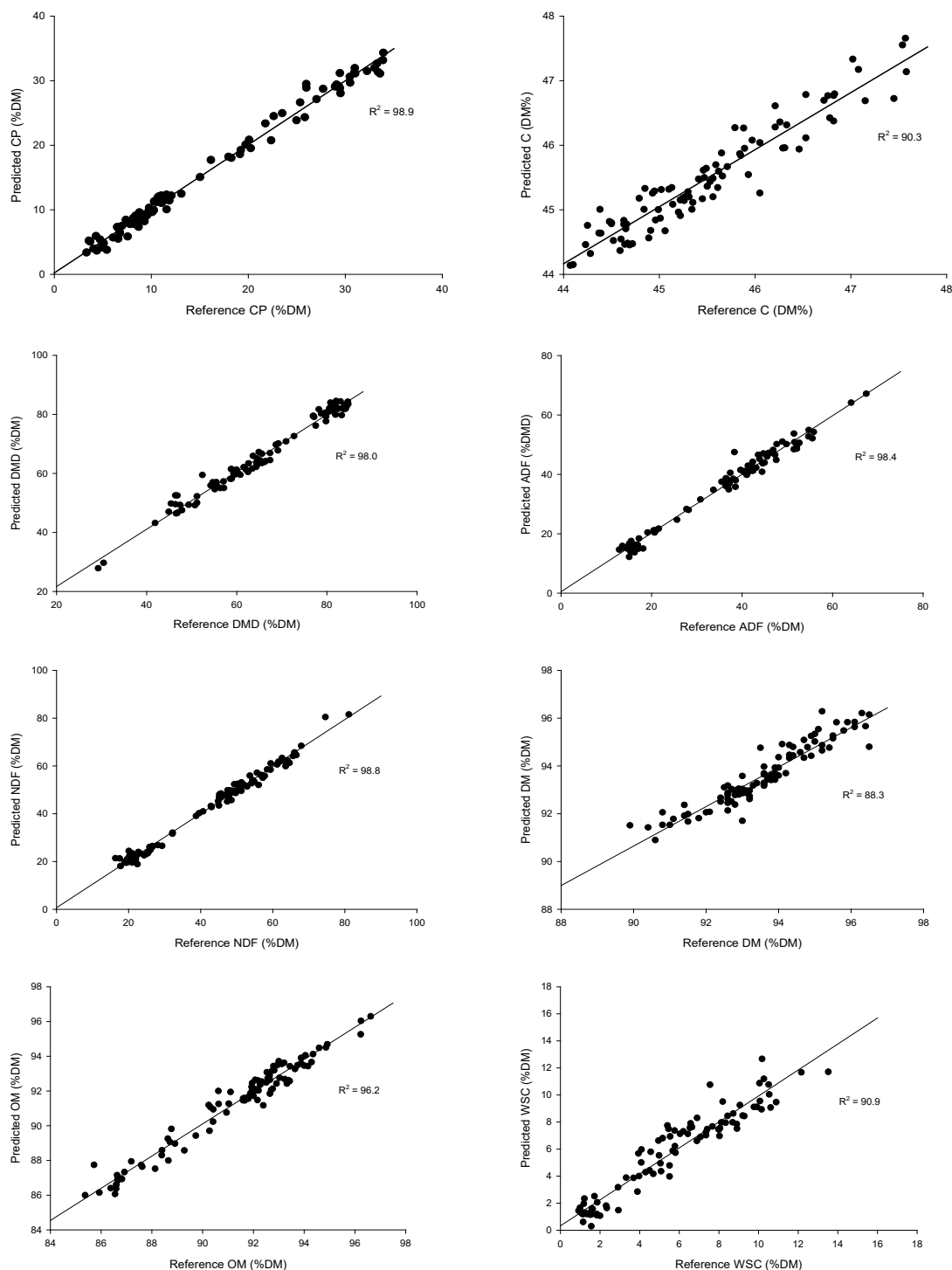
Appendix 1 Predicted values plotted against reference values for crude protein (CP), carbon (C), dry matter digestibility (DMD), acid detergent fibre (ADF), neutral detergent fibre (NDF), residual dry matter (rDM), organic matter (OM) and water soluble carbohydrate (WSC) from lamina, petiole, green stem, flower and dead material of perennial lupin.
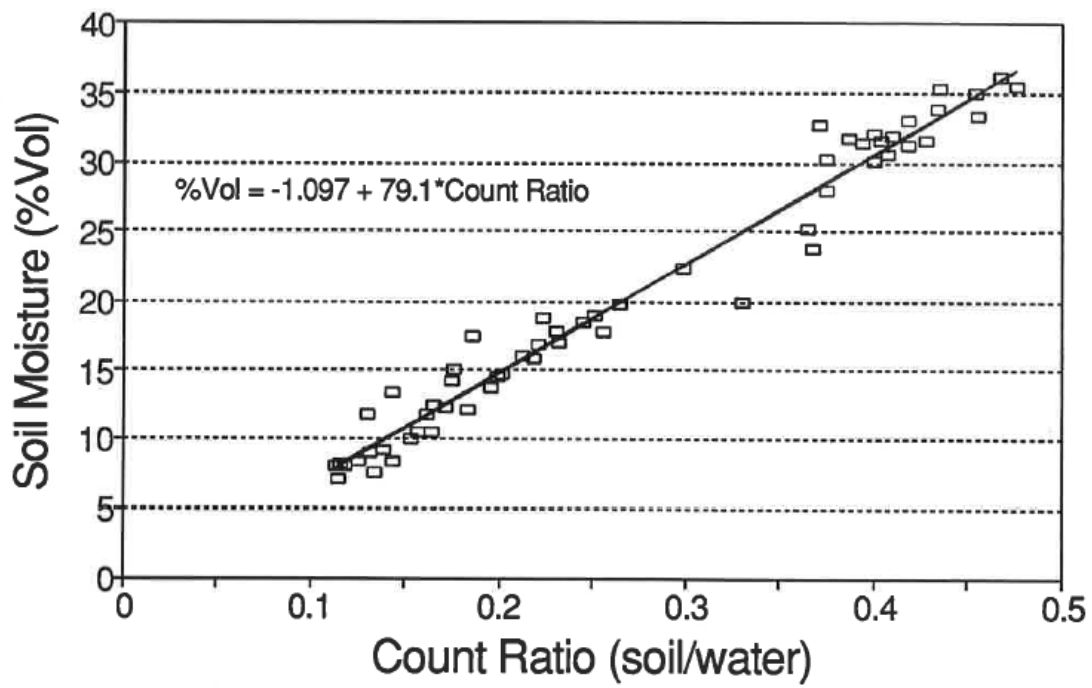
Appendix 2 Calibration data (volumetric soil moisture vs count ratio) for Troxler 4300 neutron probe. Completed by Dr Keith Pollock at Lincoln University, Canterbury.

Appendix 3 Summary of statistical outputs for Monteith framework applied to cocksfoot-lupin pastures.

Cf-Lupin	P value	R2	LL	PAWC	-kl	tc	P value	R2	LL	PAWC	-kl	tc	P value	R2	LL	PAWC	-kl	tc
Depth	R1	R1	R1	R1	R1	R1	R2	R2	R2	R2	R2	R2	R3	R3	R3	R3	R3	R3
25	<0.001	94.7	6.04	33.49	0.00969	0	<0.001	93.5	8.9	29.17	0.01188	0	<0.001	95.3	11.26	27.27	0.01274	0
35	<0.001	96.3	6.62	27.04	0.00716	0	<0.001	96.7	14.915	17.33	0.02232	32.94	<0.001	97.8	16.499	17.301	0.02061	26.81
45	<0.001	98	12.62	17.43	0.00956	26.5	<0.001	96.3	14.73	15.72	0.01319	30.2	<0.001	94.8	18.835	11.97	0.01651	33.45
55	<0.001	95.4	14.64	14.68	0.00916	45.1	<0.001	96.2	11.72	19.48	0.00599	30.18	<0.001	98.8	17.021	14.779	0.01161	33.35
65	<0.001	98	14.639	13.244	0.02248	66.38	<0.001	95.1	12.27	18.38	0.0057	34.75	<0.001	96.7	15.93	15.85	0.01272	56.13
75	<0.001	97.3	13.002	14.669	0.3833	73	<0.001	97.5	12.06	17.5	0.01321	59.51	<0.001	97.1	11.43	21.39	0.00627	58.75
85	<0.001	97.9	12.73	16.65	0.01233	67.8	<0.001	98.3	11.01	19.87	0.01477	72.59	<0.001	94.2	4.6	28.1	0.00459	76.2
95	<0.001	96.9	15.06	17.36	0.0145	104.57	<0.001	98.9	13.271	18.229	0.02632	88.44	<0.001	95.1	16.93	15.8	0.01067	79.96
105	<0.001	98.8	18.637	16.524	0.01511	130	<0.001	95.3	20.01	12.77	0.012	71.36	<0.001	97.7	15.65	17.06	0.01397	115.42
115	<0.001	93.8	19.28	15.63	0.029	145.3	<0.001	78.8	20.1	14.75	0.003073	32.28	<0.001	97.4	8.52	24.67	0.00828	118.12
125	<0.001	97.7	16.42	18	0.01023	120.95	<0.001	94.3	3.253	29.28	0.002893	138.9	<0.001	95.9	9.29	21.95	0.01475	127.89
135	<0.001	99	7.84	25.78	0.00557	92.03	<0.001	97.8	7.86	23.12	0.0078	144.78	<0.001	97.1	5.55	20.87	0.01742	123.72
145	<0.001	96.7	12.96	19.9	0.00707	94.54	<0.001	98.2	11.02	16.56	0.01776	145.91	<0.001	97.2	4.9	22.45	0.01948	123.86
155	<0.001	92.7	13.13	10.16	0.02279	147.02	<0.001	94.5	8.42	11.96	0.01425	124.94	<0.001	98.4	5.54	22.97	0.01885	124.27
165	<0.001	94.1	3.9	11.04	0.00542	93	<0.001	81.3	9.509	4.432	0.1193	161	<0.001	97.3	6.893	13.184	0.01907	124.41
175	<0.001	94.8	6.58	7.56	0.01308	123.89	<0.001	92.6	3.49	10.86	0.001946	43	<0.001	90.7	6.781	5.503	0.01894	126.01
185	<0.001	93.9	7.14	12.99	0.00513	100.4	<0.001	60.9	6.52	6.985	0.00245	94.55	<0.001	90.9	5.585	4.651	0.01413	139.95
195	<0.001	80.1	7.26	9.695	0.003967	90.96	<0.001	77.3	8.416	4.119	0.003477	98.42	<0.001	84.9	6.124	4.176	0.0174	135.2
205	<0.001	94	6.489	3.389	0.01466	132.54	<0.001	46.8	9.925	1.594	0.04579	161	<0.001	78.8	5.84	3.85	0.01254	128.2
215	<0.001	82.3	3.656	5.303	0.003417	90.42	<0.001	60.5	8.4	2.58	0.0065	133.1	<0.001	80.8	6.269	2.777	0.01125	104.5
225	<0.001	55.3	3.817	6.539	0.001726	78.82	<0.001	67.7	9.257	1.763	0.01541	161	<0.001	83.4	6.206	2.412	0.01045	103.4

Appendix 4 Summary of statistical outputs for Monteith framework applied to lucerne pastures.

lucerne	P value	R2	LL	PAWC	-kl	tc	P value	R2	LL	PAWC	-kl	tc	P value	R2	LL	PAWC	-kl	tc
Depth	R1	R1	R1	R1	R1	R1	R2	R2	R2	R2	R2	R2	R3	R3	R3	R3	R3	R3
25	<0.001	97.5	3.39	34.77	0.00798	0	<0.001	97.7	3.96	33.36	0.00796	0	<0.001	98.5	4.57	27.53	0.00928	13.68
35	<0.001	96.5	4.94	24.21	0.00633	21.88	<0.001	97.2	3.76	26.24	0.00606	29.07	<0.001	97.8	2.99	25.26	0.0066	29.6
45	<0.001	96.2	6.36	21.84	0.00544	43.21	<0.001	96.3	7.12	23.43	0.00551	21.8	<0.001	97.1	4.2	22.65	0.00571	43.47
55	<0.001	94	5.3	23.8	0.00521	50.41	<0.001	97.4	10.8	18.5	0.01035	48.68	<0.001	97.5	7.59	20.79	0.00978	59.08
65	<0.001	98.4	9.27	20.08	0.01146	46.24	<0.001	95.7	4.28	26.82	0.00692	41.24	<0.001	98.8	8.83	20.1	0.01712	73.02
75	<0.001	99	9.104	21.621	0.01972	48.23	<0.001	97.9	11.52	18.89	0.02218	80.1	<0.001	99	7.65	21.96	0.02077	78.78
85	<0.001	96.6	7.56	24.52	0.0101	54.06	<0.001	98.8	11.103	20.097	0.03105	88.46	<0.001	96.7	7.43	22.91	0.0211	93.27
95	<0.001	98	11.18	20.86	0.02463	97.95	<0.001	97.8	9.488	22.54	0.03925	101.56	<0.001	97.1	9.16	23.21	0.03294	106.34
105	<0.001	97.6	9.78	20.25	0.02681	97.26	<0.001	96.6	6.76	21.98	0.02995	93.65	<0.001	97.4	8.18	24.48	0.03642	106.42
115	<0.001	98.6	5.05	23.94	0.01799	82.24	<0.001	98	5.5	25.44	0.0115	82.14	<0.001	98.3	9.713	22.28	0.0989	127.31
125	<0.001	98.7	4.85	27.77	0.01844	85.33	<0.001	98.2	5.727	22.613	0.01623	52.8	<0.001	97.5	6.971	19.654	0.04735	116.28
135	<0.001	97.8	8.57	26.34	0.02626	121.99	<0.001	98.8	6.96	25.79	0.01539	103.68	<0.001	97.7	5.247	9.808	0.02098	106.85
145	<0.001	96.8	5.54	28.96	0.01665	136.03	<0.001	96.5	8.71	24.51	0.01642	137.58	<0.001	87.9	5.046	5.476	0.0208	135.63
155	<0.001	97.2	8.97	22.9	0.02259	142.28	<0.001	96.4	8.962	13.271	0.03041	150.72	<0.001	94.4	4.896	5.344	0.02511	150.82
165	<0.001	98.4	8.24	24.35	0.01481	152.9	<0.001	92.5	4.93	7.45	0.01446	145.17	<0.001	89.7	5.105	4.326	0.0353	159.43
175	<0.001						<0.001	87.1	3.97	7.42	0.0064	142.4	<0.001	79.4	5.08	3.5	0.0211	147.72
185	<0.001						<0.001	92.4	9.384	4.022	0.01662	131	<0.001	72.1	4.65	4.1	0.0119	149.4
195	<0.001	85.2	6.99	6.07	0.00715	115.6	<0.001	91.9	10.5	4.087	0.0207	141.95	<0.001	64.1	4.1	5.7	0.0059	142.5
205	<0.001	83.5	7.964	2.48	0.0423	159.59	<0.001	59.8	8.773	5.29	0.003072	113.6	<0.001	83.8	3.763	9.905	0.001948	102.8
215	<0.001	81.4	8.346	1.699	0.0706	176.81	<0.001	38.4	10.55	2.769	0.005182	144.7	<0.001	94.1	10.32	6.88	0.00552	36.5
225	<0.001						<0.001	32.3	10.68	2.644	0.004125	125.9	<0.001	76.8	10.98	3.58	0.0146	101.7

Appendix 5 Effect of N application on growth parameters of lupin and lucerne in Glasshouse Experiment 3 at Lincoln University, Canterbury.

Statistic	Species	P value	0	25	50	100	200	SEM	LSD
Tissue ^{15}N	lupin	<0.001	0.00	1.67	5.05	18.94	43.11	1.30	4.23
%N	lupin	0.688	3.38	3.33	3.37	3.33	3.53	0.11	0.36
DM/pot (g)	lupin	0.641	3.62	3.77	3.06	3.62	4.23	0.52	1.54
Nod score	lupin	0.718	3.08	3.33	2.83	3.00	3.00	0.25	0.74
Shoot weight (g)	lupin	0.703	0.25	0.27	0.21	0.25	0.28	0.04	0.11
Root weight (g)	lupin	0.431	1.11	1.08	0.95	1.12	1.42	0.17	0.51
Shoot:Root	lupin	0.185	2.24	2.50	2.20	2.24	2.07	0.12	0.35
Tissue ^{15}N	lucerne	<0.001	0.00	3.50	9.50	22.30	56.80	2.25	7.34
%N	lucerne	0.07	2.98	3.17	2.93	2.71	2.81	0.10	0.32
DM/pot (g)	lucerne	0.138	4.86	3.81	4.60	4.92	5.13	0.37	1.08
Nod score	lucerne	0.002	3.17	3.17	3.08	3.00	2.00	0.20	0.60
Shoot weight (g)	lucerne	0.071	0.29	0.23	0.28	0.30	0.32	0.02	0.06
Root weight (g)	lucerne	0.376	2.00	1.52	1.80	1.90	1.92	0.18	0.52
Shoot:Root	lucerne	0.32	1.42	1.55	1.55	1.67	1.73	0.11	0.32

Appendix 6 Effect of N application on growth parameters of lupin and lucerne in Glasshouse Experiment 4 at Lincoln University, Canterbury.

Statistic	Species	P value	0	50	100	200	400	600	SEM	LSD
Tissue ^{15}N	lupin	<0.001	0.10	14.60	26.30	49.70	57.50	51.70	6.03	18.18
%N	lupin	0.466	3.56	3.33	3.38	3.64	3.32	3.36	0.14	0.42
DM/pot (g)	lupin	0.423	5.17	5.09	4.94	5.32	6.16	3.41	0.87	2.63
Nod score	lupin	<0.001	4.38	3.75	3.25	3.50	2.50	2.12	0.27	0.82
Shoot weight (g)	lupin	0.487	3.58	3.54	3.40	3.81	4.12	2.35	0.62	1.87
Root weight (g)	lupin	0.246	2.26	2.27	2.18	2.55	1.96	2.28	0.15	0.47
Shoot:Root	lupin	0.279	1.59	1.55	1.54	1.51	2.04	1.06	0.27	0.80
Tissue ^{15}N	lucerne	<0.001	0.10	9.96	23.60	56.25	74.23	72.87	0.97	2.92
%N	lucerne	<0.001	3.50	3.38	3.00	2.80	3.58	3.60	0.09	0.26
DM/pot (g)	lucerne	<0.001	8.94	12.31	12.67	12.39	11.19	8.64	0.57	1.71
Nod score	lucerne	<0.001	3.38	3.63	3.25	2.50	1.25	1.38	0.20	0.61
Shoot weight (g)	lucerne	0.002	4.93	6.89	6.65	6.90	6.72	4.98	0.38	1.14
Root weight (g)	lucerne	0.026	1.24bc	1.28bc	1.1c	1.26bc	1.50a	1.38ab	0.07	0.22
Shoot:Root	lucerne	<0.001	4.01bc	5.42a	6.03a	5.48a	4.48b	3.66c	0.27	0.80

Appendix 7 16s rRNA sequences for Bradyrhizobium sp.

AP3

AGTCGAGCGGGCGTAGCAATACGTCAGCGGCAGACGGGTGAGTAACGCGTGGGAACGTACCTTTTGGTTCGGAACAACA
CAGGGAAACTTGTGCTAATACCGGATAAGCCCTTACGGGGAAAGATTTATCGCCGAAAGATCGGCCCGCGTCTGATTAGC
TAGTTGGTGAGGTAATGGCTCACCAAGGCGACGATCAGTAGCTGGTCTGAGAGGATGATCAGCCACATTGGGACTGAGA
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AP4

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AP5

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AP6
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AP7
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AP8
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AP9

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CO1

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MB2

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TA1

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Appendix 8 *nifH* sequences for *Bradyrhizobium* sp.

AP3

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TCGTGATGTC

AP4

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AP5

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AP7

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AP8

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AP9

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CO1

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TCGA

MB2

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TA1

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GATGT

Appendix 9 *nodA* sequences for *Bradyrhizobium* sp.

AP3

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AP4

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AP5

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AP6

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AP7

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