The effect of nitrogen on the yield of four pasture grass species

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Abstract of a Thesis submitted in partial fulfillment of the requirement for the Degree of Bachelor of Agricultural Science (Hons)

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This research examined the morpho-physiological attributes of four pasture grass species under two nitrogen (N) treatments. The experiment was a split plot design with main plots of N+ and N-and subplots of cocksfoot (*Dactylisglomerata*), pasture brome (*Bromusvaldivianus*), perennial ryegrass (*Loliumperenne*) and tall fescue (*Festucaarundinacea*). Leaf length, leaf area and specific leaf area (SLA) decreased by 30%, 36% and 7% under N-, whereas leaf dryness (percentage dry matter) increased by 18%. Total accumulated aboveground plant dry matter (DM) yield decreased by 13% in response to the N- treatment. Chlorophyll levels, measured as SPAD units, predicted yield under N+ conditions which was also reflected in the leaf length, leaf area and SLA measurements. The findings also suggest that species with higher green DM compensated for external N reduction by utilising higher internal available N within the plant. Brome and cocksfoot, and to a lesser degree tall fescue, showed superior morphological leaf attributes under mixed N-availability and can be proposed as suitable alternatives in dryland and low nitrogen farming systems as an alternative to perennial ryegrasses.

Keywords: nitrogen, brome, cocksfoot, perennial ryegrass, tall fescue, *Bromusvaldivianus*, *Dactylisglomerata*, *Loliumperenne*, *Festucaarundinacea*, dry matter, leaf morphology, SPAD.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

LIST OF APPENDICES

1 INTRODUCTION

Many farming systems in New Zealand operate within an environment where water and nitrogen (N) availability is often limited, especially along the east coast. This results in a period of feed deficit over summer months (Clark and Woodward, 2007). Perennial ryegrass (*Loliumperenne*) is the major pasture grass species used in New Zealand and is commonly the main dietary component of New Zealand's most intensively grazed livestock species (Easton *et al.*, 2001). Whilst it is the most commonly sown pasture grass in New Zealand (Kemp *et al.*, 2002), perennial ryegrass often struggles to persist and produce high quality pasture when exposed to dry and low nutrient conditions (Turner *et al.*, 2006). Based on recent climate change predictions, regional variation in annual rainfall will increase moisture deficits, alongside rising temperatures increasing rates of evapotranspiration experienced in the eastern and northern regions of the country (Hollis, 2014; Salinger, 2003). These changes, combined with lack of N availability in pastures are likely to decrease the survival of perennial ryegrass. Recent research has turned towards investigating alternative pasture grass species that can persist and produce high quality pasture during the hot and dry summer months when finishing young stock is paramount to farming systems profitability.

However, despite extensive knowledge on dry matter (DM) production of different grass species used in New Zealand, knowledge is less defined on plant attributes by which these species show different DM production under different N regimes. Identifying these morphological and physiological traits may give an insight into how improvements in grazing management, species and cultivar breeding development, and N application can be achieved to increase overall DM yields.

In the current study, the pasture grass species; cocksfoot (*Dactylisglomerata*), pasture brome (*Bromusvaldivianus*) and tall fescue (*Festucaarundinacea*) were compared to perennial ryegrass (*Loliumperenne*). Two nitrogen treatments were imposed during the autumn 2017 on a dryland site at Ladbrooks near Lincoln University. Through monitoring of key morpho-physiological leaf traits and aboveground plant yield under different N availability it was expected that differences in these attributes could be established between the alternative pasture grass species and perennial ryegrass.

2 REVIEW OF THE LITERATURE

2.1 New Zealand Pastures

The pastoral industry is an important component of the New Zealand economy. The production of agricultural commodities such as meat, wool and milk depend on the availability of feed from pastures (Clark and Woodward, 2007). Farmers continually seek to improve the production of their livestock systems by adopting technologies and management techniques that improve the growth and consumption of pasture (Caradus, 2008). Improving the yield and feeding value of pasture in a cost-effective way is key in maintaining New Zealand agriculture and the products it produces stays competitive on world markets. Nitrogen (N) is essential in these pastoral systems for plant growth and producing high quality pasture. Nitrogen makes the use of water more efficient (Moot *et al*., 2008), obtaining N through legumes and additions of N containing fertilisers is the primary way pastures obtain N. Finding pastures that are more efficient at using N is a way in which we can increase pasture production efficiencies.

Dryland pastoral agriculture in New Zealand has been based around perennial ryegrass (*Loliumperenne*)/white clover (*Trifoliumrepens*) based pasture mixes for the last 70 years (Mather *et al*., 1995). This has led to extensive research to adapt both species to a wide and variable range of New Zealand climatic conditions. In areas of high fertility and reliable rainfall their persistence and productivity has been high (MacFarlane, 1990). However, in dryland environments potential evapotranspiration is greater than rainfall through September to April, especially on the East Coast of New Zealand (Rickard and Radcliffe, 1976). Agricultural systems aim to take advantage of the short period of high growth in spring. At other times of the year growth is restrained by summer drought and low winter temperatures. Drought can halt growth and is more severe in free draining soils with low water holding capacity. Droughts are variable and unpredictable in duration which effects on-farm decision making (Hoglund and White, 1985). Irrigation on a large number of east coast farms is not possible due to topography, cost and environmental restraints such as leaching and water levels (Woodman *et al*., 1992).

Water is a critical limiting factor for pasture production. Plants require water to carry out a range of biophysical processes. When placed under water stress plants respond by initiating strategies to mitigate water loss, ranging from reducing leaf expansion to closing stomata pores (Brown, 2004). The duration and severity that a plant is exposed to water stress (drought), will determine the strategy used to increase the chances for plant survival and the resultant effects on plant production/crop yields (Jamieson, 1999). Growth of pasture will not occur when there is no available water. The ability of a pasture under water stress to survive, how quickly it can recover and a crops water use efficiency are the most beneficial factors to mitigate drought conditions in dryland farming environments.

The financial cost to the agricultural industry with perennial ryegrass/white clover pasture mixes in summer dry agricultural systems associated to reduced persistence and production has lead to the introduction of alternative pasture species that may be more suited to dryland environments (Mather *et al*., 1995). Increasing water use efficiency (WUE) (biomass increase per unit of water transpired), has been an area where crop yields have been looked at for improvement in recent years especially in drought prone regions and environments of New Zealand (Brown, 2004).

New Zealand's temperate climate has fewer sunlight hours and lower temperatures in the winter than in the summer which limits the quality and yield of winter forage crops. Baars and Waller (1979) identified rainfall and temperature as the main climatic variables which affect pasture production, with temperature being crucial for pasture growth especially in winter and early spring. Reduced incident solar radiation in winter means less radiation is available for crop interception in winter. Low temperatures can reduce the conversion of intercepted radiation to dry matter (DM) or radiation use efficiency (RUE) which can result in a reduced biomass (Monteith, 1977). In addition, low temperatures have been shown to slow leaf appearance and expansion, which results in canopy closure taking longer (Kirby *et al*., 1982). These factors cause seasonal variation in pasture supply which complicates management of pastoral systems. Temperature could also affect the phenological development of crops described in terms of thermal time (Tt) or growing degree days (°Cd) (Kirby *et al*., 1982). This is an important aspect of increasing the accuracy in predicting crop development to determine optimum sowing and harvest dates.

2.2 Grass species

2.2.1 Cocksfoot (*Dactylisglomerata*)

Cocksfoot (*Dactylisglomerata*) is a major pasture grass used in New Zealand in dryland farming systems. It is drought tolerant, persistent and has a main growth period that occurs during summer (Charlton and Stewart, 2000). In Canterbury annual yields have been reported from 7.5 t DM/ha to >22.5 t DM/ha (Mills *et al*., 2005). Lower yields occurred under dryland conditions with no nitrogen and the higher yields occurred under non-limiting nitrogen and water conditions. It is a valuable grass in drier regions with moderate fertility and is suited to light and free-draining soils (Charlton and Stewart, 1999).

In New Zealand, for many dryland farmers, cocksfoot (*Dactylisglomerata*) is the most persistent grass species because it survives dry periods during the summer. Cocksfoot is a persistent perennial grass that tolerates moderate soil fertility, moisture stress, insect attack and set stocking (Mills, 2007). It is a small seeded grass with a thousand seed weight of 0.65 g (Lancashire and Brock, 1983) to 1.0 g (Moloney, 1993). Perennial ryegrass has a larger thousand seed weight of 1.87-2.48 g. Thus, there are less seed reserves for cocksfoot to establish at first when compared to ryegrass, therefore a slower establishment is seen.

Cocksfoot has a relatively slow establishment when compared to perennial ryegrass (Charlton and Stewart, 1999). Stephens and Hickey (2000) reported cocksfoot pasture having a peak production four years after establishment, but measurements ceased after the fourth year. Korte*et al.* (1991) reported similar results with cocksfoot content increasing three to four years after sowing. Cocksfoot generally has lower seasonal production during spring than ryegrass but this is offset by increased production during summer and autumn. Total annual DM production of dryland cocksfoot in Canterbury was 7.6 t DM/ha compared with 4.9 t DM/ha from ryegrass pastures (Stevens *et al*., 1992). The difference in annual yield occurred because cocksfoot produced 131% more DM than ryegrass in summer and 74% more DM in autumn. Floral initiation in Cocksfoot occurs later in a growing season (late summer/autumn) (Mills, 2007). Cocksfoot becomes a valuable pasture for finishing stock as high quality pasture continues to grow over the summer.

Persistence during dry periods is a key component of dryland pastures survival to drought conditions. Cocksfoot forms a dense fibrous root in the top 0.25m of the soil profile (Ridley and Simpson, 1994). Both cocksfoot and ryegrass have been shown to extract water from similar soil profile depths (Evans, 1978). The mechanisms by which cocksfoot's superiority allows it to recover and persist when subjected to water stress may be due to differences between the two species in i) water use efficiency: ii) the ability to access and extract soil moisture and/or: iii) the mechanism used to recover from drought (Mills, 2007).

Cocksfoot is normally sown with legumes, such as white clover and has more recently been grown with Caucasian (*Trifoliumambiguum*), subterranean (*Trifoliumsubterraneum*) and balansa (*Trifoliummichelianum*) clovers (Mills *et al.*, 2008). Mills *et al*. (2008) found that annual dry matter yields of these four legumes sown with cocksfoot were higher in four of the five measurement years than annual dry matter yields of ryegrass/white clover in the 'MaxClover' experiment at Lincoln University (Fig. 2.1). Ryegrass production dropped from 8 to 4 t/ha while cocksfoot averaged about 6 t/ha in the fifth year. Cocksfoot forage quality is lower than that of perennial ryegrass, being particularly poor when seedheads are present. Grazing management is based around preventing excessive seedhead development. Under dry conditions cocksfoot may dominate white clover to such an extent that pastures become protein-deficient, unproductive and unpalatable (Moloney, 1993).

Mills *et al.* (2008), shows the sown grass component of five dryland pastures (cocksfoot/sub clover, CF/Sub; cocksfoot/white clover, CF/WC; cocksfoot/balansa clover, CF/Bal; cocksfoot/Caucasian clover, CF/CC; and ryegrass/white clover, RG/WC) (Fig. 2-1).

It shows that the sown grass component of the RG/WC declined from 7.4 t DM/ha/year in 2002/03 to slightly more than 4 t DM/ha/year in 2005/06 and 2006/07. The sown grass yield of CF/WC pastures declined from 8.7 t DM/ha/year in 2002/03 to ~6 t DM/ha/year in 2005/06 and 6.3 t DM/ha/year in 2006/07. In the CF/Sub pastures the grass component dropped from 8.5 t DM/ha/year in 2002/03 to ~5.5 t DM/ha/year in 2005-06 and 6.1 t DM/ha/year in 2006/07. These results obtained by Mills *et al.* (2008) show that cocksfoot was a more suitable grass species for dryland Canterbury where summer moisture stress occurs regularly.

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Figure 2-1: Annual dry matter yields (t/ha/yr) of the sown grass component of dryland CF/Sub (●), CF/Bal (○), CF/Wc (▼), CF/Cc (▽) and RG/Wc (■) pastures over five growth season. The error bar is the maximum SEM(Mills *et al*., 2008).

Peri *et al*. (2002) showed leaf photosynthesis in cocksfoot to be at an optimum between 19-23°C. This was similar to the 20-22°C optimum range reported in controlled environments (Mitchell and Lunacus, 1962). Thermal time is used to show the relationship between temperature and plant development between two development stages of a plant (heats unit/growing degree days (°Cd)). It shows seasonal variations in pasture growth rates and the impact of temperature on pasture growth rates (Moot *et al*., 2000). Moot *et al.* (2000) calculated cocksfoot thermal time with a base temperature of 3°C and the optimum of 23°C (growing degree days is the time spent between these two values).

2.2.2 Pasture brome (*Bromusvaldivianus*)

Brome grass is found throughout temperate New Zealand (Kon and Blacklow, 1995). The annual lifecycle of the *Bromus* species is typical of many annual grasses, including perennial ryegrass, with most germination occurring over the winter months, rapid growth during spring and seed production over the summer months (Kon and Blacklow, 1995). The root systems of brome are fibrous and concentrated in the upper soil profile (Hulbert, 1955). There is limited research around pasture brome (*Bromusvaldivianus*), used in this experiment, in New Zealand, resulting in little literature based around the characteristics of pasture brome. Most studies have focused on invasive *Bromus* species such as rigid brome (*B. rigidus*) and ripgut brome (*B. diandrus*), which are noted as being serious weeds of cereal crops and pasture (Williams, 1986). Therefore most studies concentrate on management of brome grass as a weed. However, pasture brome is described as a medium tillered, perennial brome grass species that is more persistent than prairie grass under grazing (Charlton and Stewart, 1999). It provides strong spring–summer growth with drought tolerance with moderate winter growth. Pasture brome is persistent on fertile and free-draining soils, but does not tolerate pugging or waterlogging. However, it may tolerate higher rainfall conditions than other brome grasses. It is suited to the dry East Coast and inland regions of New Zealand, where it is useful pasture for quality summer feed with a recommended sowing rate of 25–30 kg/ha (Charlton and Stewart, 1999). NZ Agriseeds cultivar 'Bareno' is used in this experiment.

Harkesset *al*. (1990) found organic matter digestibility (OMD) of brome over three years to be higher than cocksfoot, but not higher than ryegrass. In the same experiment in Edinburgh, experiments were established on a sandy loam soil that was imperfectly drained. N concentrations were ranked with cocksfoot > brome grass > perennial ryegrass over three years, with the respective means being 17.8, 17.1 and 15.3 g kg DM^{-1}.

2.2.3 Perennial ryegrass (*Loliumperenne*)

Perennial ryegrass is the most widely used temperate grass in New Zealand as it grows well in a wide range of fertile situations, is easily established and managed, and usually forms a compatible mixture with white clover and other pasture species (Charlton and Stewart, 1999). Perennial ryegrass requires moist fertile conditions and withstands hard grazing and treading. However, it has been reported to perform poorly during hot dry conditions, when many other deeper rooted grasses maintain production (Charlton and Stewart, 1999; Hogland and White, 1985).

Perennial ryegrass cultivars are classified into types according to flowering time (Charlton and Stewart, 1999). Seedhead emergence is associated with a spring growth flush, with earlier heading cultivars being more productive in August–September than later heading perennial ryegrasses. However, a decline in feed quality is associated with seedhead development, thus later flowering cultivars will retain their leafiness and pasture quality longer (Charlton and Stewart, 1999). This is useful in a dryland finishing system where stock are finished over late spring and into the summer. Cultivars can also vary according to the number of chromosomes they have with diploid and tetraploid types. Perennial ryegrasses occur naturally only as diploid plants, with a standard set of 14 chromosomes in each cell. However, plant breeders can double the number of chromosomes to form tetraploid types, which results in larger plants with bigger cells, greater water content and a larger seed (Charlton and Stewart, 1999).

The annual dry matter (DM) production of perennial ryegrass in New Zealand ranges between 9 – 20 tonnes DM ha^{-1} yr^{-1} (Easton *et al*., 2001). The large variation in production exists between regions and is determined mainly by grazing management, temperature, and soil moisture and fertility (Stewart *et al*., 2014). Significant changes in the seasonal production and morphology of perennial ryegrass pastures are based largely on temperature, and therefore the region it is grown. The use of irrigation increased dry matter production by 13% in the Waikato (Clark *et al*., 2010) and 80% in Canterbury (Mcbride, 1994). Perennial ryegrass DM production is lowest during winter.

2.2.4 Tall fescue (*Festucaarundinacea*)

Tall fescue is a deep-rooted, perennial grass that is productive and drought tolerant and best suited to heavy or wetter soils with high fertility (Charlton and Stewart, 1999). It can withstand acid, alkaline soils and poor drainage. Milne *et al.* (1998) similarly reported tall fescue required high soil fertility and responded well to nitrogen application. Tall fescue is useful in dry conditions as a summer purpose pasture that grows well in all seasons, especially summer and autumn. It is becoming increasingly popular as an alternative to ryegrass-based mixtures in drier regions and in areas where subtropical grass invasion is an increasing problem (Charlton and Stewart, 1999). Tall fescue's natural range of habitat is limited in regions of rainfall below 450 mm/yr (Easton *et al.*, 1994).

Dry matter production of tall fescue is important in dryland areas, with successful establishment being crucial to productivity and persistence. Tall fescue is slower to establish than perennial ryegrass due to its slow mobilization of seed reserves and seedling growth (Kemp *et al.*, 1999). However, once established tall fescue has a similar DM production to perennial ryegrass where water is not limiting but in dryland conditions tall fescue has greater pasture production, as it can recover quicker after a water deficit (Kemp *et al.*, 1999). Tall fescue becomes an important cool season perennial when summer moisture stress limits the persistence and yield of perennial ryegrass in the dry east coast of New Zealand (Easton *et al*., 1994). Brock (1982) showed that both tall fescue cultivars, 'Roa' and 'S170', yielded significantly higher than 'Ruanui' ryegrass by 172% and 163%, respectively, with only 55% of the normal rainfall occuring in the summer to autumn period (January-April) of the first year. Both tall fescue cultivars also out-yielded 'Ruanui' during the same period of the wetter second year, by 40% and 54%, respectively. Tall fescue was sown 1 year before the ryegrass to allow for its slower establishment.

Rollo *et al*. (1998) found that the average annual tall fescue yield over a 5 year period was greater than perennial ryegrass across three dryland sites in New Zealand (Fig. 2-2). Annual yields reflected local climatic conditions between the dryland sites. Pasture production for both grass species was greatest at the Taranaki Agricultural Research Station (TARS) which had the highest summer rainfall (290 mm) and lowest water deficit (161 mm) out of the three sites. At this site, tall fescue produced 17 t DM ha^{-1} which was 13.5% more than ryegrass. The Winchmore site had the lowest summer rainfall (256 mm), highest water deficit (235 mm), and lowest soil temperature (15.6°C) over summer, resulting in lower herbage production. Tall fescue produced an average 11.2 t DM ha^{-1} compared with 10.1 t DM ha^{-1} from perennial ryegrass at Winchmore. The Poukawa site, located in Hawke's Bay showed no significant difference in the yields of the two grasses as hot dry conditions restricted tall fescue production to 570 kg DM ha^{-1}.

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Figure 2-2: Average annual yields of perennial ryegrass and tall fescue across three dryland sites (Poukawa, Taranaki Agricultural Research Station (TARS) and Winchmore) in New Zealand over a 5 year period from 1990/91 to 1994/95 (Rollo *et al*., 1998).

Fertility and grazing management influence the persistence of tall fescue in New Zealand (Easton *et al.*, 1994). Rollo *et al*. (1998) stated that rhizome development does not begin until 2 years after autumn sowing and can be reduced by poor grazing management. Tall fescue grazing management in spring should be more frequent to prevent excessive seedhead development, to prevent the development of stemmy pastures that result in poor quality feed. Tall fescue is usually sown as the sole grass component with clover, or with low rates of erect cocksfoot types. It is not recommended to be sown in mixtures with ryegrass as ryegrass dominates and tall fescue soon disappears (Charlton and Stewart, 1999). Tall fescue sowing rates are recommended to be 15–30 kg/ha with clover. Seed establishment is faster in warm soils, so tall fescue-based mixtures should be sown in September-October, or in February–March (Charlton and Stewart, 1999).

Tall fescue cultivars vary in their flowering time, more so than perennial ryegrass. Early-flowering types grow well in August–September with a decline in quality seen earlier than in later-flowering types as seedheads develop. Cultivars also vary in establishment rate but are slow in comparison to ryegrass. Additionally, cultivars vary in tiller density. Traditional types are larger, with more broad foliage when compared to perennial ryegrass, with others as fine-leaved as perennial ryegrass. The broad-leaved types suit rotational grazing, whereas the fine-leaved dense types can withstand close continuous sheep grazing (Charlton and Stewart, 1999).

2.3 Morphological features

2.3.1 Leaf attributes

Over many years of experimentation with ryegrasses and fescues, information about leaf development in these grasses and their hybrids is still fragmentary. Sometimes leaf appearance rate and leaf extension rate have been recorded, but to the best of my knowledge there is no information on differences in leaf expansion rate. This may be due to large differences in between grasses in leaf blade width and therefore differing elongation rates. The final area and weight of individual leaf blades are further important characteristics of which there are few published comparisons of different grasses grown in field swards.

Available evidence suggests that leaf appearance rate can be expected to be higher in ryegrasses than in tall fescue (Peacock 1976; Wilman& Mohamed 1980, 1981). Also, leaf extension rate may be higher in Italian ryegrass than in tall fescue and it may be higher in Italian than in perennial ryegrass (Peacock 1976; Wilman& Mohamed 1981; Davies *et al*., 1989). It appears that tall fescue, in established swards, can be expected to have larger, heavier leaf blades than ryegrasses (Wilman& Mohamed 1980, 1981). The leaf blades of Italian ryegrass may be larger and sometimes heavier than those of perennial ryegrass (Wilman*et al*., 1977; Wilman& Mohamed 1981).

Gao and Wilman (1994) studied eight related grasses, grown in field swards cut at 5-week intervals over two years in Wales, UK. The rate of leaf expansion was recorded in the order of Westerwolds ryegrass > Italian ryegrass (*Loliummultifloruni*), Italian ryegrass x meadow fescue > hybrid ryegrass > perennial ryegrass x meadow fescue, meadow fescue (*Festucapratensis*), tall fescue and perennial ryegrass (Fig. 2-3). This is similar to the findings of Peacock (1976), Wilman& Mohamed (1981), and Davies *et al*., (1989). Gao and Wilman (1994) found the order of grasses for rate of leaf appearance, rate of leaf extension, weight of leaf blade emerging per shoot per week and rate of increase in length of the exposed leaf sheath to be similar to that of the order of rate of leaf expansion, the order was approximately the reverse for weight per unit area of emerging leaf blade. The area per leaf blade in tall fescue increased greatly between May-July of the year of sowing, and May-July of the following year.

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Figure 2-3: Fully expanded area (cm^2) per blade of leaf 2 in six periods of study in Westerwolds ryegrass (○), Italian ryegrass (●), perennial ryegrass (Δ), meadow fescue (▲) and tall fescue (□) (Gao &Wilman, 1994).

Expanded leaves constitute the yield a pasture has and also the means of which it has to capture light energy through photosynthesis (Ryle, 1964). It has been reported that the final leaf lamina size in grasses is influenced by temperature and N (Ryle, 1964). An increased supply of N generally promoted all aspects of growth, while temperature was noted to have a more complex effect. Leaf length was increased and leaf width decreased by raising the temperature. Light intensity, and especially day length are also known to influence leaf size. Ryle (1964) also found fescues and cocksfoot to have the largest leaf area above ryegrass. A high rate of leaf production and a large number of living leaves, rather than leaf size, were presumed to be responsible for the performance of cocksfoot, in contrast to the fescues which appeared to attain a larger leaf area per shoot due to their ability to develop large leaves. A significant effect of N application was seen with an increase of leaf area of main shoots by about 25%. In conclusion, perennial ryegrass and meadow fescue were found to produce more leaves more quickly than tall fescue, while tall fescue had larger leaves than perennial ryegrass, with fescue in between. The experiment was carried out in glasshouses.

Leaf percentage DM (PDM), or relative water content, is the amount of water a plant leaf contains. Relative water content can provide an indication of the severity of a water deficit and the degree of stress a plant is under.Plants that maintain a high leaf percentage DMunder water deficits are likely to have an improved performance under drought conditions (Pask*et al.,* 2011). Perennial ryegrass has been reported to have a decline in leaf expansion rate when relative water content is below 88% (Wilson, 1975). There are gaps in literature in regards pasture grass species relative water content in leaves.

Specific leaf area (SLA) is a predictor of effective plant productivity. A higher SLA means more leaf area, resulting in more light interception for photosynthesis per leaf DM. Leaf thickness is another leaf attribute calculated by $(SLA * PDM)^{-1}$. There is no literature on leaf thickness measurements in pasture grass species to the best of my knowledge but it has been discussed that thicker leaves are a sign that leaves are more stressed and less palatable(Pask*et al.,* 2011).

2.3.2 Leaf DM

Turner *et al*. (2012) reported on leaf DM production in a glasshouse trial of tall fescue, perennial ryegrass and cocksfoot, under different water treatments. The experiment used applications of 133, 100, 66 and 33% of the replacement water required to maintain volumetric soil water content at 0.255 mm^3 mm^{-3}, similar to the experiment that will be carried out at Ladbrooks where water was non-limiting over the course of the experiment (April-June). Table 1 shows the leaf DM production by the three species was consistently in the order: tall fescue > perennial ryegrass > cocksfoot. Stubble DM of cocksfoot was far greater than that of perennial ryegrass and tall fescue throughout the study.

Table 1: Mean leaf and stubble dry matter (DM; g plant^{-1}) of cocksfoot (CF), perennial ryegrass (PR) and tall fescue (TF) under 33, 66, 100 and 133% of the replacement water required to maintain a soil water potential of −10 kPa, during two regrowth phases and subsequent recovery phase. Adapted from Turner *et al*. (2012).

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While leaf DM was consistently lower for cocksfoot than for perennial ryegrass and tall fescue in Turner *et al.* (2012), cocksfoot was the least drought sensitive of the three species, and had the smallest relative reduction in DM due to moisture stress. While perennial ryegrass suffered a 78% reduction in leaf DM owing to the 33% water treatment (compared with the 100% water treatment), cocksfoot leaf DM only decreased by 52%. Similarly, in the recovery phase, there was a 58% difference in leaf DM between perennial ryegrass plants previously watered at 33% compared with 100%, versus a 35% difference for cocksfoot. Tall fescue displayed an intermediate drought response in this regard. For tall fescue and perennial ryegrass, there was a pattern of increased leaf DM with increased prior water allocations during the recovery phase of this experiment. Leaf extension rate was found to respond to water availability with a pattern of decreased leaf extension with decreased water availability.

2.3.3 Roots

Rooting depth and water use efficiency (WUE) can determine how productive a pasture is. Moot *et al*. (2008) calculated the water use efficiencies (WUE) of a range of temperate pasture species from several different dryland and irrigated pastures in Canterbury. Nine different data sets were summarised from 2000-2007 and the data collated. The data set is comprehensive and gives a greater importance to the research as it was spread out over 7 years with multiple experiments showing similar results. The annual WUE found ranged from 6.7 kg DM/ ha/mm for a dryland cocksfoot pasture to 18 kg DM/ha/mm for perennial ryegrass grown on a Wakanui silt loam soil. Perennial ryegrass extracted 243 mm of water to 1.5 m depth. On a stony Lismore soil, perennial ryegrass extracted 129 mm of water to a depth of 1.5 m.

In the same study (Moot *et al.,* 2008) during the year WUE of the ryegrass pasture ranged from 3 to 22 kg DM/ha/mm. This showed how pasture growth was affected by seasonal variability in soil moisture deficits, soil evaporation and drainage. When moisture was non-limiting (spring), clover monocultures and pasture mixtures had higher WUEs than pure grass swards due to higher herbage nitrogen (N). The results found suggested that WUE can be maximised annually and seasonally by adopting grazing management strategies to enhance clover production, strategic applications of N fertiliser and growing monocultures of legumes, such as lucerne, to maximize growth when soil moisture is available. This was further backed up by a cocksfoot monoculture having an annual WUE of 38 kg DM/ha/mm when fertilised with N but only 17 kg DM/ha/mm when unfertilised (Moot *et al.*, 2008). Cocksfoot forms a dense fibrous root in the top 0.25m of the soil profile (Ridley and Simpson, 1994), maximising water extraction. Both cocksfoot and ryegrass have been shown to extract water from similar soil profile depths (Evans, 1978).

2.4 ENVIRONMENT

2.4.1 Climate

Pasture production is variable throughout New Zealand with temperature and rainfall being the two most important environmental factors affecting pasture growth. Seasonal variability in temperature, nutrient and soil water availability, pests and diseases and solar radiation (Biscoe and Gallagher, 1977), creates inconsistency in feed supply (Baars and Waller, 1979). For instance, pasture production in Central Otago is ~5.0 t DM ha^{-1} yr^{-1} compared with 18.0 t DM ha^{-1} yr^{-1} for Northland and the Waikato (Matthews *et al*., 1999). Annual pasture production is mostly affected by low temperature in winter and soil moisture content in summer. Annual herbage yield in the Waikato were 11.7 t DM ha^{-1} yr^{-1}, 10.9 t DM ha^{-1} yr^{-1} in the Wairarapa, 5.9 and 10.2 t DM ha^{-1} yr^{-1} for dryland and under irrigation, respectively, in Canterbury (Valentine and Kemp, 2007). Soil fertility also has a major effect on productivity. However, a key driver of annual productivity for perennial pastures is radiation interception (Valentine and Kemp, 2007). After canopy closure, solar radiation (light interception) will be the main factor responsible for DM production but prior to canopy closure, temperature will affect the rate at which the canopy expands (Radcliffe and Baars, 1987). At suboptimal temperatures the photosynthetic rate of plants is reduced. These environmental variations show how difficult it can be to balance feed supply and demand of pasture in dryland systems for growing animals.

The influence of environment and climate on the yield of a pasture or crop can be broken into four levels (de Wit, 1986). The first level, the potential yield for a crop in a region, determined by local solar radiation and temperature influences on radiation interception and radiation use efficiency (RUE). Solar radiation and temperature are correlated so potential yield changes with season and latitude (Monteith, 1977). The second level relates to the impact of water limitations on potential yield (Jamieson, 1999). Levels three and four relate to soil conditions in relation to mineral availability/toxicity (Fageria*et al*., 1997). A combination of all four levels contribute to season and location specific yield of any pasture or crop.

2.4.1 Temperature and light

Temperature influences the rate of leaf appearance and expansion (Thomas, 1975). Light influences the rate of photosynthesis as well as the rate of tiller and leaf expansion and it controls the timing of floral development and associated seed production and hence the distribution of dry matter in the plant (Cooper, 1972). Peacock (1975) reported higher rates of leaf expansion in spring compared to autumn due to growth from reproductive rather than vegetative tillers and not to a temperature response. Thomas & Norris (1977) also found that both leaf appearance and leaf extension at a given temperature were greater in spring than autumn.

2.4.2 Moisture and wind

Pasture growth is often restricted by suboptimal levels of soil moisture and nitrogen. Gates (1974) reported on the effects of water stress on plants, emphasising that moisture stress lowered the demand and uptake of nutrients by plants and as synthesis rates declined, the demand for nutrients decreased further. Once water became available again, young tissues developed rapidly and increased demand for nutrient uptake, particularly phosphorus.

Gloyne (1976) reviewed crop responses, drawing attention to the lack of experimental work on pastures. Shelter was shown to increase plant growth in many situations probably because evapotranspiration may be reduced and sheltered plants have better root development with a more effective utilization of water. Wind may not necessarily increase rates of transpiration (Grace 1975). It may even lower leaf temperatures and decrease the leaf to air water vapour pressure difference so that transpiration rates are decreased. Plant physiological processes in response to wind are difficult to measure in on farm scenarios.

2.4.3 Soil

A lot New Zealand of dryland farm systems are based on hill and high country topography. The high country of New Zealand is largely made up of yellow grey and yellow brown earths. These soils tend to be more acidic, predominantly with a pH equal to or less than 5.5. High country soils are acidic due to being shallow and exposed, heavily leached, low temperatures, high altitudes and exposed slopes and steep terrain all contribute to acidic soils (McClaren& Cameron, 1996). Because of these factors, high country areas are less productive than lower rolling country. With soil acidity, pasture productivity rapidly declines, especially legumes (Edmeades*et al.,* 1985).

Nutrient availability is affected by soil pH (McClaren& Cameron, 1996). New Zealand soils vary over very short distances which can restrict nutrient supply to pastures (Moir*et al*., 2010). Thus, plants can be limited in growth and productivity at varying soil pH. Fig. 2-4 shows as pH levels increase, nitrogen, phosphorus, sulphur, molybdeum, magnesium and calcium availability increase. At lower pH levels (<5.5), zinc, manganese, iron and copper availability increase as well as Al levels which can lead to Al toxicity and reduction in pasture production, especially legumes (Moir*et al*., 2010). Liming is a management practice that can raise pH levels in soils and has been shown to increase pH by 0.15 per tonne of lime application in the 0-7.5 cm soil horizon (Moir*et al*., 2010). This reduces the risk of Al toxicity and increases the availability of essential nutrients such as N, P and S. Applying 1.25 t/ha of lime was reported by Edmeades (1985) to be economical on dryland farms.

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Figure 2-4: The effect of soil pH on nutrient availability (McClaren and Cameron, 1996).

2.4.4 Water/rainfall/drought

New Zealand has a temperate climate (White, 1999), characterised by a low evaporation and reduced likelihood of water shortages affecting pasture growth when compared to tropical and Mediterranean climates. However, the east coast of New Zealand is in the rain shadow of the Alpine mountain ranges and predominant westerly winds. Therefore, from Gisborne to North Otago, and in inland Central Otago and the McKenzie Basin, there is a sub-humid climate (400-800 mm rainfall) with dry periods restricting pasture production during late spring, summer and autumn months (White, 1999). For example, Lincoln (Canterbury) has an evenly distributed annual rainfall (long-term) of 460 mm per month, but potential evaporation exceeds this from September-April, reaching a peak of 150 mm per month in December/January. Pasture production in these regions is usually greatest during the spring, which then declines in the dry summer due to water stress, increasing again with autumn rainfall and decreases with a decreased temperature in winter (Radcliffe and Baars, 1987).

Sheep breeding systems occur in theses dryland East Coast areas, where lambs are born outside in late winter and grown on spring pasture. Surplus stock are then sold in late spring/early summer so only the breeding stock are carried through the dry summer period. Breeding stock are generally mated on fresh autumn pasture growth and wintered on pasture or green-feed crops carried over from the autumn. Alternatively, stock can be wintered on pasture conserved from a spring surplus or purchased supplementary feed. 8-16 stock units to a hectare are run on high intensive farms in these regions. Reductions in pasture production due to rainfall variability each year impacts on the productivity of stock and farming businesses (Young, 1989). A dry spring will reduce pasture production and availability to lambs, and in turn reduce sale weights and values (Rattray *et al*., 1987). A very dry summer/autumn will reduce the body weight of ewes, which can effect mating success and conception rates and the number of lambs born in the following spring.

Woodman *et al*. (1992) reported on grass performance in dryland environment in Omarama assessing through visual scoring on a 1-5 scale: where 1 = extremely poor and 5

= excellent. The pastures were mob grazed twice a year. Cocksfoot performed the best with a drought tolerance score of 4.25 (Table 2). This is most likely due to the deeper rooting plant having a greater access to water for a longer period of time during a dry period when compared to the other grasses. Brome and tall fescue performed fairly well with a score of 3.50, although were noted to not persist as well during the dry. Perennial ryegrass had a poor drought tolerance score of 2.50 during the experiment, highlighting the issue of persistence during dry periods. It must be noted however, that during the experiment that winter and seasonal rainfall were below averages for the area during the trial.

Table 2: Overall survival of grasses with mean plant survival ≥20% on lower sunny slopes at Tara Hills, Omarama. Adapted from Woodman *et al*., 1992.

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2.5 Nitrogen

Nitrogen is an essential macro-element that can limit growth and development in plants (Andrews *et al.*, 2013). It is important in agricultural systems for pasture and crop growth. Nitrogen is a component of DNA, RNA, protein and therefore enzymes, chlorophyll, ATP and cytokines. Thus, it is a part of all biological and chemical processes in a plant. Nitrate

(NO_3^-) is the main form of nitrogen taken up by plants that is readily available and assimilated by most crop plants in cultivated soils. Nitrate can be assimilated in either the root or shoot of plants depending on plant species and form of nitrogen supply. Ammonia (NH_4^+) can also be the main form of nitrogen taken up by plants in undisturbed soils. (Andrews *et al.*, 2013).

Nitrogen is assimilated by plants (Andrews*et al.*, 2013). Nitrogen assimilation is the process where NO_3^- and NH_4^+ in the soil, through nitrification and nitrogen fixation, are incorporated into the shoots and the roots of the plant. Once inside the plant metabolism occurs, normally starts with reduction of nitrate to nitrite, and then reduces to form ammonium with the presence of relevant enzymes. This reaction occurs more rapidly in leaves in the presence of light as photo-reduction of nitrate occurs as the plant uses energy from the light reactions of photosynthesis in chloroplasts to fuel nitrate assimilation (CO_2 is fixed in chloroplast as well) (Andrews *et al.*, 2013). If nitrate is assimilated in roots, it is respiration driven, requiring sugars to be transported from the shoots, down the phloem to the roots.

Increased nitrogen supply will increase the leaf area and chlorophyll pigment density, hence increasing the rate of photosynthesis. This is important with crop plants due to the high growth rates shown in the shoot of the plants with high N (Rogers *et al*., 1996). After ammonia is formed, it enters into the biosynthetic pathways of plant cells (via transpiration) to produce different amino acids. Amino acids form proteins and other nitrogenous compounds that help in body building (increasing cell size, leaf expansion). Radiation, gaseous factors, soil pH, the presence of metals and the amount of nitrate in the soil are environmental factors that affect absorption and reduction of nitrogen in plants (Mokhele*et al*., 2012).

An indication of pasture or crop N status can be measured by a chlorophyll meter, measuring leaf greenness and is based on the relationship between plant chlorophyll concentration and plant nitrogen status. Every chlorophyll molecule contains four nitrogen atoms and as leaf nitrogen concentration increases, the density of chlorophyll molecules within leaf chloroplasts also increases (Wood *et al*., 1993). Therefore measurement of leaf greenness can be a way of determining leaf N status which in turn is a good predictor of crop N status and yield of pasture (Rowarth& Archie, 1996; Griffiths & Thomson, 1996). Kantety*et* al. (1996) reported that the chlorophyll meter is an easy and efficient method of detecting tall fescue N status.

Based on this literature review the following conclusions have been drawn:

- Cocksfoot, tall fescue and brome are viable alternate pasture species that can be used in dryland systems in New Zealand.
- Pasture production is variable throughout New Zealand with temperature and rainfall being the two most important environmental factors affecting pasture growth.
- Leaf elongation and area is influenced by temperature, water and nitrogen.
- Rooting depth and water use efficiency (WUE) can determine pasture productivity.
- Nitrogen is essential for maintaining high quality pasture growth.

3 MATERIALS AND METHODS

This chapter describes the methodology and data analysis for the field experiment which provided the results presented in Chapter 4. The experiments focus on the production of cocksfoot, tall fescue, brome, and perennial ryegrass monocultures under two levels of soil moisture and two levels of nitrogen fertilizer. The plants were established from the seeds sown in October 2014.

3.1 Experimental site

Ladbrooks (43.37'S, 172°.30'E, 12 m.a.s.l) is the site where the experiment was established on 0.357 ha of a flat land in 2014. It is a Field Research Farm for Seed Force Limited and was monitored from 1st April through to 30th June 2017.

Plate 3-1: Ladbrooks experiment site.

3.2 Paddock history

In 2012 Ladbrooks was planted in kale (*Brassica oleracea*) and in rape (*Brassica napus*). In March 2013, annual ryegrass (*L. multflorum*) was sown and died out in January of 2014.

When annual ryegrass was sown in March 2013, a mixture of Urea and 200 kg/ha/year of Cropmaster 20 (19.3, 10, and 12.5) was applied to the entire area. From January to September 2014, immediately prior to this experiment, the area was uncultivated. It was sprayed with Roundup (glyphosate) at 1.4 kg a.i/ha, at the end of September 2014.

3.3 Soil characteristics

Ladbrooks site is flat to very gently undulating land with severe drainage/permeability restrictions. The experimental site is located over the boundary of an imperfectly drained Wakanui silt loam (Mottled Immature Pallic) soil at the northeast end and a poorly drained Temuka clay (TypicOrthicGley) at the southwest (http://smap.landcareresearch.co.nz). Ladbrooks soil is deep (>1m) and stone less with 50-100 cm potential for rooting depth (http://smap.landcareresearch.co.nz). It has very poor drainage and very limited aeration in the root zone. Water logging vulnerability at Ladbrooks site is high and the drought vulnerability (if irrigated) is low.

3.4 Meteorological conditions

Long term average for meteorological data from 1975-2012 are presented in Table 3. Canterbury’s climate is categorised as cool and temperate with an average annual rainfall of 636.5 mm equally spread over the year. Annual mean temperature is 12°C, ranging from an average of 6.3°C in July to 17.1°C in January (Table 3). Annual average Penman (EP) is 76.7 mm which normally surpasses precipitation from September to April.

Table 3: Monthly means from 1975 to 2012 for total solar radiation (Ro), maximum (Tmax), minimum (Tmin) and mean (Tmean) air temperatures (°C), rainfall (mm), Penman potential evapotranspiration (EP), wind run (km/day), and vapour pressure deficit (VPD). From 1975-2000 measurements were taken at EdlBroadfields Meteorological Station, Lincoln, Canterbury, New Zealand. From 2000-2012 measurements were taken from EwsBroadfields Meteorological Station, Lincoln, Canterbury, New Zealand.

Month	R_o MJ/m^2	T_{max}(°C)	T_{min} (°C)	T_{mean}(°C)	Rainfall (mm)	EP (mm)	Windrun km	VPD (KPa)
Jan	22.7	21.1	11.6	17.1	45.2	140	398	1.4
Feb	19.5	22	11.9	17	43	112	387	1.4
Mar	14.9	20.6	9.6	15.1	51.3	89.3	359	1.3
Apr	9.4	17.2	6.7	12	48.2	46.3	318	1.1
May	6.1	14.4	4.3	9.4	51.5	27.1	296	0.9
Jun	4.6	11.8	2	6.9	61.6	16.4	271	0.8
Jul	5.4	11	1.5	6.3	69	18.4	274	0.7
Aug	8	12.5	3	7.9	63	34.5	321	0.8
Sep	12.7	14.8	4.8	9.9	42	59.7	353	1.0
Oct	17.7	17.3	6.6	12.1	50.5	97.8	385	1.1
Nov	22.3	18.7	8.3	13.6	54.4	126	391	1.2
Dec	23.1	21	10.4	15.7	54.1	144	389	1.3
Annual	13.9	16.9	6.7	11.9	637	76.7	345	1.1

3.5 Agronomic management

3.5.1 Experimental design and treatments

In the first year of the study (2014/15), both Experiments 1 and 2 were a Latin square design with four grasses and four replicates. This was due to the environmental gradients including a ditch in the Northern corner of the paddock and variability in soil nitrogen background which was extended from the North to South of the paddock at Ladbrooks. In the second year (2015/16), once nitrogen background was utilised by the grasses, the experimental design was modified to include ±N fertiliser treatments. The 16 plots at each site were halved to accommodate the additional treatments and the experiment was subsequently analysed as a strip-plot designed experiment with ±N as the rows and pasture species as columns (Fig 3-1). The 2017 experiment carried out at Ladbrooks is the same as the 2015/16 design. Nitrogen was applied as strip lines in each block and randomized within each block. There were 32 plots and individual plot size for the strip-plot experiment 6.3 x 9 m for Experiment 1 at Ladbrooks. There was limited available area at the Ladbrooks site, therefore there was no boundary line between the blocks.

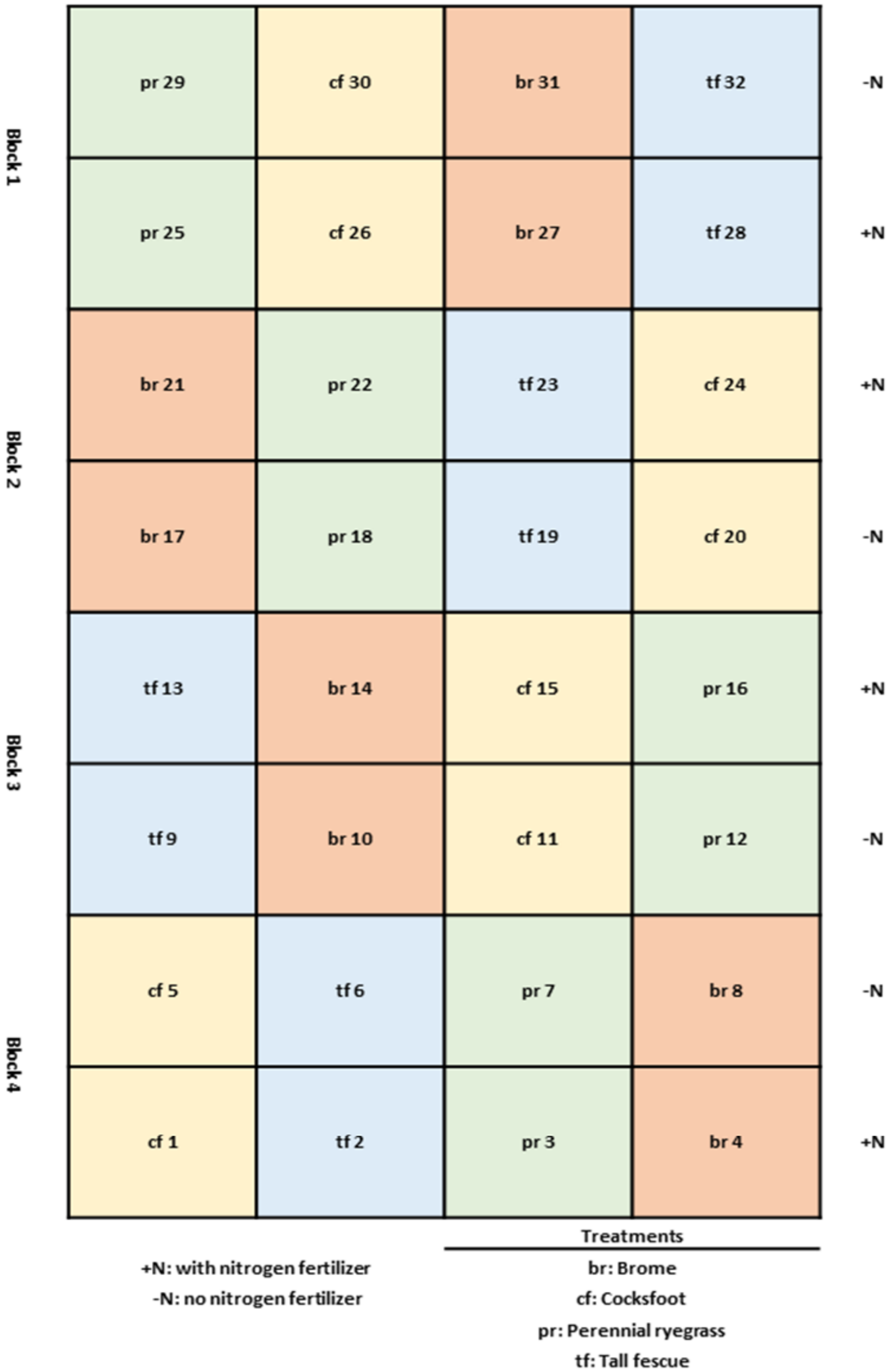

Figure 3-1: Ladbrooks experimental site. The blocks in which nitrogen fertilizer was applied are shown by +N and the blocks which no nitrogen was applied on them are shown by N-.

3.5.2 Seedbed preparation

MCPB (4-(4-chloro-o-tolyloxy) butyric acid; 1.5 kg a.i./ha) was used to control weeds on 4/8/2014. The main weeds at Ladbrooks were fathen (*Chenopodium album*), wire weed (*polygonumarenastrum*), stinging nettle (*Urticadioica*), shepherd's-purse (*Capsella bursa-pastoris*), and white clover (*T. repens*).

3.5.3 Establishment

A single application of 350 kg/ha of Cropzeal 20 (NPKS) was applied to the site on 9/9/2014. On 10/10/2014 cultivation took place at Ladbrooks. On 16/10/2014 the site was Cambridge-rolled just before sowing. Sowing rates are found below in Table 4.

Table 4: Cultivars, sowing rate (kg/ha) and germination percentage of four grass species at Ladbrooks.

Species	Cultivar	Sowing rate (kg/ha)	Germinatio n %
P. ryegrass	Stellar AR1	20	96%
Cocksfoot	Sfr36-009	10	88%
Tall fescue	Finesse Q	25	95%
Brome	Bareno (9045D)	35	98%

3.5.4 Soil fertility

Soil samples were taken on 30/7/2014 at Ladbrooks. The samples contained 12 cores taken randomly to a depth of 150 mm of the top soil from each experimental site (Table 5).

To assess the amount of nitrogen fertilizer required to be applied to the nitrogen fertilised (N+) sub-plots, on 9/9/2015 soil samples were taken from the both sites. In 2015, once plants established at Ladbrooks, uneven growth was visible within different plots due to having nitrogen background. To alleviate amount of nitrogen in those parts of the paddock, separate soil samples were taken from areas with nitrogen background as well as other areas. Based on soil test results (Table 5), in the first application of nitrogen fertilizer, less nitrogen fertilizer was applied (Appendix 1) to the area with nitrogen background.

Table 5: Soil test results (0-150 mm) from Ladbrooks site, Canterbury, New Zealand.

Year	Site	pH	Olsen P (µg/ml)	SO4-S (µg/g)	Ca^{2+}	K^{+}	Mg^{2+}(meq/100g)	Na^{+}	Available N (kg/ha)
2014	Ladbrooks	6.5	83	25	18.4	0.51	2.34	0.39	-
2015	Ladbrooks(a)	6.1	47	-	13.8	0.32	2.23	0.41	103
2015	Ladbrooks(b)	5.9	73	-	18.5	0.53	2.72	0.48	128

3.5.5 Weed control after establishment

On 1/1/2015 site was sprayed with Trimec (600g/L mecoprop, 150g/L MCPA and 18.7g/L dicamba; 13.30 kg/a.i/ha) to control fathen, wire weed, stinging nettle and shepherd's-purse.

3.5.6 Mowing and defoliation management

At the end of each growth cycle, herbage was mown to a residual cutting height of ~30 mm by a Field Master Forage Harvester with a collection cage. This machine was unable to mow around and between the neutron access tubes. So, an area of 2 m^2 around each tube was hand cut followed by the use of a lawn mower to cut the grass area between the tubes. On all mowing dates, mown herbage mass was carried with the collection cage and removing out of the experimental site. The frequency of mowing and regrowth periods for the experiment are given in Appendix 2.

3.5.7 Nitrogen fertilizer

In 2015/16, a total annual application of 900 kg N/ha/y was applied in eight split applications of 100 kg N/ha and one application of 200 kg N/ha, at the beginning of each regrowth cycle.

Based on the soil test results, six sub-plots at Ladbrooks (plots 22, 23, 24, 26, 27, 28) showed a background nitrogen (Table 5). Therefore, on 18/9/2015, 50 kg N /ha was applied to the +N sub-plots located in those areas. 100 kg N/ha was applied to the rest of +N sub-plots at the same date. 50 kg N/hanitrogen fertilizer was applied to all N+ plots on 10th March 2017.

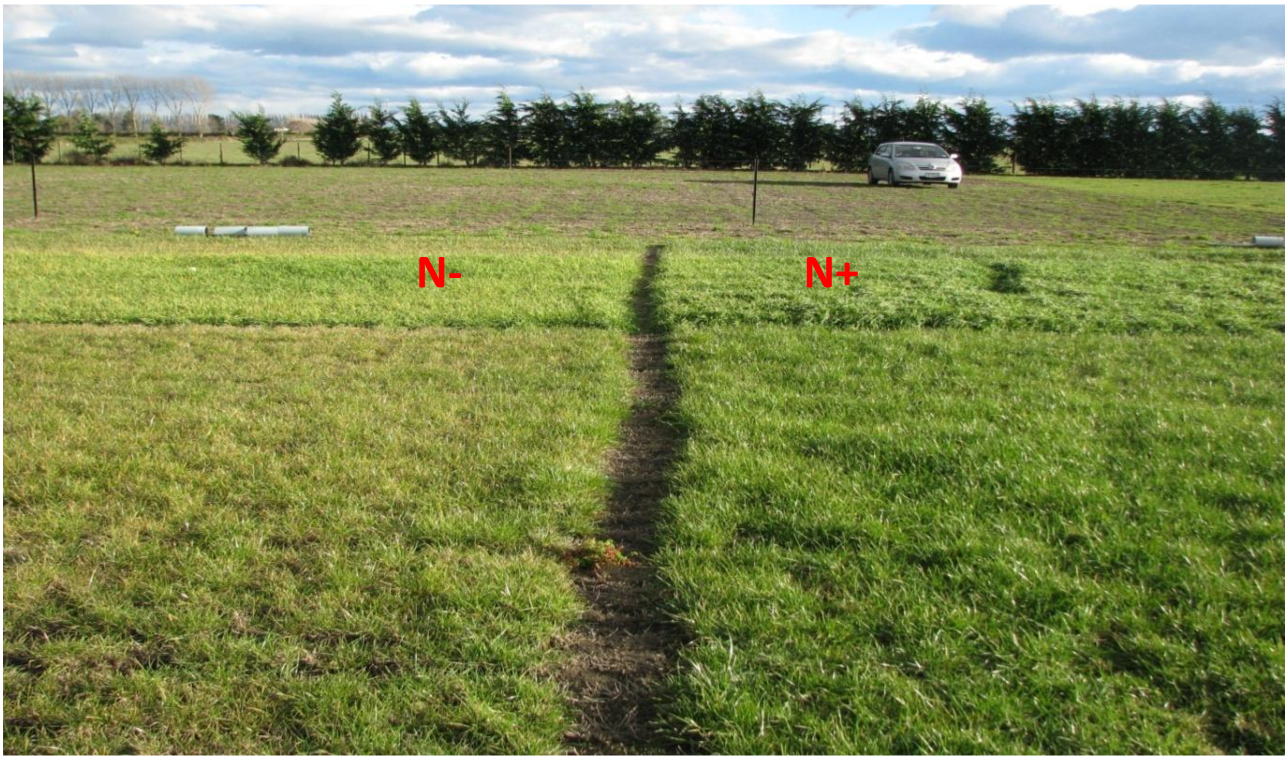

Plate 3-2: Nitrogen treatment split plot design at Ladbrooks 2017.

3.6 Measurements

3.6.1 Dry matter production

3.6.1.1 Destructive harvests

Regrowth periods were 35 to 46 days during the experiment. Samples for dry matter measurements were taken from a 0.2m^2 quadrat with a set of electric shears to a residual pasture height of ~30 mm. The area harvested at the previous sampling was excluded. After cutting, sample bags were taken to the Field Service Centre and left in an oven (~45°C) for at least 48 hours and dry weight of each sample measured.

3.6.2 Botanical composition

For each destructive harvest, botanical composition was measured from the sub-samples which were taken randomly from each sub-plot using a quartering technique (Cayley & Bird, 1996). The harvested materials were transferred to the laboratory and sorted into sown grass, weeds and senesced herbage. If leaves were more than 50% dead, they were included in the senesced category. Each botanical component was bagged, and placed in a tray along with the rest of the sample (bulk). The samples were then dried in a forced draft oven at 50-60°C for at least 48 hours until reaching a constant weight. Samples were then removed and weighed. Data on botanical composition and dry matter (DM) yields were recorded.

3.6.3 SPAD chlorophyll meter

On the 8th of April 2017 chlorophyll content readings were taken non-destructively on three fully unfolded green leaves per plot of each pasture species. This was done with a SPAD -502 Plus Chlorophyll Meter (Konica Minolta, obtained from Thermofisher Scientific, Auckland) which records chlorophyll content in SPAD units.

3.6.4 Morphological leaf measurements

Before each destructive harvest a number of morphological measurements were conducted on all four pasture species. Three individual tillers were marked in each plot. At each marked tiller leaf elongation was measured at the beginning of the regrowth period and again at the end of the regrowth period. On each tiller the green blade length of individual fully expanded leaves was measured from the tip to its own ligule, or of growing leaves from the ligule of the previous fully expanded leaf with a 1 mm graduated ruler.Leaf elongation was deemed to begin at appearance of the first new leaf after mowing, the leaf was deemed to have finished elongation once its ligule appeared, at which point leaf elongation continued to be measured on the next new leaf. The total leaf elongation was then recorded for a growth period. Three leaf elongation measurements were taken between 10/3/2017 and 30/6/2017.

Each marked tiller was then cut at the base, sorted and placed in sample bags. Each sampled tiller then had the newest fully elongated leaf measured for length (L) and width (W). The length of the tiller sample leaf was taken in two measurements. One to the beginning of leaf tapering (L1), the second from the beginning of leaf tapering to the tip of the leaf (L2). This allowed leaf lamina area to be calculated from the width and length measurements. The formula used for this was:

Leaf lamina area = (L1*W) + (L2*W/2)

A total of 96 leaves were measured each harvest. There were three harvests on the 9th April, 15th May, and 20th June 2017.

The fresh weight of each leaf lamina was recorded on a three decimal point scale (grams). The laminae were then put back into sample bags and dried for 48 hours at 50-60°C. The leaf lamina dry weight weights were then recorded and water content(percentage DM) of the leaves was then calculated as lamina (FW/DM)*100.

Leaf thickness was calculated as:$(SLA * PDM)^{-1}$ (mm)

Specific leaf area was calculated as:leaf area/ (leaf DW (g) * 1000)($mm^2\ g^{-1}$)

3.7 Statistical analysis

Results were analysed by Anova with the General Linear Model function in Genstat, using replicates as blocks. Where necessary, data were transformed to satisfy the assumptions of homogeneity of variances (Table 4-1). Data were examined in a 2 factorial analysis, including all possible interactions. Interactions are presented here where P<0.05 in the Anova. No three way interaction was significant at that probability level and thus only the significant main effects and two-way interactions are presented in the Results section. All statistical significances from the ANOVA are presented in Table 4-1 and are thus not mentioned further in the Results section. Table 4-1 also summarizes differences in response to the various treatments and treatment interactions.Data were further examined using Pearson correlation coefficients, regression analysis and linear trendlines.

Plate 3-3: Ladbrooksplots pre-harvest.

4 RESULTS

Table 6: Summary of statistical analyses carried out on Ladbrooks data (PDM = percentage dry matter; DM = dry matter; SLA = specific leaf area, ↑ = increase, ↓ = decrease).

Trait	Time		N		Sp		Time x N		Time x Sp		N x Sp		Time x N x Sp	Tr
Leaf length	***	T1=T2>T3	***	N↓	***	Br>Cf>Tf>Pr	***	T1,T2,T3:N↓	ns		*	N-: Br, Cf, Pr, Tf↓	ns	
Leaf area	***	T1=T2>T3	***	N↓	***	Cf>Br>Tf>Pr	***	T1, T2: N↓	***	T1: Cf> Br> Tf> Pr T2: Cf= Br> Tf> Pr T3: Cf= Br> Tf= Pr	***	N-: Br, Cf, Pr, Tf↓	ns	
Leaf DM	***	T1=T2>T3	***	N↓	***	Cf>Br=Tf>Pr	***	T1,T2:N↑ T3:N↓	***	T1: Cf> Tf= Br> Pr T2: Cf= Tf> Br> Pr T3: Br= Cf> Tf= Pr	**	N-: Br, Cf↓	ns	
Leaf PDM	**	T1<T2= T3	***	N↑	**	Pr=Tf=Cf>Br	ns		ns		ns		ns	
SLA	ns		***	N↓	***	Br>Cf>Pr>Tf	*	T1,T2,T3:N↓	*	T1: Br= Cf= Pr> Tf T2: Br> Cf> Pr= Tf T3: Br> Cf> Pr= Tf	***	N-: Br, Cf, Pr, Tf↓	ns	LN
Leaf thickness	***	T3> T1 >T2	ns		***	Pr>Tf> Br = Cf	***	T1,T2: N- ↑ T3: N- ↓	*	T1: Pr> Tf> Br> Cf T2: Pr> Tf> Br= Cf T3: Pr> Tf> Br= Cf	*	N-: Br↑	ns	SQ
Total aboveground DM	***	T1> T2>T3	***	N↓	ns		*	T1,T2,T3:N↓	ns		*	N-: Br, Cf, Pr, Tf↓	ns	LN
Green aboveground DM	***	T1> T2>T3	***	N↓	ns		***	T1,T2,T3:N↓	*	T1: Cf= Tf= Br= Pr T2: Pr= Tf= Br= Cf T3: Pr= Tf= Br= Cf	+	N-: Br, Cf, Pr, Tf↓	ns	SQ
Dead aboveground DM	***	T1> T2>T3	ns		ns		ns		ns		ns		ns	

*** P < 0.001 = "very highly significant"
** P < 0.01 = "highly significant"
* P < 0.05 = "significant"
ns ≥ 0.05 = non-significant

N = nitrogen
Sp = species (Pr, Perennial ryegrass; Tf, Tall fescue; Br, Brome; Cf, Cocksfoot)
Tr = transformation (SQ, LN)
T1, T2, T3 = harvest 1, 2 and 3, respectively

4.1 Leaf length

Time

Averaged across treatments and species, leaf length (mm) was highest at the second harvest (175.0 mm), 36.7% higher than the third harvest (110.8 mm), harvest 1 wasintermediate (166.4 mm) (Fig. 4-2).

Species

Measured across time and treatments, leaf length was highest in brome (174.1 mm), followed by cocksfoot (160.3 mm) and lowest in perennial ryegrass (126.1 mm) (Fig. 4-2).

Nitrogen

Across time and species, leaf length decreased under the N- treatment by 30% from 177.7 mm to 123.8 mm (Fig. 4-2).

Time x nitrogen

N- treatment-induced differences in leaf length depended on harvest date. Leaf length decreased at all harvests, by 33% in harvest, 37% at harvest 2,and by 14% at harvest 3 (Fig. 4-3).

Nitrogen x species

Measured over time, there were N- treatment-induced differences in leaf length among the four species with brome showing a decrease in leaf length by 35%, perennial ryegrass and tall fescue both decreased by 29%, and cocksfoot by 27% (Fig. 4-2).

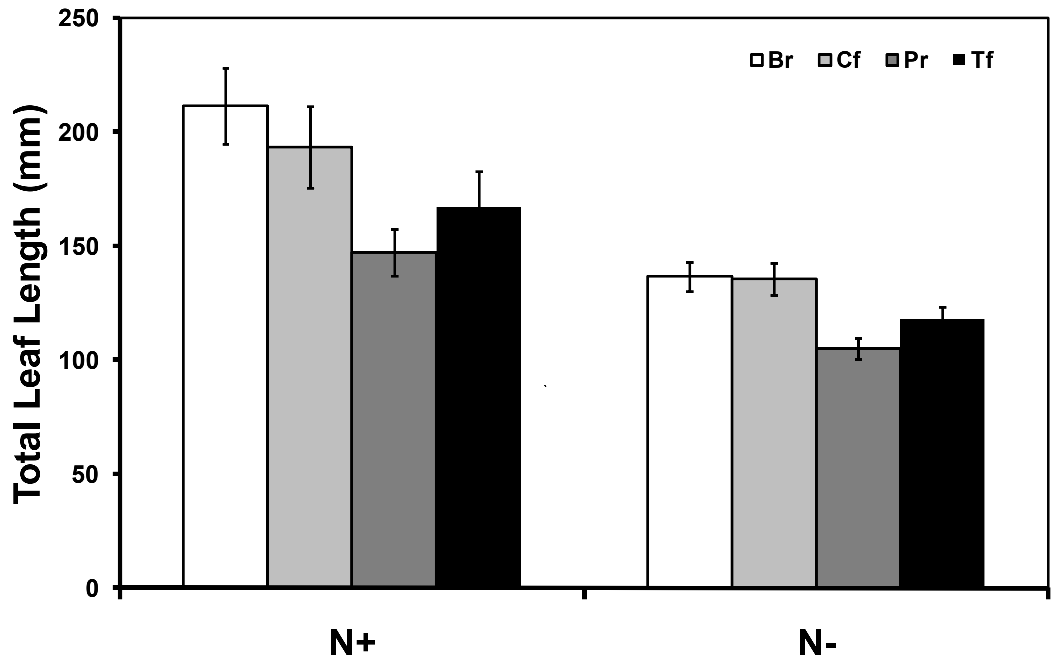

Figure 4-1: Total leaf length in response to nitrogen (N) treatments of four pasture grass species (brome, Br; cocksfoot, Cf; perennial ryegrass, Pr and tall fescue, Tf) averaged across three harvests (April, May and June 2017).

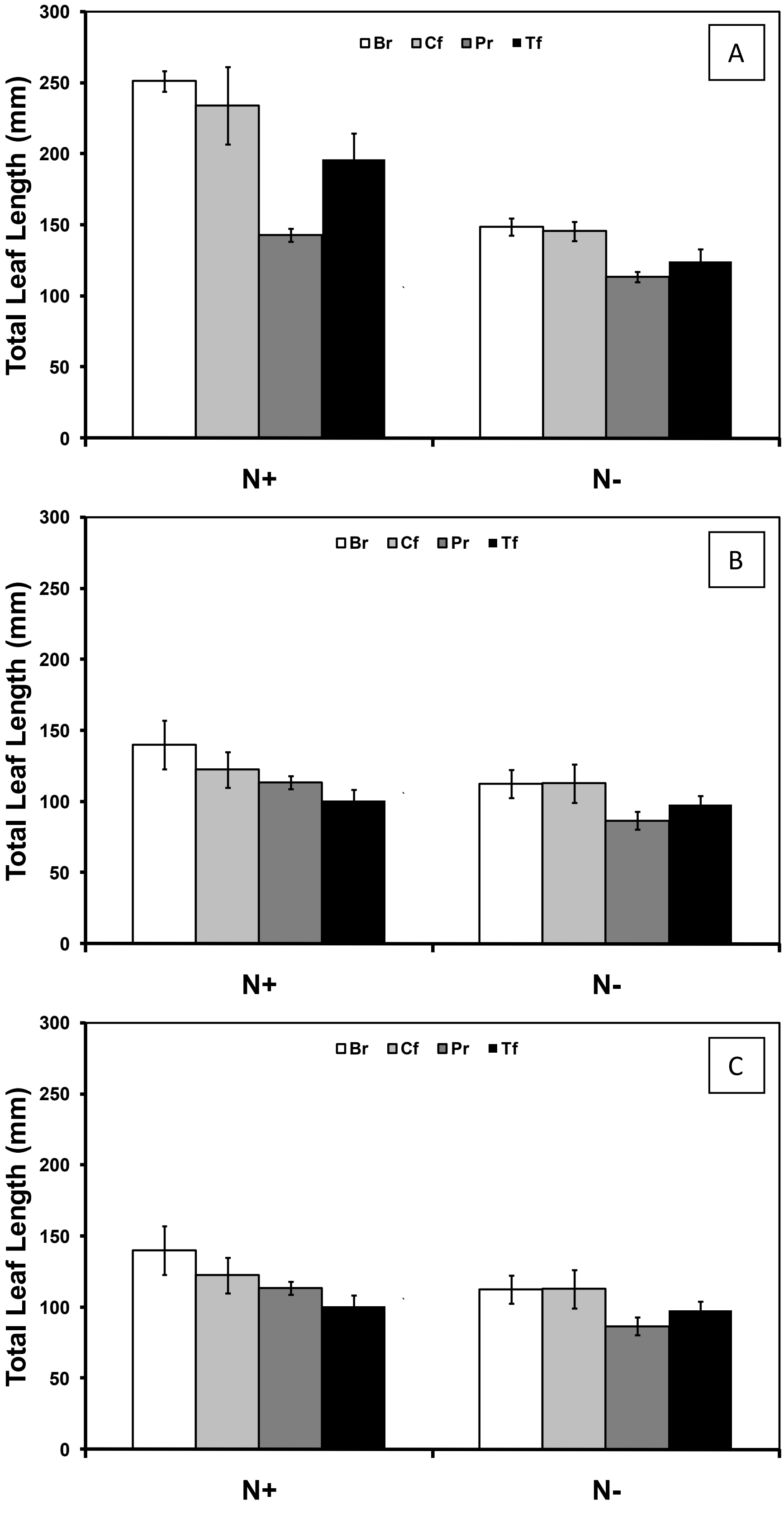

Figure 4-2: Total leaf thickness in response to nitrogen (N) treatments of four pasture grass species (brome, Br; cocksfoot, Cf; perennial ryegrass, Pr and tall fescue, Tf) in April (A), May (B) and June (C) 2017.

4.2 Leaf area

Time

Averaged across treatments and species, leaf area (mm^2) was highest at the second harvest (908 mm^2), nearly double (97%) the third harvest (462 mm^2), harvest 1 was intermediate at 849 mm^2 (Fig. 4-4).

Species

Measured across time and treatments, leaf area was highest in cocksfoot (1020 mm^2), followed by brome (912 mm^2) and lowest in perennial ryegrass (361 mm^2) (Fig. 4-4).

Nitrogen

Across time and species, leaf area decreased under the N- treatment by 36% from 901 mm^2 to 579 mm^2(Fig. 4-4).

Time x nitrogen

N- treatment-induced differences in leaf area depended on harvest date. Leaf area decreased at the first two harvests, by 40% in harvest 1 and 46% at harvest 2. However, leaf area only decreased by 1% at harvest 3 (Fig. 4-5).

Nitrogen x species

Measured over time, there were N- treatment-induced differences in leaf area among the four species with all four species showing a decrease in leaf area, brome by 41%, cocksfoot by 35%, perennial ryegrass by 32%, and tall fescue by 31% (Fig. 4-4).

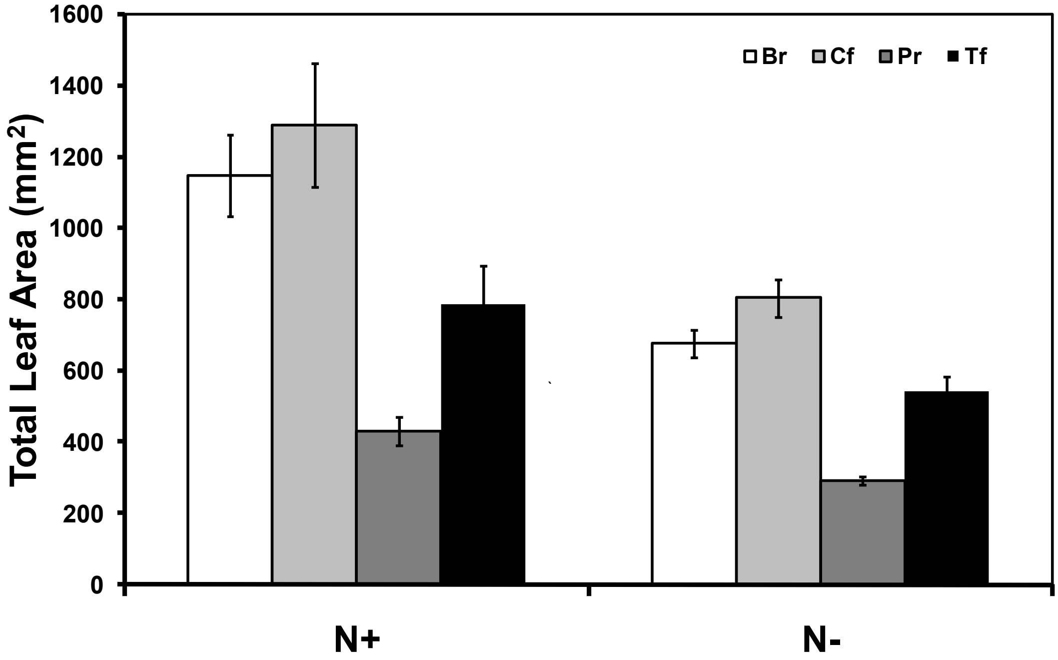

Figure 4-3: Total leaf area in response to nitrogen (N) treatments of four pasture grass species (brome, Br; cocksfoot, Cf; perennial ryegrass, Pr and tall fescue, Tf) averaged across three harvests (April, May and June 2017).

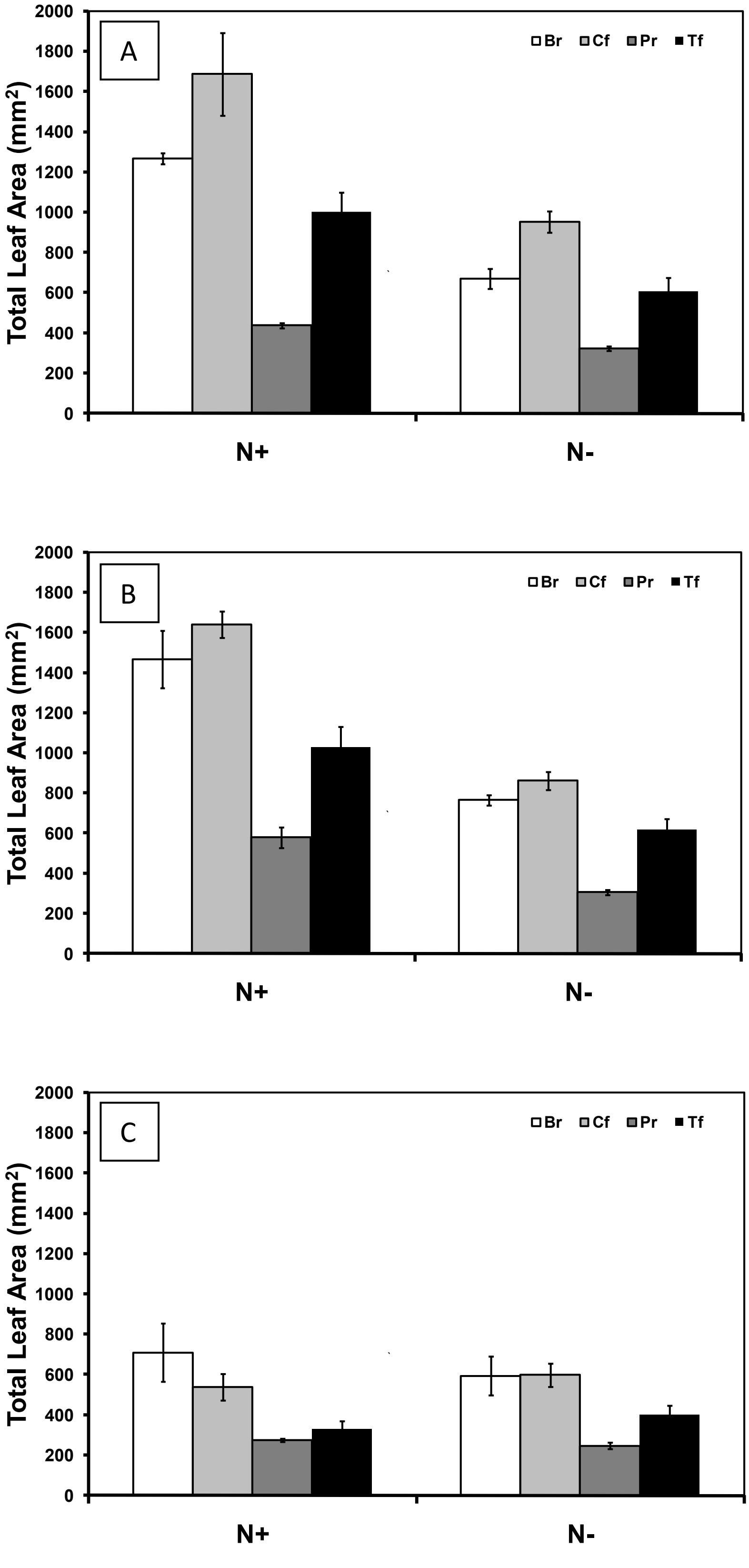

Figure 4-4:Total leaf area in response to nitrogen (N) treatments of four pasture grass species (brome, Br; cocksfoot, Cf; perennial ryegrass, Pr and tall fescue, Tf) in April (A), May (B) and June (C) 2017.

4.3 Leaf DW

Time

Averaged across treatments and species, leaf DW (g) was lowest at the third harvest (0.01625 g). The second harvest (0.03254 g) was double the third, and slightly higher than the first harvest (0.03039 g) (Fig. 4-6).

Species

Measured across time and treatments, leaf DW was highest in cocksfoot (0.03430 g), followed by brome (0.02742 g) and lowest in perennial ryegrass (0.01415 g) (Fig. 4-6).

Nitrogen

Across time and species, leaf DW decreased under the N- treatment by 19% (from 0.02922 g to 0.02356 g) (Fig. 4-7).

Time x nitrogen

N- treatment-induced differences in leaf DW depended on harvest date. Leaf DW decreased at the first two harvests, by 29% in harvest 1 and 31% at harvest 2. However, leaf DW decreased by 37% at harvest 3 (Fig. 4-7).

Nitrogen x species

Measured over time, there were N- treatment-induced differences in leaf DW among the four species with all four species showing a decrease in leaf area, brome by 24%, cocksfoot by 30%, perennial ryegrass by 14%, and tall fescue by 4% (Fig. 4-6).

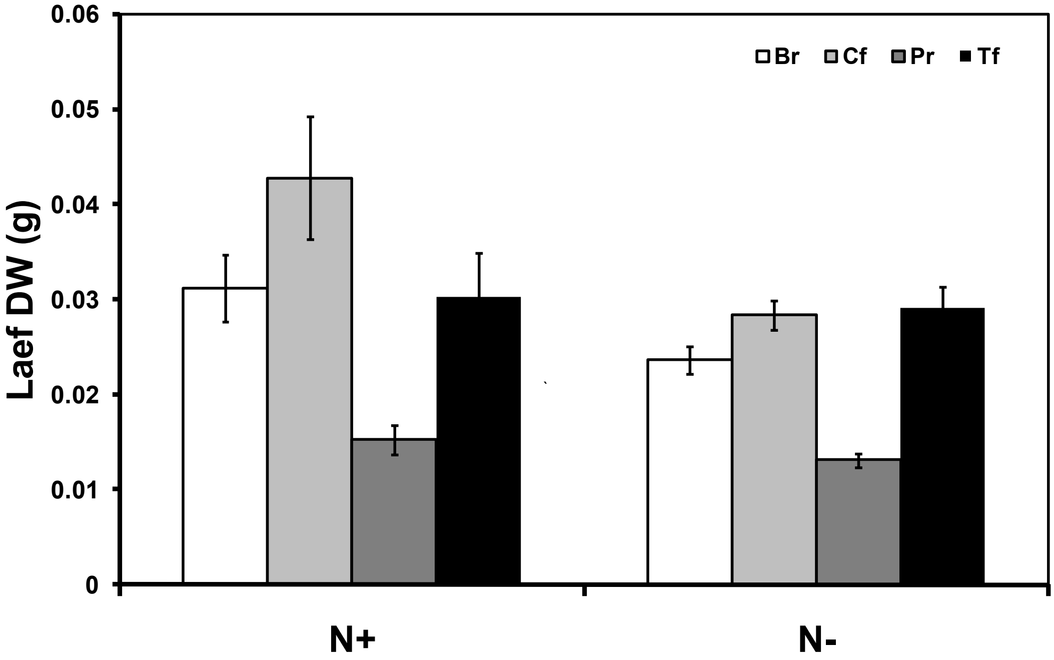

Figure 4-5:Total leaf DW in response to nitrogen (N) treatments of four pasture grass species (brome, Br; cocksfoot, Cf; perennial ryegrass, Pr and tall fescue, Tf) from April, May and June 2017.

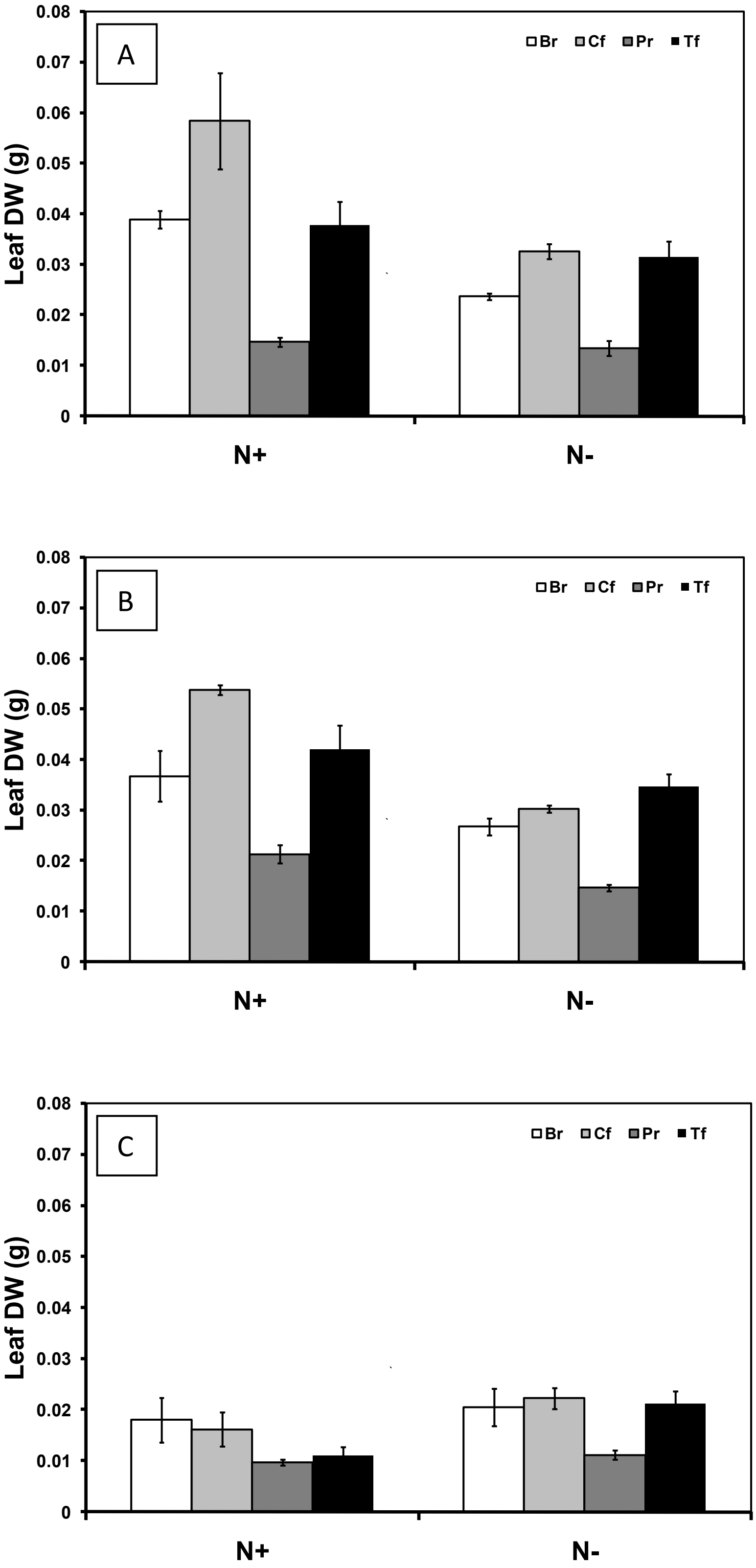

Figure 4-6: Total leaf DW in response to nitrogen (N) treatments of four pasture grass species (brome, Br; cocksfoot, Cf; perennial ryegrass, Pr and tall fescue, Tf) in April (A), May (B) and June (C) 2017.

4.4 Leaf percentage DM

Time

Averaged across treatments and species, leaf percentage DM (%) was lowest at the first harvest (21.5%) and higher at the second (23.3%) and third harvest (23.2%) (Fig. 4-8).

Species

Measured across time and treatments, leaf percentage DM was highest in tall fescue (23.9%), cocksfoot (23.7%) and perennial ryegrass (22.8%) and lowest in brome (21.5%) (Fig. 4-8).

Nitrogen

Across time and species, leaf percentage DM increased under the N- treatment by 18% from 0.2084 g to 0.2463 g (Fig. 4-8).

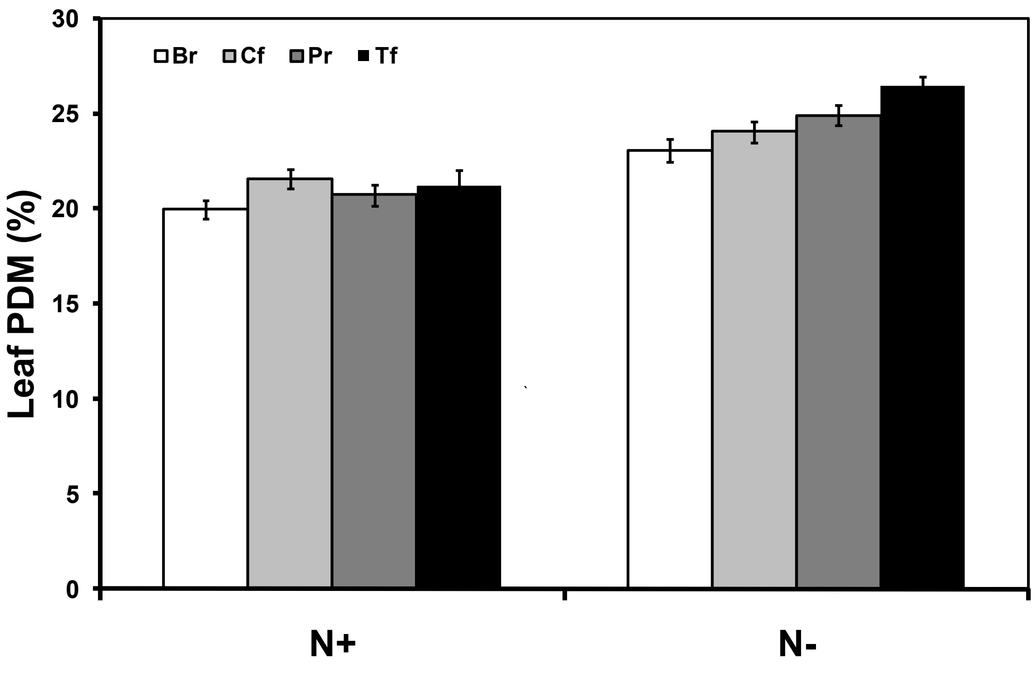

Figure 4-7: Total leaf percentage DM response to nitrogen (N) treatments of four pasture grass species (brome, Br; cocksfoot, Cf; perennial ryegrass, Pr and tall fescue, Tf) averaged across three harvests (April, May and June 2017).

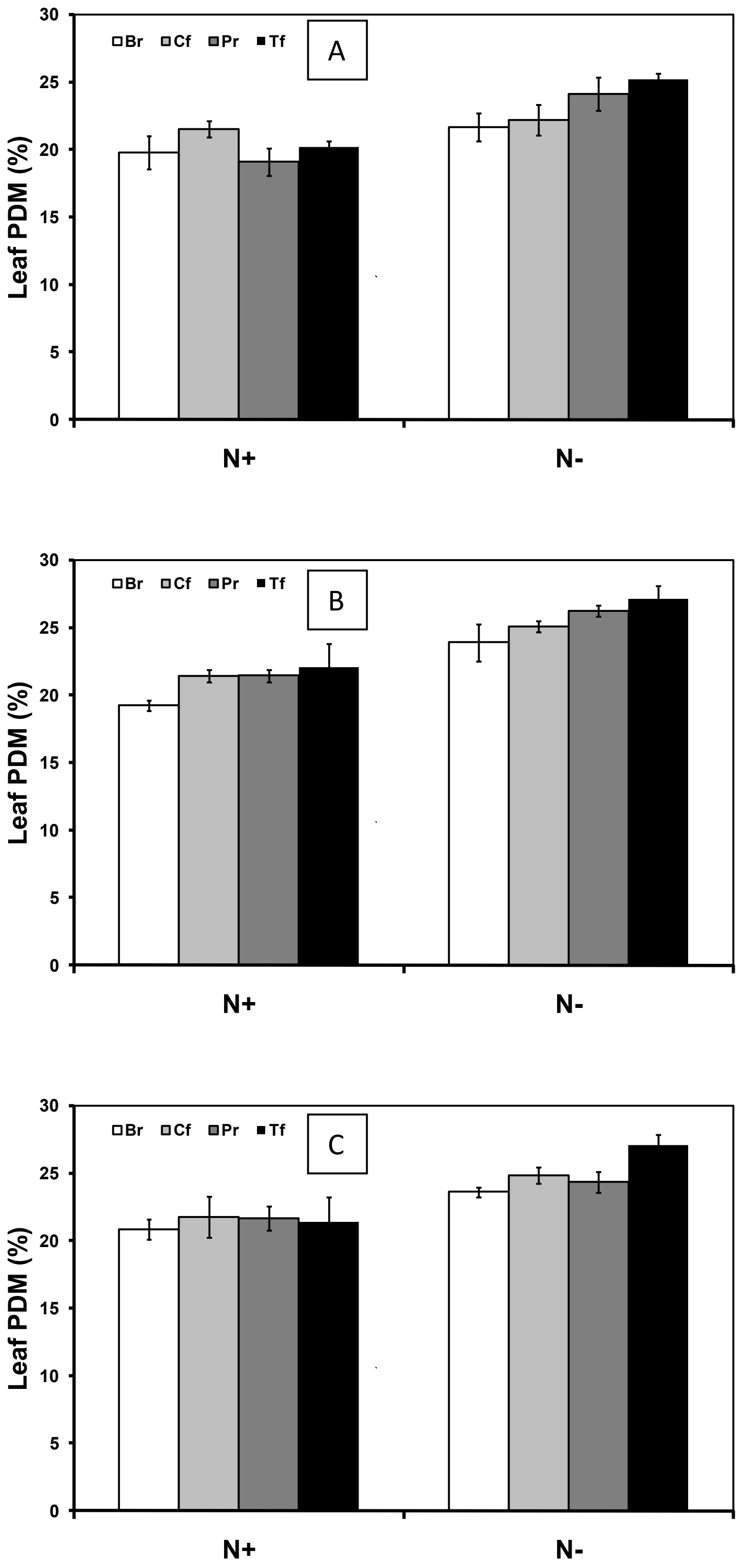

Figure 4-8: Leaf percentage DM in response to nitrogen (N) treatments of four pasture grass species (brome, Br; cocksfoot, Cf; perennial ryegrass, Pr and tall fescue, Tf) in April (A), May (B) and June (C) 2017.

4.5 Specific Leaf Area (SLA)

Species

Measured across time and treatments, SLA was highest in brome (3.495 $mm^2\ g^{-1}$) and cocksfoot (3.400 $mm^2\ g^{-1}$), and lowest in perennial ryegrass and tall fescue (3.231 $mm^2\ g^{-1}$and 3.114 $mm^2\ g^{-1}$, respectively) (Fig. 4-10).

Nitrogen

Across time and species, SLA decreased under the N- treatment by 7%, from 3.437 $mm^2\ g^{-1}$ to 3.183 $mm^2\ g^{-1}$ (Fig. 4-10).

Time x nitrogen

N- treatment-induced differences in SLA depended on harvest date. SLA decreased at all harvests, by 5% in harvest 1, by 7% at harvest 2, and by 9% at harvest 3 (Fig. 4-11).

Nitrogen x species

Measured over time, there were N- treatment-induced differences in SLA among the four species. All four species showed a decrease in SLA with tall fescue decreasing by 12%, perennial ryegrass by 7%, cocksfoot by 4%, and brome by 7.5% (Fig. 4-10).

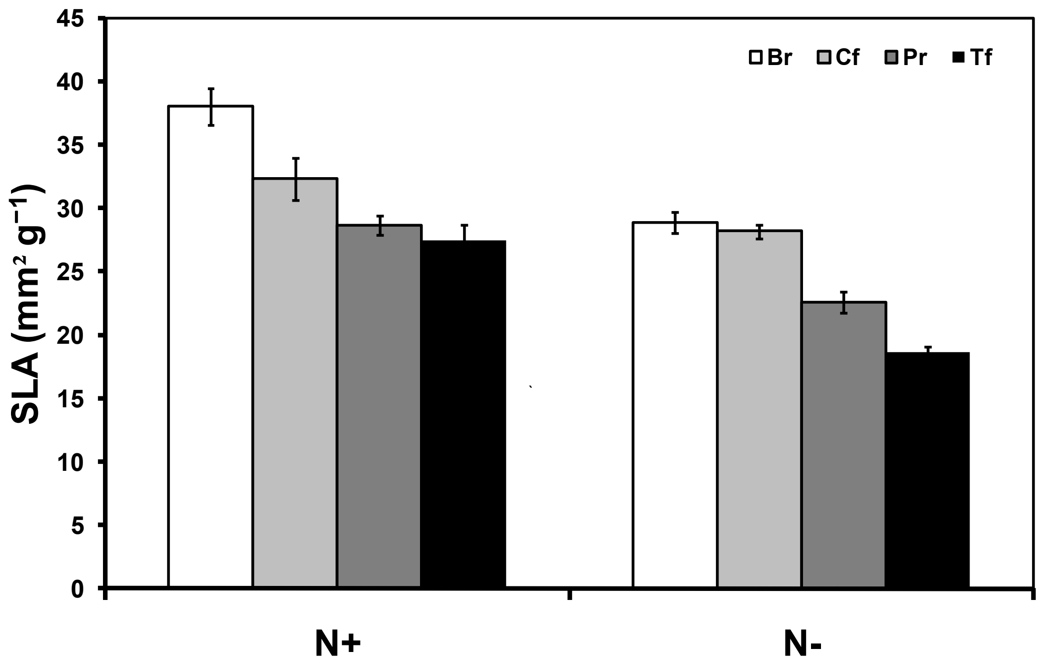

Figure 4-9:SLA in response to nitrogen (N) treatments of four pasture grass species (brome, Br; cocksfoot, Cf; perennial ryegrass, Pr and tall fescue, Tf) averaged across three harvests (April, May and June 2017).

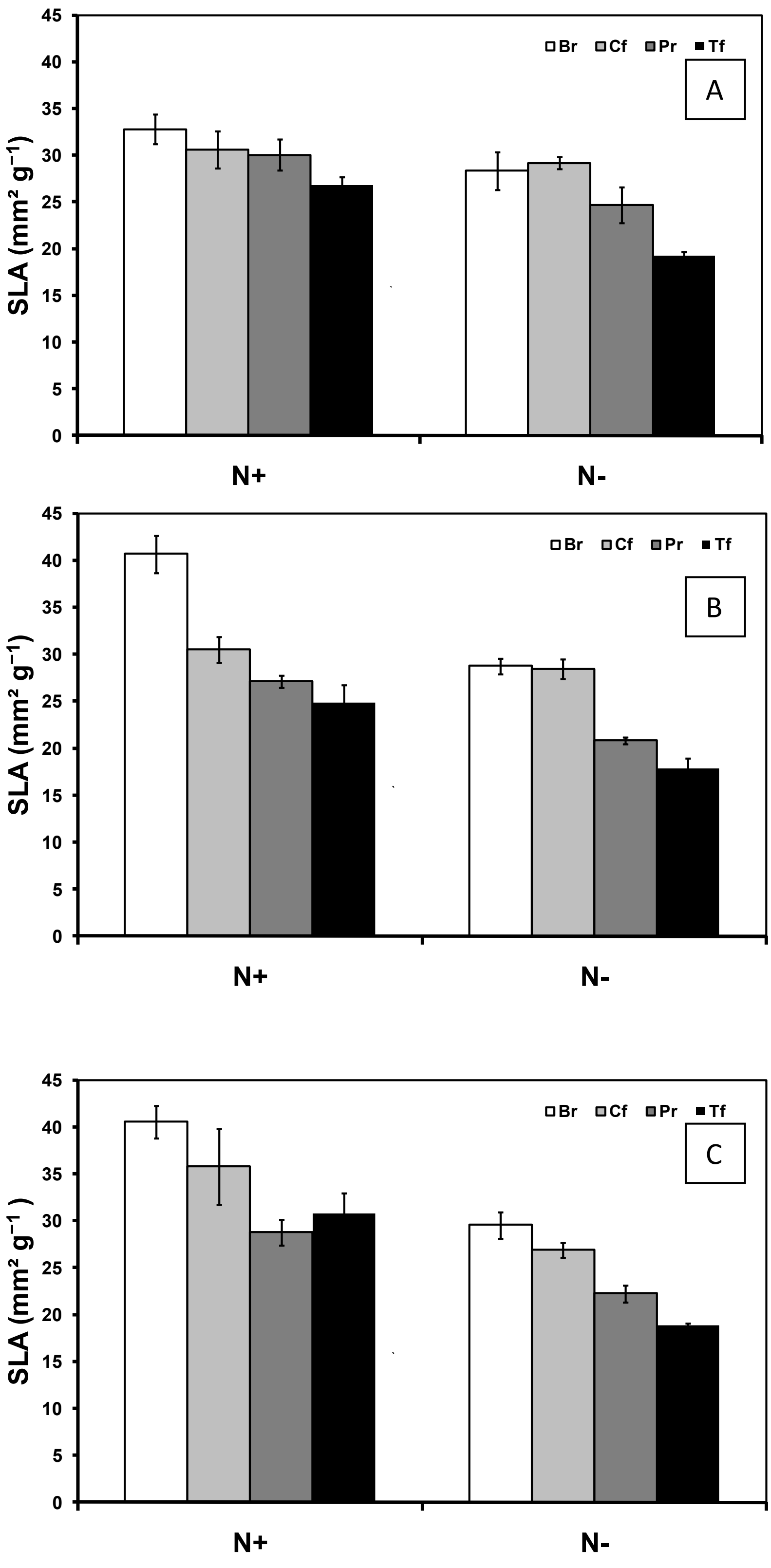

Figure 4-10:SLA in response to nitrogen (N) treatments of four pasture grass species (brome, Br; cocksfoot, Cf; perennial ryegrass, Pr and tall fescue, Tf) in April (A), May (B) and June (C) 2017.

4.6 Leaf thickness

Time

Averaged across treatments and species, leaf thickness was lowest at the second harvest (0.0064 mm) and nearly double that at the third harvest (0.0124 mm), harvest 1 was intermediate at 0.0076 mm (Fig. 4-13).

Species

Measured across time and treatments, leaf thickness was highest in perennial ryegrass (0.014mm) and lowest in brome and cocksfoot (0.0063 mm and 0.0058 mm, respectively) (Fig. 4-12).

Time x nitrogen

N- treatment-induced differences in leaf thickness depended on harvest date. Leaf thickness increased at the first two harvests, by 26% in harvest 1 and 45% at harvest 2. However, leaf thickness decreased by 21% at harvest 3 (Fig. 4-13).

Nitrogen x species

Measured over time, there were N- treatment-induced differences in leaf thickness among the four species with brome showing an increase in leaf thickness by one third and perennial ryegrass increased by 13% under N- treatment. Cocksfoot stayed the same between the two treatments (0.57 mm). Tall fescue decreased by 21% under N- treatment (Fig. 4-12).

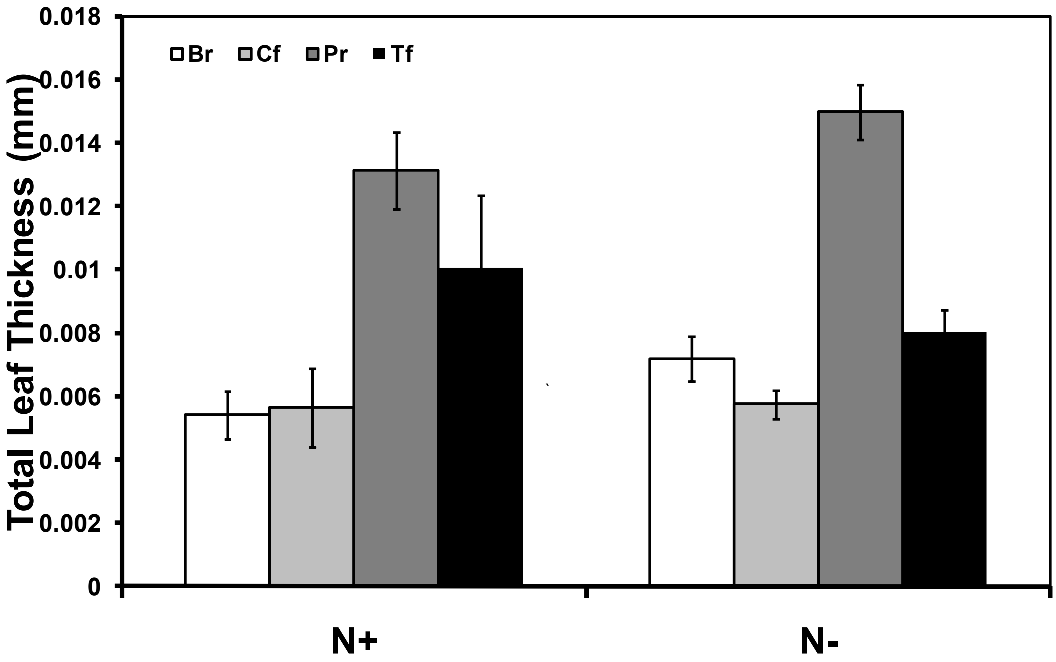

Figure 4-11: Total leaf thickness in response to nitrogen (N) treatments of four pasture grass species (brome, Br; cocksfoot, Cf; perennial ryegrass, Pr and tall fescue, Tf) averaged across three harvests (April, May and June 2017).

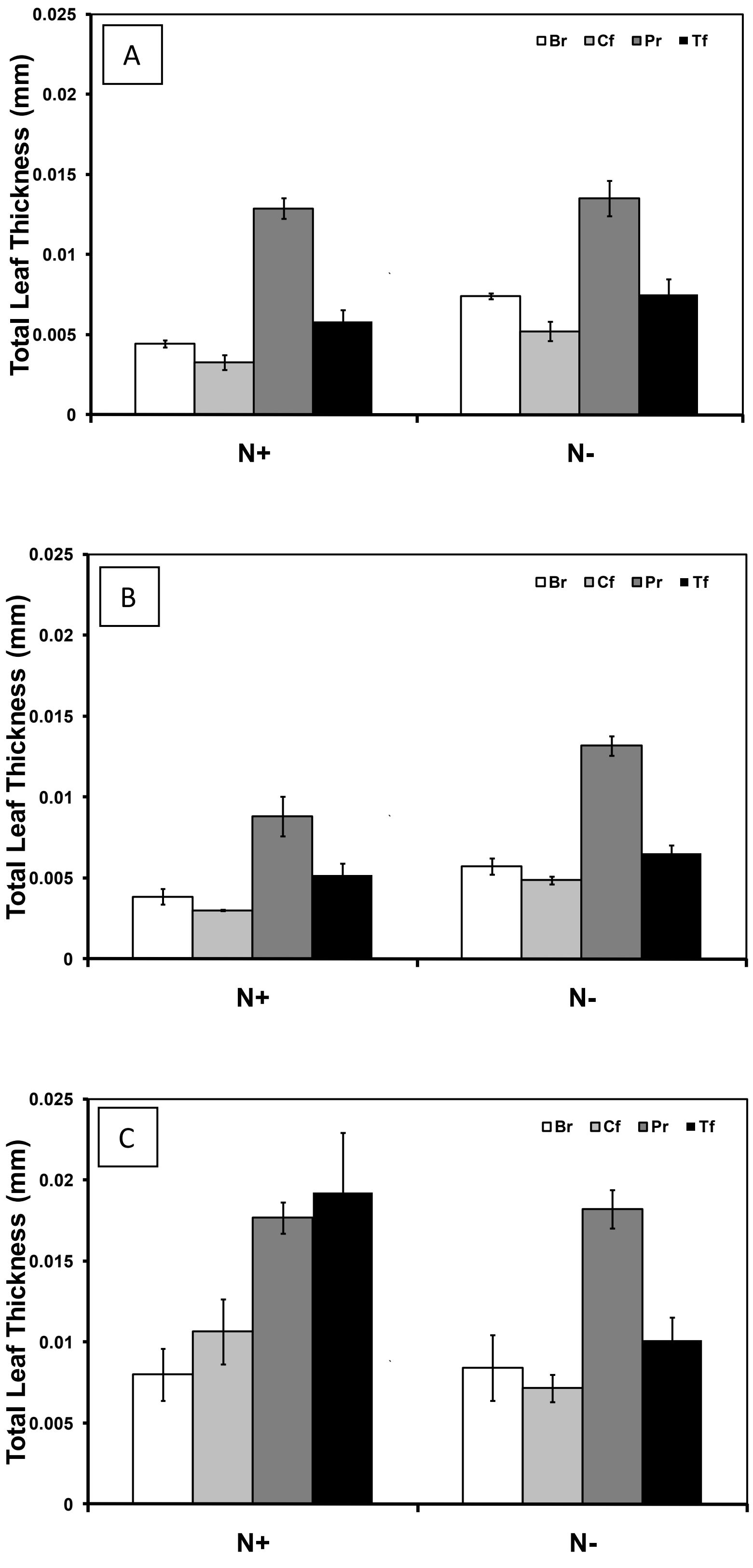

Figure 4-12: Total leaf thickness in response to nitrogen (N) treatments of four pasture grass species (brome, Br; cocksfoot, Cf; perennial ryegrass, Pr and tall fescue, Tf) in April (A), May (B) and June (C) 2017.

4.7 SPAD

Nitrogen

Nitrogen treatment-induced differences in SPADwere seen between N treatments, with a 15% reduction in SPAD under N-from 43.54 SPAD units to 37.15 SPAD units (Fig. 4-14).

Species

Measured across species, SPAD levels were highest in tall fescue (42.0 SPAD units) and cocksfoot (42.0 SPAD units), 11.6% and 4.8% higher than perennial ryegrass (37.1 SPAD units) and brome (40.2 SPAD units), respectively (Fig. 4-14).

Nitrogen x species

There were nitrogen treatment-induced differences in SPAD among the four species with brome showing adecrease in SPAD by 15%, cocksfoot and perennial ryegrass decreased by 17%, and tall fescue decreased by 10% under N- treatment(Fig. 4-14).

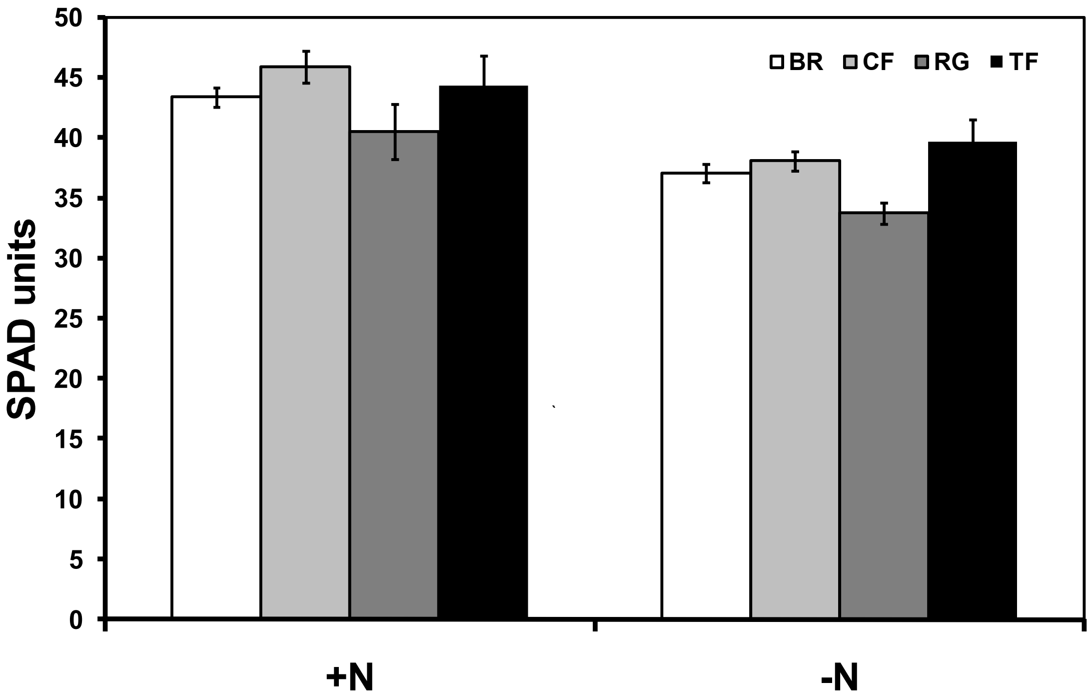

Figure 4-13:SPAD in response to nitrogen (N) treatments of four pasture grass species (brome, Br; cocksfoot, Cf; perennial ryegrass, Pr and tall fescue, Tf), 9th April 2017.

4.8 Total aboveground DM

Time

Averaged across treatments and species, DM was lowest at the third harvest (5.906 kg ha^{-1}) which was 17% lower than at the first harvest (6.913 kg ha^{-1}), the second harvest was intermediate at 6.583 kg ha^{-1} (Fig. 4-15).

Nitrogen

Across time and species, DM decreased under the N- treatment by 13%, from 6.926 kg ha^{-1} under N+, to 6.009 kg ha^{-1} under N- treatment (Fig. 4-15).

Time x nitrogen

N- treatment-induced differences in DM depended on harvest date. DM decreased across all harvests under N- treatment, by 14% in harvest 1, 9% at harvest 2, and by 17% at harvest 3 (Fig. 4-16).

Nitrogen x species

Measured over time, there were N- treatment-induced differences in DM among the four species with brome, cocksfoot and tall fescue all showing a decrease in DM by 11%, 14% and 19%, respectively under N- treatments, similarly perennial ryegrass decreased by 7% (Fig. 4-16).

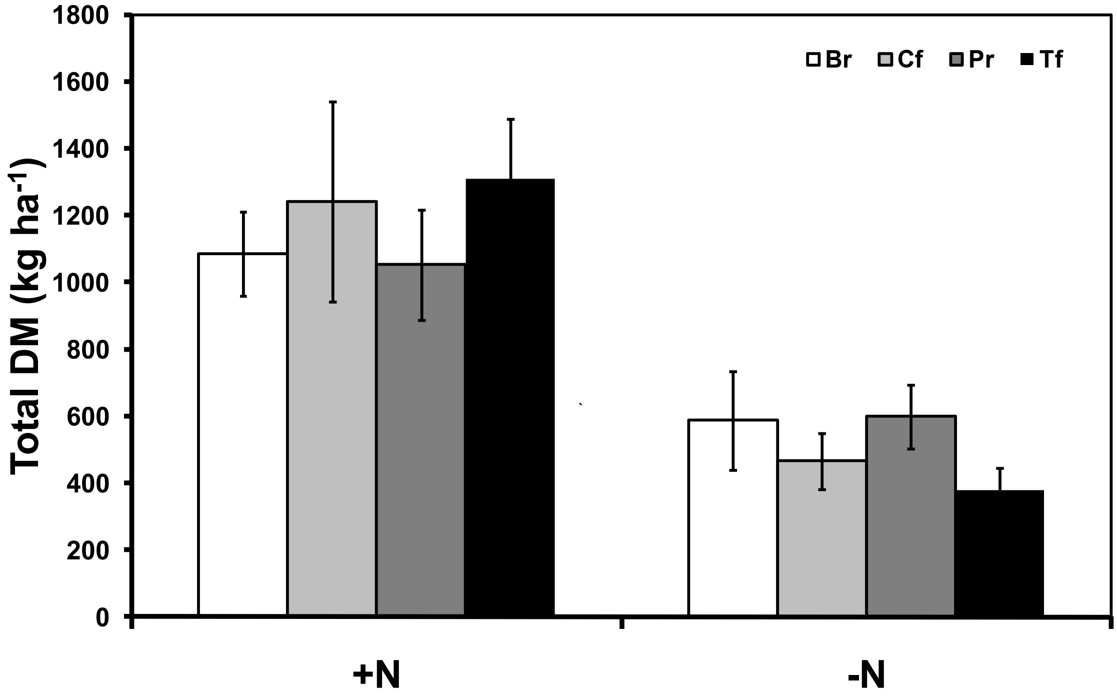

Figure 4-14: Total dry matter in response to nitrogen (N) treatments of four pasture grass species (brome, Br; cocksfoot, Cf; perennial ryegrass, Pr and tall fescue, Tf) averaged across three harvests (April, May and June 2017).

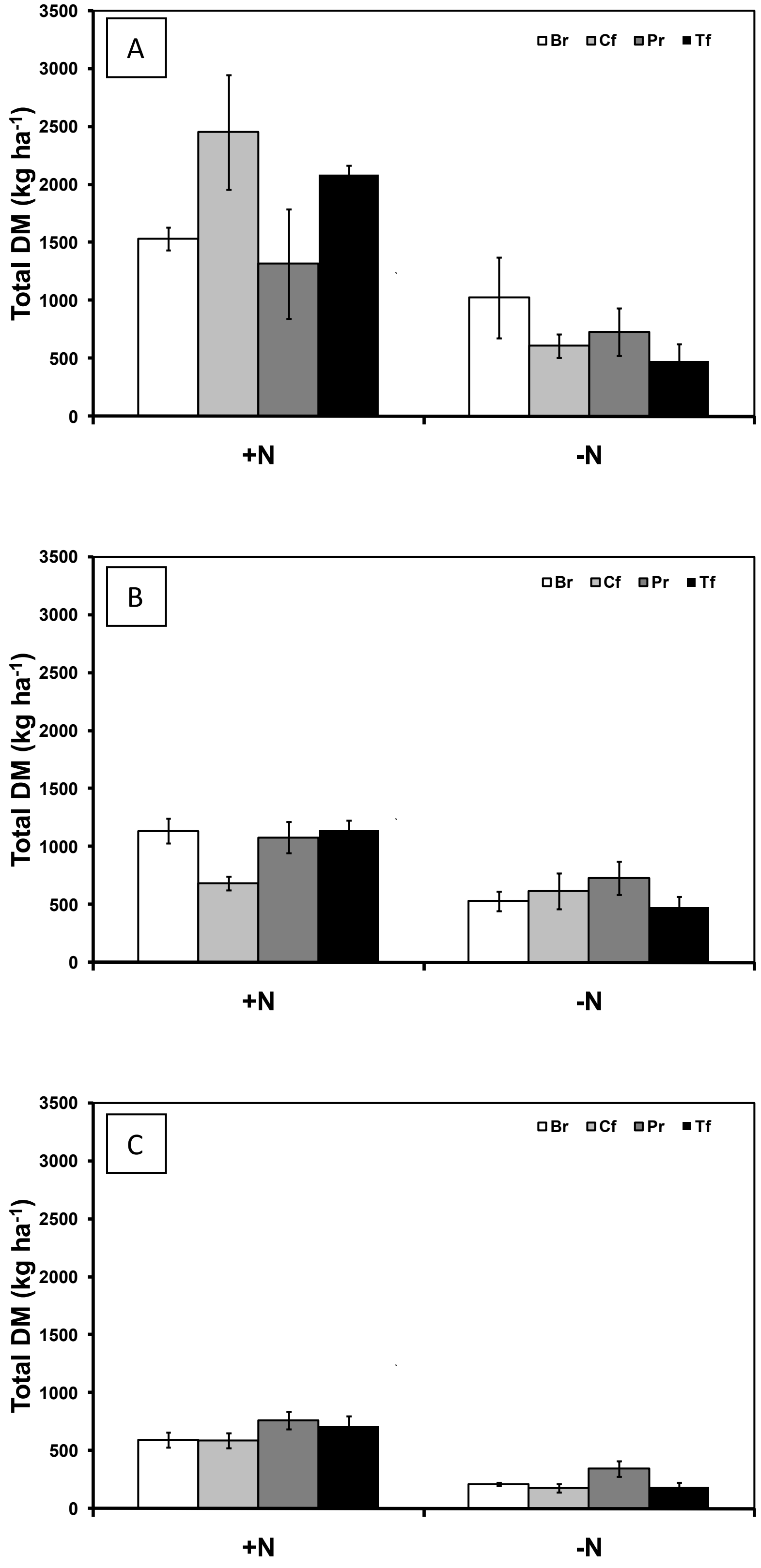

Figure 4-15: Total dry matter in response to nitrogen (N) treatments of four pasture grass species (brome, Br; cocksfoot, Cf; perennial ryegrass, Pr and tall fescue, Tf) in April (A), May (B) and June (C) 2017.

4.9 Green aboveground DM

Time

Averaged across treatments and species, green DM was lowest at the third harvest (19.54 kg ha^{-1}) which was 25% lower than at the second harvest (26.1 kg ha^{-1}), which was 14% lower than the first harvest (30.27 kg ha^{-1}) (Fig. 4-18).

Nitrogen

Across time and species, green DM decreased under the N- treatment by 42%, from 31.94 kg ha^{-1} to 18.66 kg ha^{-1} (Fig. 4-17).

Time x nitrogen

N- treatment-induced differences in green DM depended on harvest date. Green DM decreased in all harvests under the N- treatment, by half in harvest 1, 29% at harvest 2, and by 44% at harvest 3 (Fig. 4-18).

Nitrogen x species

Measured over time, there were N- treatment-induced differences in green DM among the four species. All species decreased in green DM under N- treatment; tall fescue by half, brome by 42%, cocksfoot by 41%, and perennial ryegrass by 32% (Fig. 4-17).

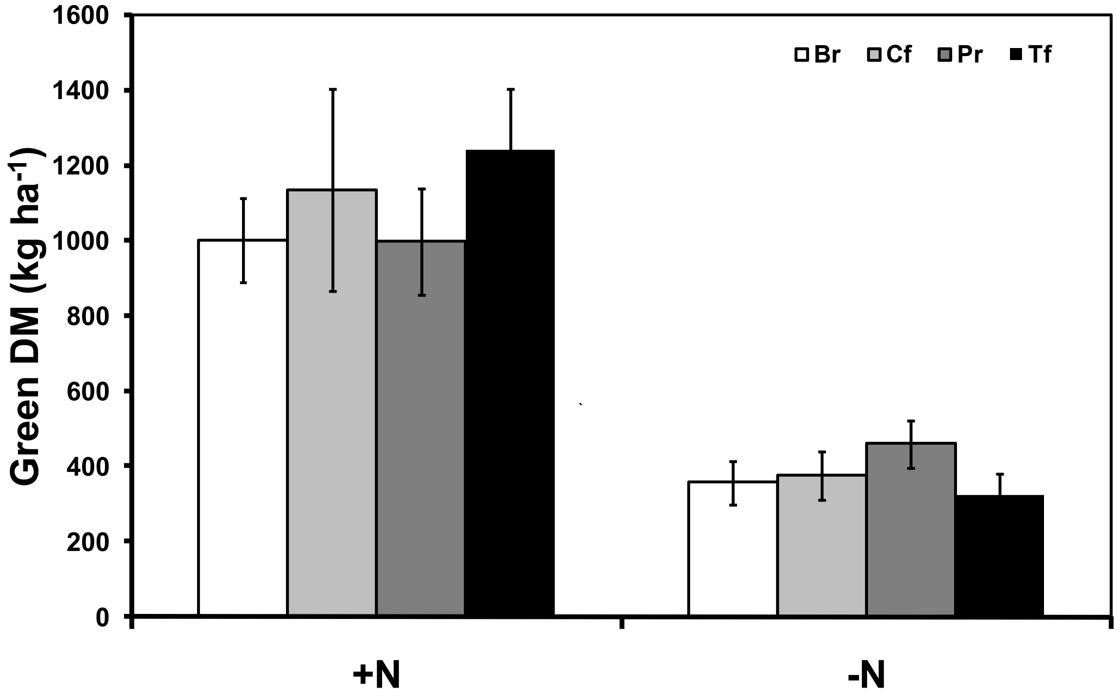

Figure 4-16: Total green dry matter in response to nitrogen (N) treatments of four pasture grass species (brome, Br; cocksfoot, Cf; perennial ryegrass, Pr and tall fescue, Tf) averaged across three harvests (April, May and June 2017).

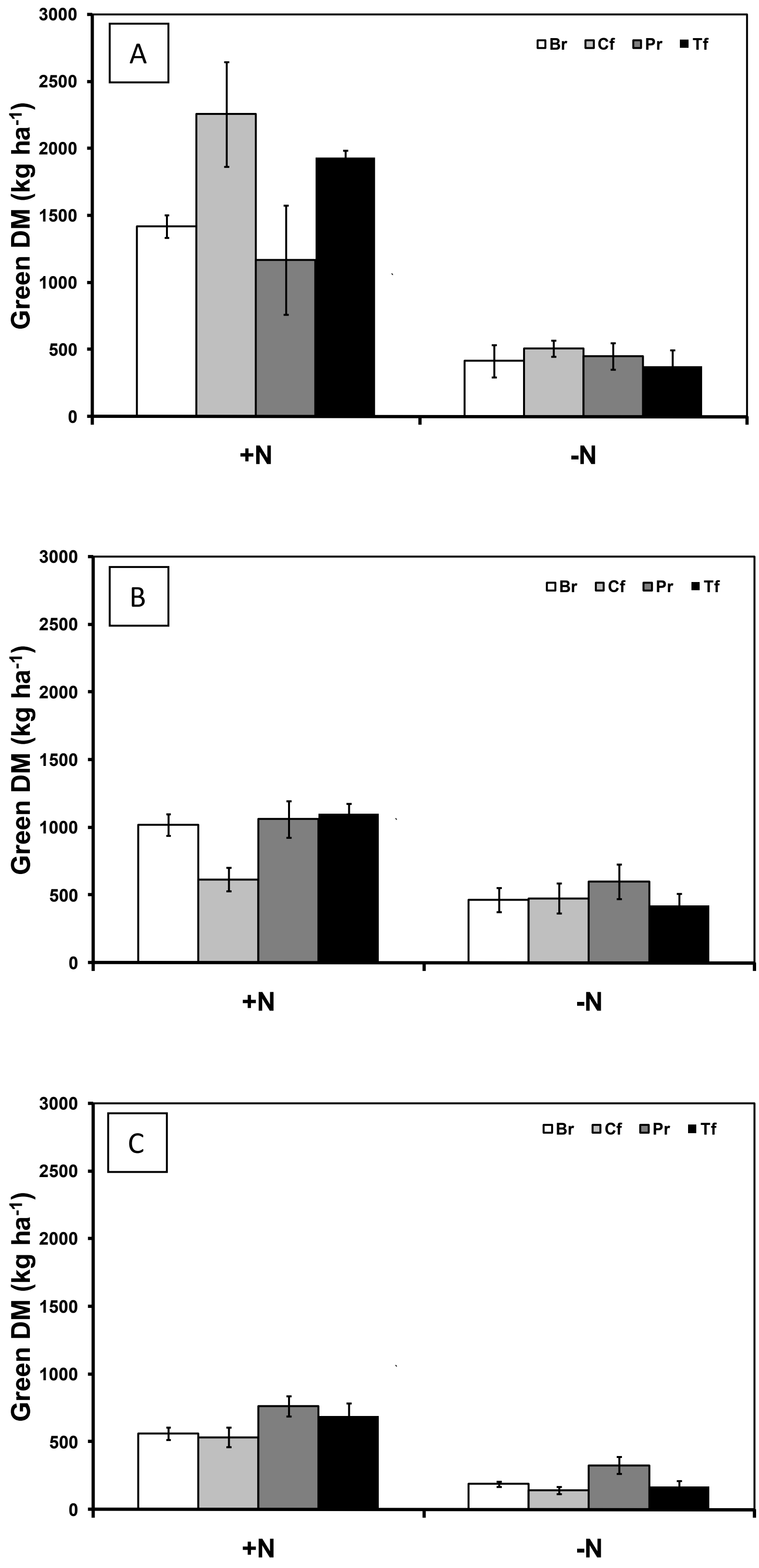

Figure 4-17: Total green dry matter in response to nitrogen (N) treatments of four pasture grass species (brome, Br; cocksfoot, Cf; perennial ryegrass, Pr and tall fescue, Tf) in April (A), May (B) and June (C) 2017.

4.10 Senesced aboveground DM

Time

Averaged across treatments and species, dead DM was lowest at the third harvest (23 kg ha^{-1}) and triple at the second harvest (75 kg ha^{-1}), and double at the first harvest (152 kg ha^{-1}) (Fig. 4-20).

Time x nitrogen

N- treatment-induced differences in dead DM depended on harvest date. Dead DM increased at the first two harvests, by 3% in harvest 1 and 52% at harvest 2. However, dead DM decreased by 16% at harvest 3 (Fig. 4-19).

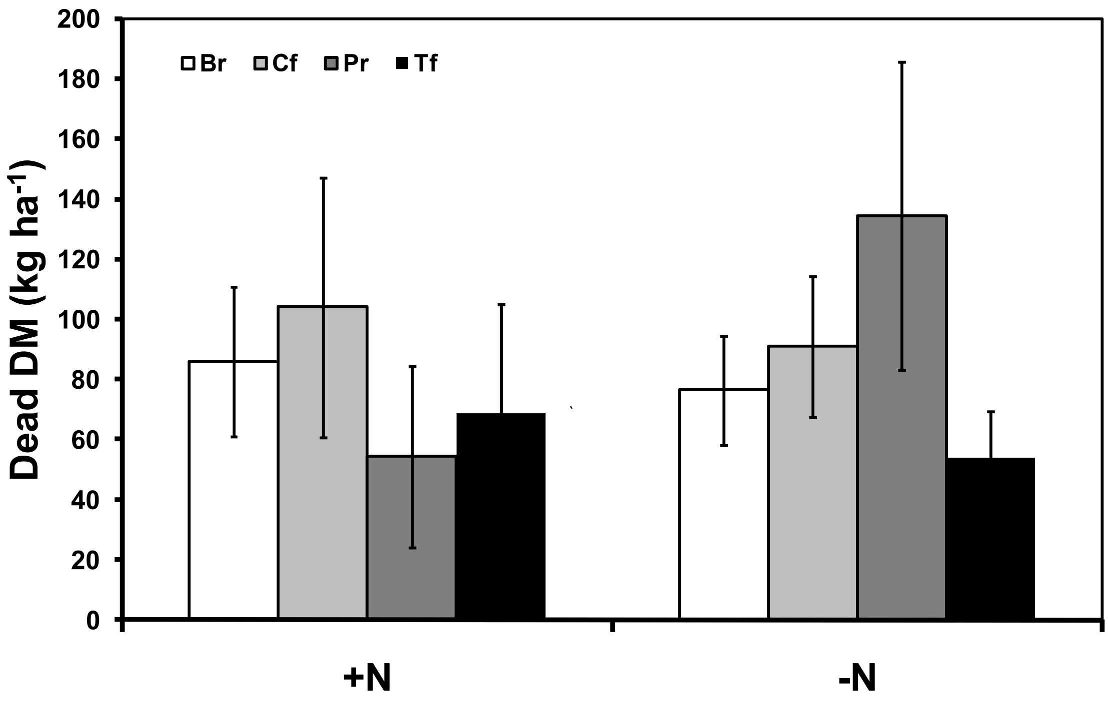

Figure 4-18: Total dead dry matter in response to nitrogen (N) treatments of four pasture grass species (brome, Br; cocksfoot, Cf; perennial ryegrass, Pr and tall fescue, Tf) averaged across three harvests (April, May and June 2017).

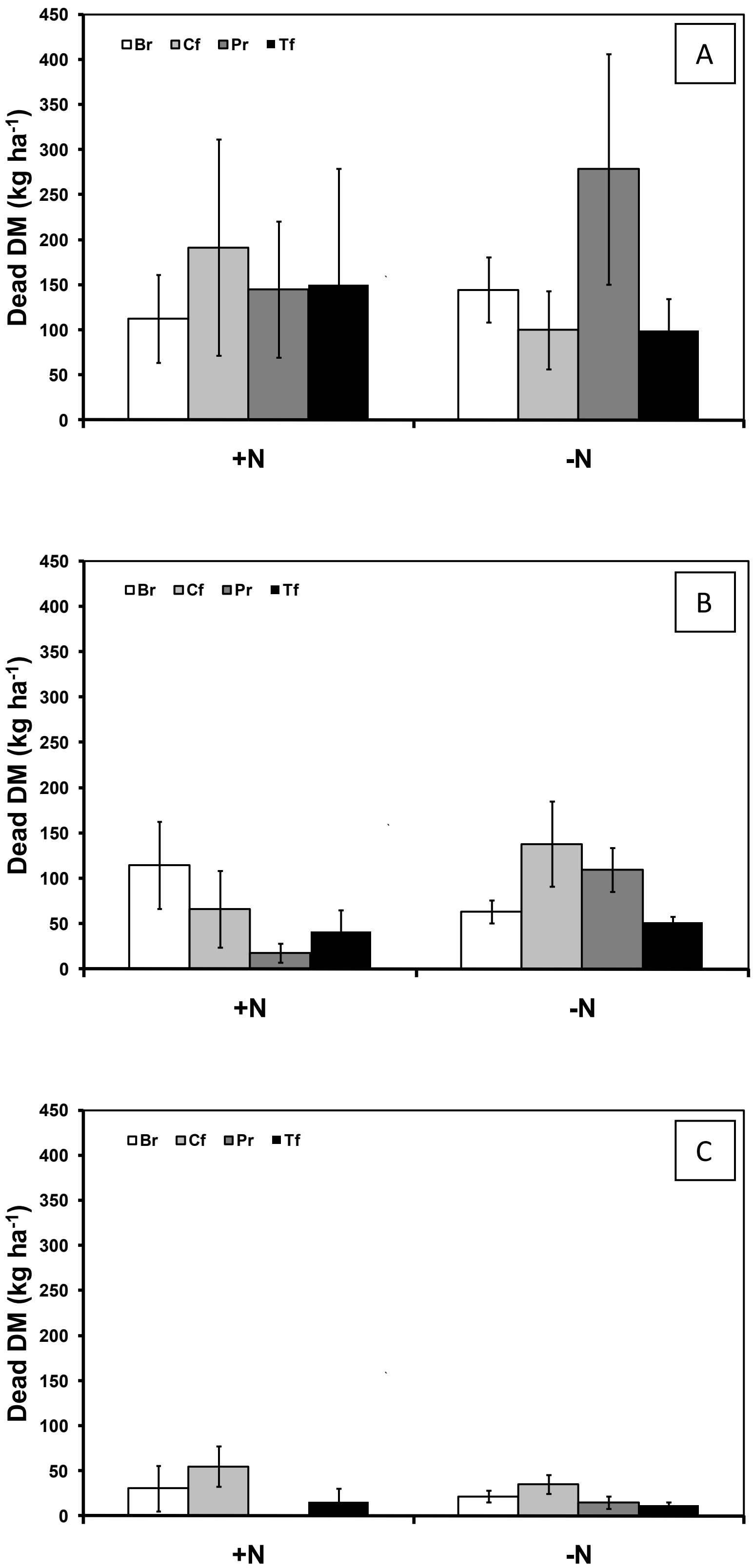

Figure 4-19: Total dead dry matter in response to nitrogen (N) treatments of four pasture grass species (brome, Br; cocksfoot, Cf; perennial ryegrass, Pr and tall fescue, Tf) in April (A), May (B) and June (C) 2017.

4.11 Relationships

Total DM yield (kg/ha) increased with increasing SPAD levels under N+ treatments. This treatment was removed under N- conditions (Fig. 4-21 A and B).

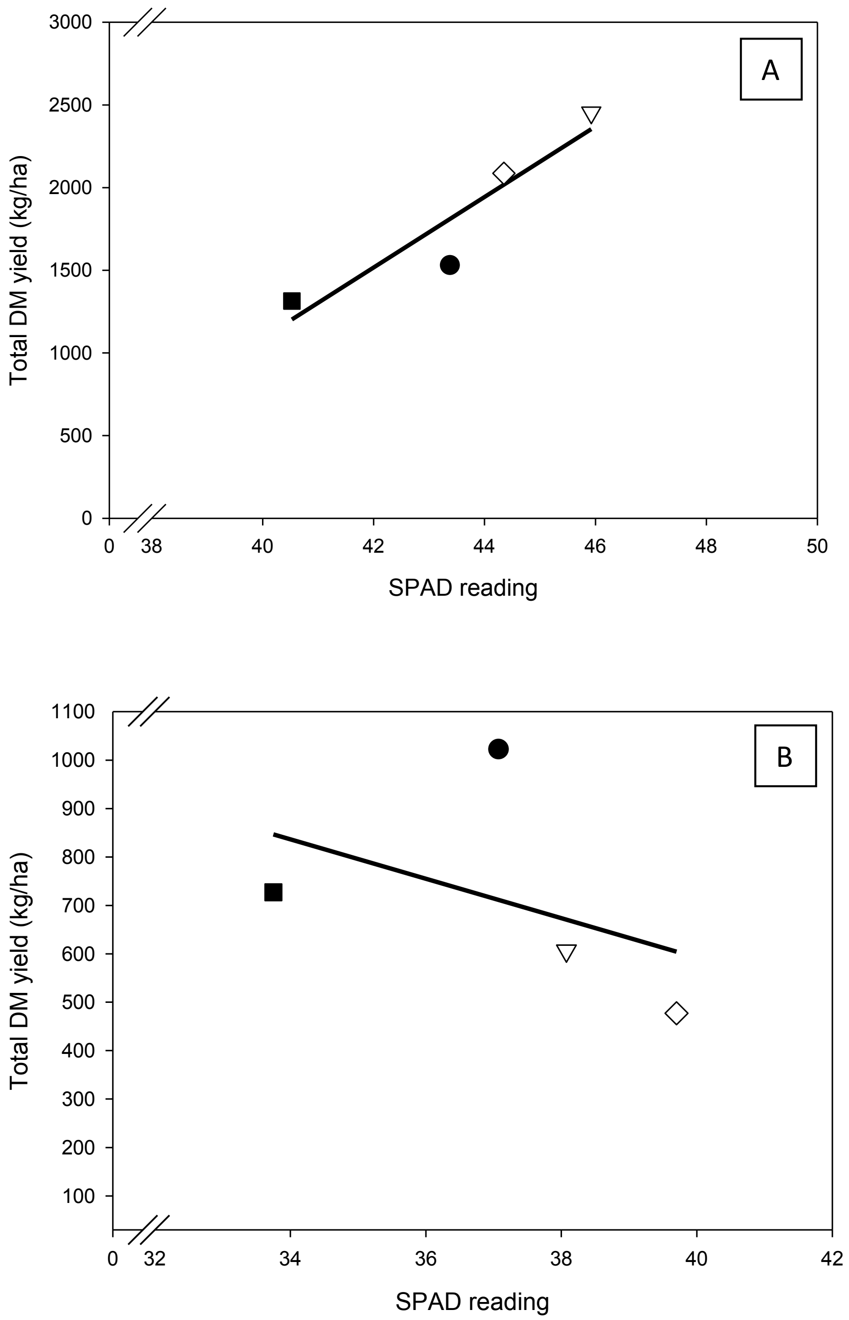

Figure 4-20: Relationship between total DM yield and leaf greenness (SPAD reading) forfour pasture grass species (■ = perennial ryegrass, ● = brome, ◊ = tall ▽ fescue, = cocksfoot) in N+ conditions (A) and N- conditions (B) in April 2017. Equation A: Total yield = 213.4 (±58.3)x - 7444 (±2548) (R^2 = 80.5), Equation B: Total yield = -40.7 (±58.9)x + 2221 (±2193.5) (R^2 = 0.19).

Yield reduction under N- was inversely related to the reduction in leaf elongation in response to the N- treatment (Fig. 4-22).

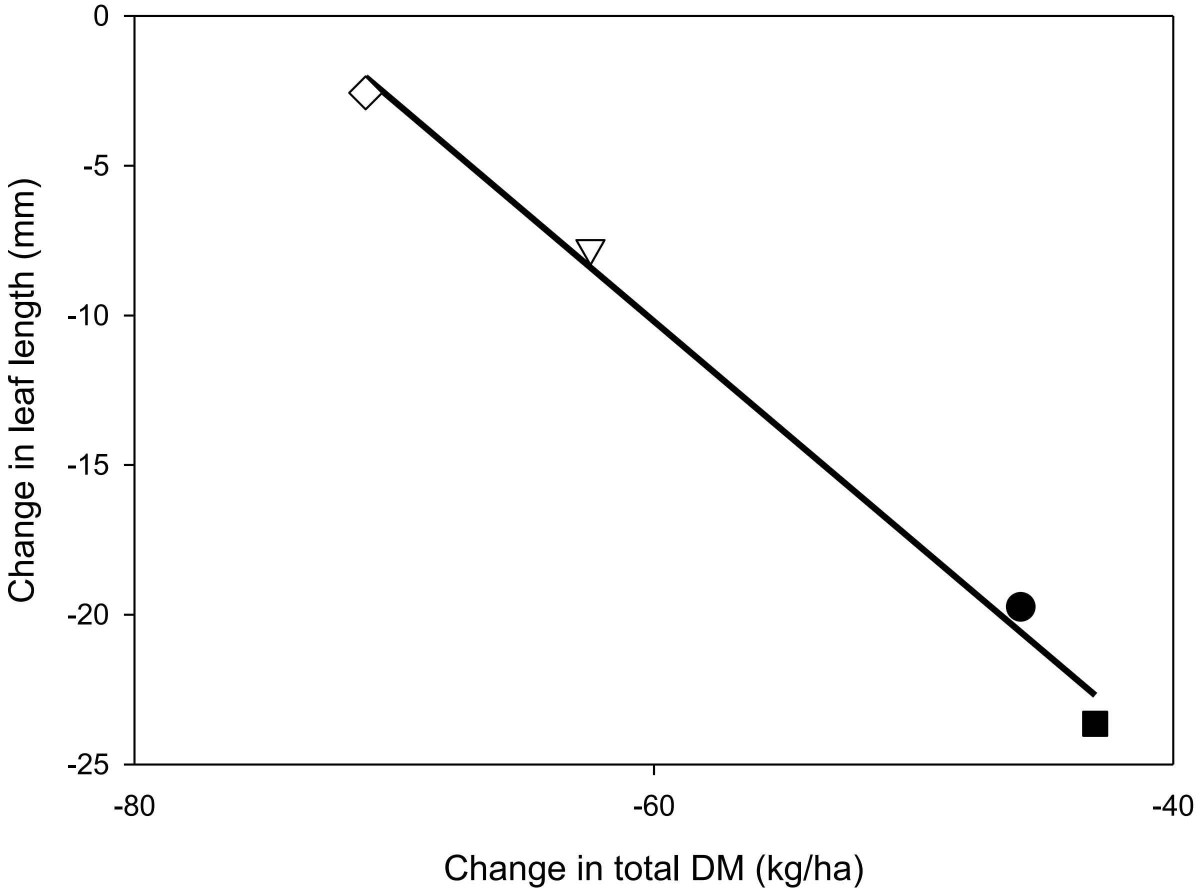

Figure 4-21: Relationship between change in leaf length and the change in total DM yield in four pasture grass species (■ = perennial ryegrass, ● = brome, ◊ = tall fescue, ▽ = cocksfoot) in N+ conditions and N- conditions. Total yield = -0.735 (±0.003)x - 54.30 (±3.36) (R^2 = 99.6).

Species with high green leaf DM showed the least reduction in leaf elongation in response to N- treatments. Cocksfoot and tall fescue had the highest green DM yields (1135 and 1242 kg/ha respectively) and the lowest change in leaf length at or lower than -5%, compared to brome and perennial ryegrass yields at ~1000 kg/ha and at a leaf reduction of -20-25% (Fig. 4-23).

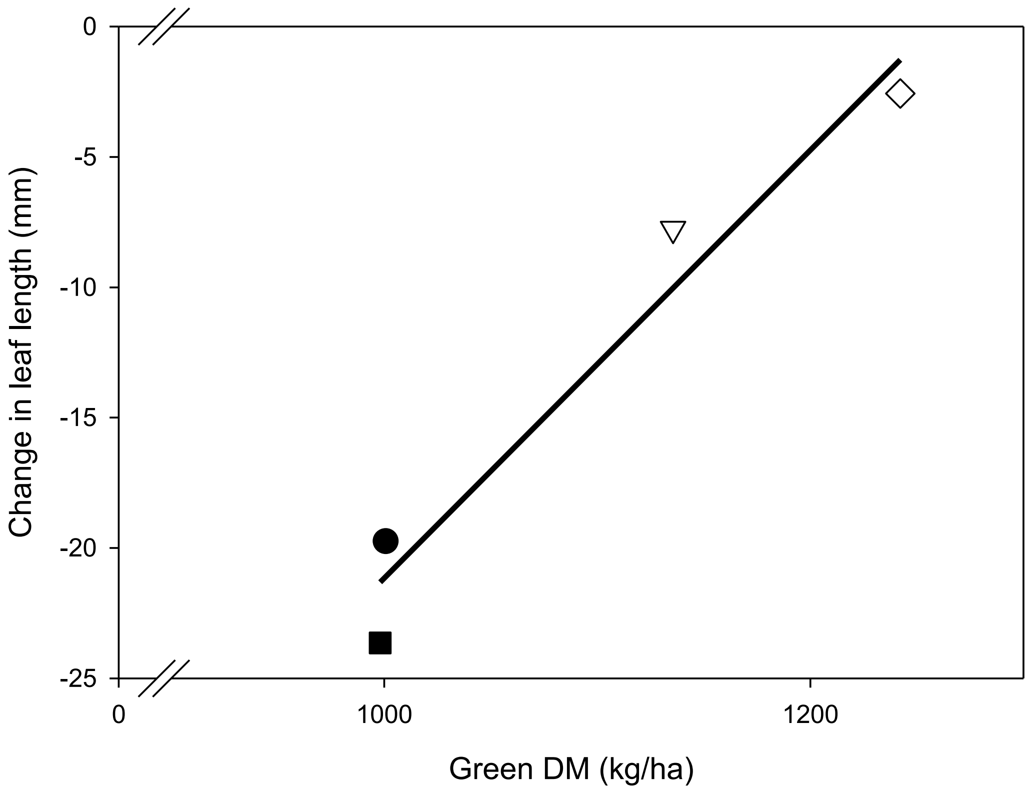

Figure 4-22: Relationship between change in leaf length and the green DM yield in four pasture grass species (■ = perennial ryegrass, ● = brome, ◊ = tall fescue, ▽= cocksfoot)in N+ conditions and N- conditions. Total yield = 0.082 (±0.01)x - 103.2 (±14.3) (R^2 = 92.8).

5 DISCUSSION

5.1 Leaf length

The observation of highest leaf length in brome and cocksfoot and lowest in perennial ryegrass (Fig. 4-2) reflects the conclusions by Berone*et al.* (2007) that *B. stamineus* has a greater capacity to elongate leaf tissue at moderately low temperatures than perennial ryegrass. Furthermore, differences in leaf length between these species are temperature-dependent and small below 5°C (Berone*et al.*, 2007), which is reflective of this current experiment where relatively small differences were seen in June between species and N treatments with temperatures decreasing into winter (Fig. 4-2).

Under non-limiting N conditions, brome had the highest leaf length,followed by cocksfoot, then tall fescue and finally perennial ryegrass (Fig. 4-2). Wilman and Mohamed (1980, 1981) similarly found in established swards, similar to this experiment, tall fescue to have larger leaf blades than perennial ryegrass. This may reflect the higher leaf appearance rate (Peacock, 1976; Wilman& Mohamed, 1980, 1981)in ryegrass which was higher than in tall fescue, reducing leaf expansion time in ryegrass and overall leaf length. Leaf appearance rate would have been another viable measurement to take during the duration of the experiment to provide an explanation as to the leaf length results relationships between grass species used in the experiment.

5.2 Leaf area

The observation of highest leaf area in cocksfoot, followed by brome and tall fescue (Fig. 4-3) reflects the findings of Gao and Wilman (1994) that leaf expansion was greater in tall fescue than perennial ryegrass. This is also in agreement with Ryle (1964) who found cocksfoot and tall fescue had a greater leaf area than perennial ryegrass. The observed increase in leaf area under the N+ treatment is in line with Ryle's (1964) observations that final leaf lamina size increased by 25% with increased supply of N. Leaf area and leaf length are closely related with increased leaf length in cocksfoot and brome,resulting in a corresponding increase in leaf area. An expanded leaf length and area gives a greater radiation interception potential, increasing growth and overall pasture grass sward yields.

The selection of a pasture grass that has a greater leaf area will provide greater DM yields in a dryland situation.

5.3 Leaf DW

The observation that cocksfoot and tall fescue had higher leaf dry weights than perennial ryegrass were similar to other studies where tall fescue was observed to have larger, heavier leaf blades than ryegrass (Wilman& Mohamed, 1980, 1981; Gao &Wilman, 1994). In contrast, Turner *et al.* (2012) found leaf DM was greater in tall fescue than perennial ryegrass, and that it was higher in perennial ryegrass than in cocksfoot. The Turner *et al.* (2012) experiment was carried out in glasshouses where in comparison to this field experiment. Stubble DM of cocksfoot was much greater than that of perennial ryegrass and tall fescue throughout in the Turner *et al.* (2012) study, perhaps eluding to the conversion of sugars into storage carbohydrates by cocksfoot when the plant was not under water stress.

5.4 Leaf percentage DM

Plants that maintain a low leaf percentage DM, or high water content, under water deficits are likely to have an improved performance under drought conditions (Pask*et al.,* 2011). Leaf PDM was lowest in brome compared to the other pasture grass species (Fig. 4-8). This higher water content in brome may be of advantage for leafelongation in this species. Perennial ryegrass has been reported have a decline in leaf expansion rate when relative water content is below 88% (Wilson, 1975), which would explain the lower leaf lengths observed in this species during this experiment. Further work needs to be conducted that links water content with leaf elongation in pasture grass species.

5.5 Specific leaf area (SLA)

Specific leaf area is a predictor of effective plant productivity: higher SLA means more leaf area for the capture of light for photosynthesis per leaf DM (i.e. carbon investment).Thus a higher SLA reduces the carbon cost of a plant and increases the relative potential yield of a pasture sward.SLA related directly to higher leaf area in the pasture grass species in this study and there was an increased SLA under N+ treatments. Cocksfoot and brome had the highest SLA,reflecting advantages for resource acquisition in these species under N+ as well as N- (Fig. 4-10).

5.6 Leaf thickness

Leaf thickness was similar across treatments,greatest in perennial ryegrass and lowest in brome and cocksfoot in both N treatments (Fig. 4-12). There is little informationin the literature on leaf thickness in pasture grass species. It can be suggested that thicker leaves are a sign that the leaves are less palatable.In essence this measurement is an inverse reflection of SLA: thicker leaves are less efficient in resource utilisation as there is more biomass invested per unit area. Future work should investigate the nature of differential leaf thickness in these pasture grass species: e.g. does a thicker leaf mean thicker cuticles/epidermal cells or more mesophyll cells in these leaves?

5.7 SPAD chlorophyll reading

A significant N effect across all cultivars was seen with cultivars differing in SPAD levels, again showing lowest values in perennial ryegrass (Fig. 4-14). As N content of grass pastures increased, yield increased, reflecting the findings of Rowarth and Archie, 1996, Griffiths and Thomson, 1996, and Kantety*et al*., 1996. This reflects an increased N supply which increases chlorophyll pigment density, which increases light interception resulting in more ATP for plant growth. The higher SPAD readings corresponded to higher leaf areas,further contributing to increased light capture. SPAD readings were taken once at the beginning of the experiment to confirm that the N treatment was effective. Future studies should measure SPAD throughout the duration of the trial.

5.8 Aboveground DM

Aboveground DM was decreasedby the N- treatment in all pasture grass species in this study (Fig. 4-15). This experimentcontradicts other studies where cocksfoot outperformed other pasture grass species (Mills *et al*., 2005). Cocksfoot is noted for being able to persist when other pasture species such as perennial ryegrass do not in dry conditions (Ridley & Simpson, 1994). However, the depletion of N may have reached a point where any advantage for cocksfoot may have been removed. This may reflect the long-term nature this experiment,with N levels under the N- treatments becoming more depleted over the years with no additional N fertiliser applied. This is evident from the low pasture cut yields over successive harvests,and the pasture species appearing stunted and N-deficient throughout the growth period, especially as temperatures cooled moving into winter. The higher yields of cocksfoot and tall fescue under N+ conditions give evidence that they will outperform in environments where soil N content is more prominent. Future soil tests on N levels in N- plots need to be carried out to determine N levels remaining in soil substrates and to observe if N levels are within a range that would be expected on a managed dryland farm.

Pasture grass species were not subjected to water stress over the time period of this experiment as frequent precipitation held soil water reserves. The Ladbrooks soil is an imperfectly drained Wakanui silt loam and poorly drained Temuka clay (Section 3.3), with a depth >1m, as well as being stone-less. This may reflect the lesser performance of cocksfoot as it is suited to more free-draining soils (Charlton and Stewart, 1999).

5.9 Aboveground green and senesced DM

Aboveground green DM was related to total aboveground DM, the total leaf and shoot mass of pasture grass swards whichis increased by greater leaf area. The more N supplied to a plant the greater the green leaf DM which is why the N+ treatments yielded higher. Aboveground senesced DM did not differ between N treatments and species, showing similar senescence rates of these species in the experiment.

5.10 Correlations

As expected, SPAD predicted yield under N+ conditions (Fig. 4-21A). The surprising result that the reduction of leaf elongation under N- was negatively related to the reduction of accumulated plant DM (Fig. 4-22) can be explained as follows: nitrogen is a mobile nutrient in plants and it is possible that species with higher green DM are able to compensate for external N reduction by utilising the higher amount of N available within the plant.This means that overall there are higher internal N levels available to the more productive species, even under low external N supply. Thus the more productive species reduce their leaf elongation less under the N- treatment. This is evidenced by: i) the greenest species showed the least N- generated DM reductions and leaf elongation reductions (Fig. 4-23), and ii) the original positive relationship between SPAD and DM was removed after N- application, where high and low productivity species had similar amounts of N, i.e. SPADlevels (Fig. 4-21B). Nitrogen levels should be measured in future work.

6 GENERAL DISCUSSION AND CONCLUSIONS

Averaged across treatments and time, brome and cocksfoot had highest levels in most leaf traits measured here, with leaves of these two species being longer, larger, heavier, more moist (lowest PDM), with higher SLA as well as being thinner.Averaged across treatments and time, there were no differences in the aboveground DM traits among the four species. This means they perform similarly well under the two nitrogen treatments. However, the superior leaf performance traits suggest potential advantages for brome and cocksfoot under long-term N- stress, with a higher potential for leaf growth, resource acquisition and palatability. The use of cocksfoot in dryland farming systems is plausible as it performs well in water deficit environments (Mills, 2007), as well as having itsmain growth period during summer (Charlton & Stewart, 2000). Cocksfoot has consistently been well documented to have an increased yield over summer compared to perennial ryegrass (Mills *et al.,* 2005; Stephens& Hickey, 2000; Stevens *et al*., 1992),and also to have reduced yields under dryland conditions and limited N (Charlton & Stewart, 1999) which this study eludes to.

The observed reduction in productivity under N- conditions needs to be further investigated. Future work with staggered levels of N treatments may provide an insight into differences between the two treatments. The N+ treatment used N application rates normally used on a dryland farm. The timing of the experiment was over autumn into early winter, meaning application to a study over summer would provide a greater understanding of the influence of water deficit and the persistence and recovery of pasture grass species used.

Floral initiation in cocksfoot occurs later in a growing season (Mills *et* al., 2005) which means it becomes a valuable pasture species for finishing stock as it maintains high quality pasture that grows over the summer. Cocksfoot forage quality is lower than that of perennial ryegrass, and particularly so when seedheads are present (Moloney, 1993). This means that grazing management on a dryland farm needs to prevent seedhead development to preserve pasture quality. Brome provides strong spring–summer growth with drought tolerance (Charlton & Stewart, 1999), and can have a higher N concentration similar to cocksfoot that is greater than perennial ryegrass (Harkess*et al*., 1990). This improves the quality of a pasture and makes cocksfoot and brome viable candidates for a dryland system.

Tall fescue is drought tolerant and performed better than perennial ryegrass in most leaf traits in this study. The difference between tall fescue to brome and cocksfoot may be due to the fact tall fescue is not well adapted to water logged soils (Easton *et al.*, 1994) resulting in reduced yield at Ladbrooks where the soils were fairly saturated. Future work could include relating pastures to differing water treatments as well to see the influence on pasture yields.

Overall, there are potential advantages for brome and cocksfoot, and to a lesser degree for tall fescue, under mixed N-supply due to superior morphological leaf attributes. The use of these species would fit in well in a dryland farming system as an alternative to the poorer performing perennial ryegrasses over the winter period. Continued study on these pasture species regarding their leaf morpho-physiology, quality attributes and performance over summer and under contrasting N availability would allow more concise conclusions.

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APPENDICES

Appendix 1: Application date and rate of nitrogen fertilizer (urea (46,0,0,0)) at Ladbrooks.

Date	Site	Fertilizer	Plots	Rate (kg N /ha)
18/9/2015	Ladbrooks	urea (46,0,0,0)	+N sub-plots except plots 22, 23, 24, 26, 27and 28	100
18/9/2015	Ladbrooks	urea (46,0,0,0)	Plots 22, 23, 24, 26, 27, 28	50
15/10/2015	Ladbrooks	urea (46,0,0,0)	+N sub-plots	200
16/12/2015	Ladbrooks	urea (46,0,0,0)	+N sub-plots	100
13/1/2016	Ladbrooks	urea (46,0,0,0)	+N sub-plots	100
11/2/2016	Ladbrooks	urea (46,0,0,0)	+N sub-plots	100
14/3/2016	Ladbrooks	urea (46,0,0,0)	+N sub-plots	100
20/4/2016	Ladbrooks	urea (46,0,0,0)	+N sub-plots	100
10/3/2017	Ladbrooks	urea (46,0,0,0)	+N sub-plots	50

Note: Based on soil test results, in the first application of nitrogen fertilizer, 50kg N/ha of nitrogen fertilizer was applied to the sub-plots with nitrogen background.

Appendix 2: Mowing time and date, growth/regrowth period (days) at Ladbrooks.

Site	Mowing order	Mowing date	Regrowth period (days)
Ladbrooks	1	9/4/2017	46
Ladbrooks	2	15/5/2017	36
Ladbrooks	3	20/6/2017	36