Nitrogen management strategies for mixed pastures grown under irrigation in the Winelands sub-region of the Western Cape

by

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Declaration

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Abstract

Three different pasture mixtures were established under irrigation at the Elsenburg research farm with the aim of devising nitrogen (N) management strategies for pastures in the Winelands sub-region of South Africa. The pasture mixtures were as follows: i) a mixed grass pasture consisting of perennial ryegrass *(Lolium perenne)*, tall fescue (*Festuca arundinaceae)* and cocksfoot (*Dactylis glomerata*); ii) a grass-clover pasture consisting of perennial ryegrass, tall fescue, cocksfoot and red and white clover (*Trifolium pratense* and *Trifolium repens*); and iii) a grass-lucerne pasture consisting of perennial ryegrass, tall fescue and lucerne (*Medicago sativa*). The effect of fertiliser N on selected nutritive characteristics was also evaluated. The grass-legume pastures were subjected to two management strategies: the once-off application of N and the consecutive application of N over the autumn-early spring period.

The reaction of the mixed grass pasture to applied N was mostly characterised by an interaction between the season of N application and N application rate. The productivity of the pasture in terms of the primary dry matter production (PDMP) and the total dry matter production (TDMP) was highest in spring and summer with the application of 60 – 80 kg N ha^{-1}, and decreased in autumn and winter. There was a strong response of the winter residual dry matter production (RDMP) to N, which indicated that not all applied N was utilised during the first regrowth cycle, which might present a risk of nitrate being leached below the root zone.

The botanical composition of the mixed grass pasture was determined by season of N application, and not N application rate. The tall fescue content was low over all seasons, presumably due to poor establishment and strong competition from accompanying species. In the cooler months perennial ryegrass and tall fescue was the dominant species, while in the warmer months cocksfoot was the main species. Nitrogen application also had a significant effect on the quality of the pasture, most notably the crude protein (CP) content. The response of the CP content was characterised by a strong interaction between season of N application and N application rate. Crude protein levels in excess of 22 % were recorded in autumn

and winter with the application of 40 – 80 kg N ha^{-1}. Other characteristics remained within the expected range.

The response of the grass-clover and grass-lucerne pastures in terms of productivity and nutritive characteristics were mainly determined by the season of N application, and not N application rate. Productivity tended to be highest in autumn and early spring for both the once-off and the consecutive N application strategies, emphasizing the effect of temperature on pasture growth.

The effect of season of N application and the N application rate on the botanical composition of the respective pastures were inconsistent over the two years of the study. The clover content tended to decrease in response to increasing rates of N, while the grass fraction was stimulated. Lucerne productivity decreased from autumn through winter and reached minimum levels in early spring, and was unaffected by fertiliser N rate. The legume component in both the grass-clover and grass-lucerne pastures remained above recommended levels of 20 – 40 % for optimum animal production, even when N was applied consecutively.

The nutritive characteristics measured (dry matter (DM) content, CP, *in vitro* organic matter digestibility (IVOMD)) remained within the expected range, except the total CP content which was very high in the first year (> 30 %), although N application rate did not have a significant effect.

Based on these findings, preliminary recommendations for N fertilisation (on low carbon soils) for a mixed grass pasture is 40 kg N ha^{-1} during autumn and winter and 60 kg N ha^{-1} in spring and summer. Based on the poor response of the grass-legume pastures to applied N it is doubtful whether fertilisation will lead to an economical advantage, but low rates of approximately 40 kg N ha^{-1} could be beneficial in a grass-clover pasture during autumn and late winter/early spring based on the relatively strong response of PDMP to N during this period.

Uittreksel

Drie verskillende weidingsmengsels is onder besproeiing te Elsenburg proefplaas gevestig met die doel om stikstof (N) bestuurstrategieë te ontwikkel vir aangeplante weidings in die Wynland distrik van die Wes-Kaap van Suid Afrika. Die weidingsmengsels was as volg: i) 'n gemengde gras weiding bestaande uit meerjarige raaigras *(Lolium perenne)*, langswenkgras (*Festuca arundinaceae)* en kropaargras (*Dactylis glomerata*), ii) 'n gras-klawer weiding bestaande uit meerjarige raaigras, kropaargras, langswenkgras, wit - en rooi klawer (*Trifolium pratense* en *Trifolium repens*), en iii) 'n gras-lusern weiding bestaande uit meerjarige raaigras, langswenkgras en lusern (*Medicago sativa*). Die effek van stikstof bemesting op sekere kwaliteitsaspekte van die onderskeie weidings was ook geëvalueer. Die gras-peulplant weidings was onderworpe aan twee bestuurstrategieë, naamlik die eenmalige toediening van N en die agtereenvolgende toediening van N bemesting tydens die herfs – lente periode.

Die reaksie van die gemengde gras weiding op N bemesting was hoofsaaklik gekenmerk deur 'n interaksie tussen die N bemestingspeil en die seisoen van N toediening. Die produktiwiteit van die weidings i.t.v. die primêre droeëmateriaal produksie (PDMP) en die totale droeëmateriaal produksie (TDMP) was die hoogste in die lente en somer met die toediening van 60 – 80 kg N ha^{-1} en het in herfs en winter afgeneem. Daar was n sterk respons van die winter residuele droeëmateriaal produksie (RDMP) teenoor N, wat aandui dat nie alle toegediende N tydens die eerste hergroei periode benut was nie en dus 'n moontlike risiko van loging inhou.

Die botaniese samestelling van die gemengde gras weiding was deur die seisoen van N toediening bepaal, en nie die N bemestingspeil nie. Die langswenkgras inhoud was baie laag in alle seisoene, vermoedelik a.g.v. swak vestiging en sterk kompetisie van gepaardgaande spesies in die mengsel. Tydens die koeler seisoene van die jaar was meerjarige raaigras en langswenkgras die dominerende spesies, terwyl kropaargras tydens die warmer maande gedomineer het.

Stikstof toediening het ook 'n betekenisvolle effek op die kwaliteit van die weiding gehad, veral die ru-proteien (RP) inhoud. Die respons van RP was weereens

gekenmerk deur 'n betekenisvolle interaksie tussen die seisoen van N toediening en die N peil. Ru- proteien vlakke hoër as 22% was tydens herfs en winter waargeneem met die toedieningspyle van 40 – 80 kg N ha^{-1}. Ander kwaliteitseienskappe het binne normale perke gebly.

Die respons van die gras-klawer en gras-lusern weidings in terme van produktiwiteit en kwaliteitseienskappe was hoofsaaklik deur die seisoen van N toediening bepaal, en nie deur die N bemestingspeil nie. Die produktiwiteit was die hoogste tydens herfs en vroeë lente vir beide die eenmalige en die herhaalde N toedieningsstrategieë. Hierdie bevindinge beklemtoon die belangrike effek van temperatuur op die groei en produksie van weidingsgewasse.

Die effek van seisoen van N toediening en N peil op die botaniese samestelling van die gras-peulgewas weidings was inkonsekwent oor die twee jare van die studie. Die klawer-fraksie was geneig om af te neem soos wat die N peil toegeneem het, terwyl die gras-fraksie toegeneem het. Die lusern-inhoud het van herfs tot lente afgeneem en was ongeaffekteer deur die N peil. Die peulgewas-inhoud van beide weidingsmengsels was deurentyd hoër as die voorgeskrewe minimum vlak van 20 – 40%, selfs met opeenvolgende N-toediening.

Die kwaliteitseienskappe gemeet in die studie (droeëmateriaal (DM) inhoud, RP en *in vitro* organiese materiaal verteerbaarheid (IVOMV)) het binne normale perke gebly, behalwe die totale ru-proteien (TRP) inhoud wat baie hoog was tydens die eerste jaar (>30%), alhoewel dit nie deur die N peil beinvloed was nie.

Aan die lig van bogenoemde bevindinge is die voorlopige aanbeveling vir N-bemesting (op lae koolstof gronde) van 'n gemengde grasweiding 40 kg N ha^{-1} tydens die herfs en winter en 60 kg N ha^{-1} tydens lente en somer. Gebasseer op die swak respons van die gras-peulgewas weidings op toegediende N, is dit twyfelagtig of N toediening enige ekonomiese voordeel vir die boer sal inhou. Gebaseer op die relatiewe sterk respons van die gras-klawer PDMP op toegediende N tydens herfs en laat winter/vroeë lente kan dit moontlik voordelig wees om lae N-vlakke van ongeveer 40 kg ha^{-1} tydens hierdie seisoene toe te dien.

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Abbreviations

ADMP	Annual dry matter production
Ca	Calcium
CF	Cocksfoot
CP	Crude protein
DM	Dry matter
g	gram
ha	hectare
IVOMD	*In vitro* organic matter digestibility
kg	kilogram
LAN	Limestone ammonium nitrate
mm	millimetre
N	Nitrogen
NPN	Non protein nitrogen
NUE	Nitrogen Use Efficiency
P	Phosphorous
PDMP	Primary dry matter production
PRTF	Perennial ryegrass + tall fescue
RDMP	Residual dry matter production
t	ton
TCP	Total crude protein

TDMP	Total dry matter production
˚C	Degrees Celsius

Table of Contents

CHAPTER 1

Introduction

Nitrogen (N) is one of the most important elements for plant growth and even though it is one of the most abundant elements, comprising approximately 78% of the earth's atmosphere, it is most often the first limiting nutrient for crop production (Hardarson 1993). The development of N fertilisers was a major feat for increasing crop production per hectare as it led to the doubling and even trebling of yields in the 1960's, but currently it represents one of the major costs in crop production as well as being a serious source of environmental pollution (Olson 1977).

In the past little attention was given to the efficiency of fertiliser use: Nitrogen fertiliser was cheap enough for farmers to fertilise liberally, at rates calculated for maximum productivity, plus some extra as 'insurance' to ensure no loss in yield and maximum economic return. However, agricultural policies around the world are changing with regards to the amount of fertilisers that may be applied to agricultural fields and there is a tendency towards more environmentally friendly farming practices (Crews and Peoples 2005).

Nitrogen can also be supplied by legume crops through the process of biological nitrogen fixation (BNF) (Hardarson 1993). The use of legumes to supply a proportion of a crops' N requirement through BNF is in fact the original way that farmers were able to improve soil fertility (Jarvis et al. 1995). Prior to the commercialisation of inorganic fertiliser N, a portion of farmland was always kept under legume rich pastures or cover crops to increase the soil's 'fertility' (Crews and Peoples 2004) and in New Zealand and Australia white clover-based pastures are still used to supply the majority of N for dairy operations (Ledgard and Steele 1992, Ledgard and Giller 1995). Legumes can thus fulfill an important role in the development of sustainable pastoral agricultural systems as they can potentially reduce N fertiliser use and thereby improve the profitability of a farming enterprise.

Simultaneously legumes will reduce the potential for groundwater pollution and decrease the reliance on non-renewable fossil fuels (Hardarson 1993).

1.1 Strategies to maximise pasture productivity

The aim of any pasture-based production system is to maximise animal performance throughout the year, but both the availability of forage and the quality thereof can be a limiting factor for livestock production. These constraints can potentially be mitigated by two strategies, firstly, by the establishment of diverse pasture mixtures where species with complementary growth curves are combined to produce a more consistent amount of dry matter throughout the year (Haynes 1980, Sleugh et al. 2000) and secondly, by the strategic application of fertiliser N which aims to boost pasture production during the cooler months of the year normally characterised by low pasture productivity (Labuschagne and Agenbag 2006). This strategy is specifically suited for use on grass-legume pastures where the majority of N for the summer months is supplied as biologically fixed N by the legume component and winter production stimulated by fertiliser N when the legume component is essentially dormant (Simpson 1987, Eckard 1994).

1.1.1 Advantages and disadvantages of mixed pastures

Temperate grasses typically produce 45 to 50% of their annual yield during spring, whereafter production decreases during summer when pastures become more mature (Figure 1.1). In autumn there is often a flush of new growth, but yields reach their lowest levels during winter (Christian 1987, Santini et al. 1975 as cited by Rearte and Pieroni 2001).

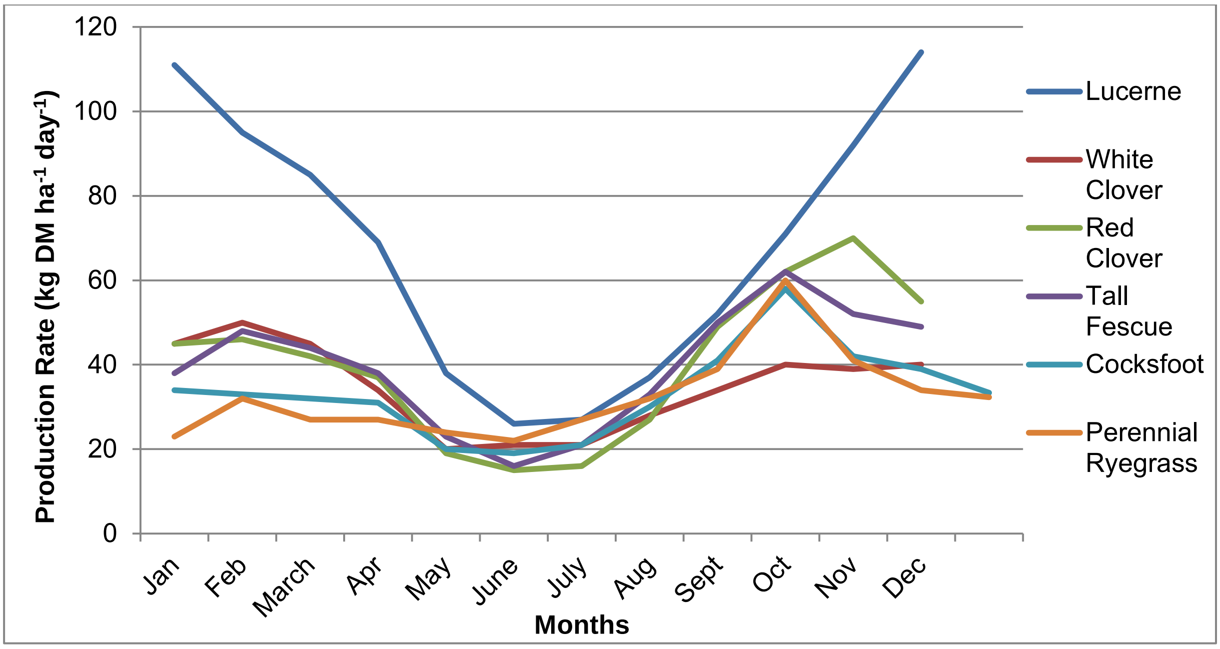

Figure 1.1 Dry matter production rate (kg ha^{-1} day^{-1}) of various pasture species under irrigation in the Winelands sub-region of the Western Cape. Adapted from van Heerden (1986)

The inclusion of legumes in a pasture mixture (in the Western Cape Province of South Africa) may improve the distribution of fodder throughout the season (Sleugh et al. 2000) as they typically produce most of their biomass during the summer (Ledgard et al. 1996), with grasses producing at higher levels during winter.

Other advantages of legume mixtures are:

a. **A reduction in the requirement for inorganic fertiliser N due to their ability to fix atmospheric N (Wu and McGechan 1999).** Frame (1992) reported that unfertilised grass-clover pastures were able to produce 6-9 t DM. ha^{-1} y^{-1}, while grass monoculture swards could only produce 2-5 t DM ha^{-1} y^{-1} under the same conditions.

b. **An improvement in pasture quality which leads to increased animal intake and performance (Peoples and Baldock 2001)**. Legumes are nutritionally superior to grasses - specifically with regards to protein, calcium, phosphorous, magnesium, copper and cobalt content (McDonald et al. 2002). Legumes also have a lower cell wall concentration (Rearte and Pieroni 2001) and there is a slower decline in nutritive value with age (McDonald et al. 2002) which means higher fodder digestibility. The consequence of the high nutritional value and digestibility of legumes is that livestock generally prefer legumes and consume it in greater quantities than grasses (McDonald et al, 2002).

c. **Legumes typically produce less dry matter than highly fertilised grass monocultures (Botha 2002) and therefore mixed grass-legume pastures often produce higher yields, are more persistent and reduce the occurrence of bloat in ruminants (Baylor 1974)**.

d. **Nitrate poisoning in livestock is less likely to occur in legume-grass mixtures**. A legume such as white clover contains much less nitrate than grass at the same level of N fertilisation and thus grass-clover pastures may contain less nitrate than grass only pastures of similar yields (Shiel et al. 1997).

e. **Reproductive disturbances associated with phyto-estrogens occurring in certain clovers are also reduced when these clovers are planted with grasses (Rochon et al. 2004)**.

Important disadvantages include:

a. **The often poor predictability of legume growth and the maintenance of a sufficient legume fraction to achieve high levels of nitrogen fixation (Miles and Manson 2000).**

1.2 Strategic nitrogen fertilisation of grass-legume pastures

During the cooler months of the year (late autumn – early spring) pasture growth is often limited by the amount of N made available through mineralisation and the amount of N contributed by fixation due to low soil temperatures (McKenzie et al. 1999). It is during this period that the addition of small amounts of fertiliser N is capable of boosting pasture growth without negatively affecting the legume component (Eckard and Franks 1998, McKenzie et al. 1999). White clover growth and N fixation requires temperatures > 9˚C (Frame and Newbould 1986), while perennial ryegrass is still able to grow and respond to N fertiliser at soil temperatures of 5ºC (Frame 1992). It could therefore be anticipated that application of N fertiliser can potentially increase fodder production during seasons of decreased fodder availability as a result of low temperatures (McKenzie et al. 1999, Miles and Manson 2000). However, the addition of fertiliser N will only be beneficial if it doesn't compromise the yield of the pasture or the ability of the legume to fix N later in the season (Stout and Weaver 2001).

Results of Labuschagne et al. (2006) indicate that N fertiliser application to a white clover-perennial ryegrass pasture increased total pasture dry matter production, but decreased the total clover content. Total clover content was especially sensitive to increasing levels of N fertiliser during early and late spring, and N levels of 150 kg N ha^{-1} resulted in a decrease in clover percentage below 30% (which is considered the minimum threshold for maintenance of the benefits associated with the legume) even when applied in winter.

1.3 Problem statement and aim

Agricultural production in the Winelands sub-region of the Western Cape, South Africa is dominated by horticultural crops such as vines and stone fruit, but there is an increasing area of land where patches of pastures under irrigation are being established to diversify farming operations (van Heerden and Tainton 1988). Mixed pastures, commonly consisting of species such as perennial and annual ryegrass (*Lolium* spp.), tall fescue (*Festuca arundinaceae*), cocksfoot (*Dactylis glomerata*) and legumes such as red (*Trifolium pratense*) and white (*Trifolium repens*) clover or lucerne (*Medicago sativa*) have traditionally been the backbone of pasture production in the Winelands region of the Western Cape (van Heerden and Tainton 1989).

Research regarding fertilisation norms for pastures under irrigation in the Winelands region of the Western Cape is very limited as pasture research has invariably been focused on the dairying regions in the southern Cape of South Africa. Due to economic pressure it is becoming increasingly important to improve the efficiency and precision, (which includes both the timing and the amount) of N fertiliser use. This will not only maximise pasture productivity, but also yield and minimise the shortage of available fodder during the cooler seasons, commonly known as the winter gap.

The aim of this research was to develop seasonal guidelines for the application of N fertiliser to mixed grass and grass-legume pastures grown under irrigation in the Winelands sub-region of the Western Cape Province. Specific objectives include i) the evaluation of production potential and response to N fertiliser of a grass-clover, grass-lucerne-clover and a grass-only pasture during different seasons, ii) the effect of N on the botanical composition of these pastures and iii) the effect of N on selected quality (nutritive) parameters.

1.4 Lay-out of the thesis

The layout of the thesis is as follows:

Chapter 1: Introduction and aim

Chapter 2: Literature review

Chapter 3: Materials and methods

Chapter 4: The effect of nitrogen fertiliser application on the yield, botanical composition and selected nutritive characteristics of a mixed grass pasture in the Winelands sub-region of the Western Cape

Chapter 5: The effect of nitrogen fertiliser application on the yield, botanical composition and selected nutritive characteristics of a mixed grass-clover pasture in the Winelands sub-region of the Western Cape

Chapter 6: The effect of nitrogen fertiliser application on the yield, botanical composition and selected nutritive characteristics of a mixed grass-lucerne pasture in the Winelands sub-region of the Western Cape

Chapter 7: Summary

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CHAPTER 2

Literature Review

2.1 Nitrogen uptake and metabolism

Nitrogen (N) is an essential element for all organisms as it is the foundation block of proteins, amino acids and nucleic acids (Ohyama 2010). Protein is a vital component of the chlorophyll molecule and thus N influences both photosynthesis and growth (Wedin, 1974). The first sign of an N deficiency is the gradual chlorosis of the older leaves, stunted growth and eventually abscission (Sueyoshi et al. 2010).

Fertiliser N, manure, urine and soil organic matter (SOM) are the main sources of nitrogen in soil (Vance 2001). The latter constitutes the largest pool of nitrogen but it must first be released through the process of mineralisation, mediated by soil micro-organisms, (Figure. 2.1) before it can be utilised by plants (Ohyama 2010).

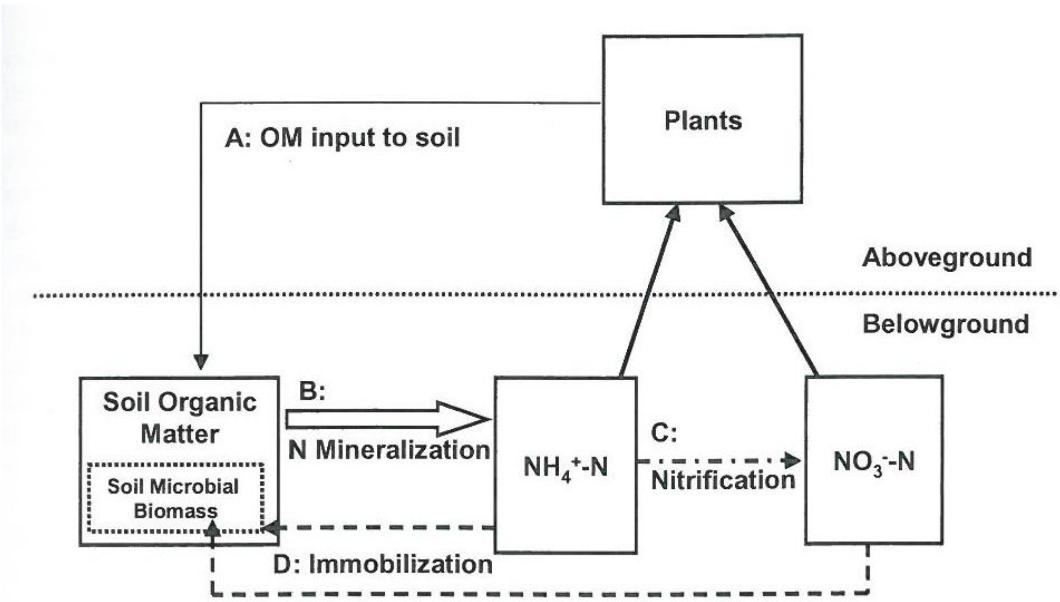

Figure 2.1: Soil N cycling as driven by the input of soil organic matter (SOM). Taken from Matsushima et al. (2010)

Nitrogen can be taken up by plants in different forms: Nitrate (NO_3^-), ammonium (NH_4^+), urea and organic compounds such as amino acids. The latter is normally not a major source of N for plants (Bidwell 1979). Very few plants are able to grow under high NH_4^+ conditions and will quickly develop toxicity symptoms which will consequently lead to low productivity. Therefore nitrate is the predominant inorganic form in which N is taken up in most grassland ecosystems, although uptake can also be affected by other factors such as soil pH and plant species (Ohyama 2010).

After uptake, NO_3^- is reduced to NH_3 and incorporated into amino acids which are then used for protein synthesis (Buxton and Fales 1994). This reduction of nitrate is a two-step process involving the enzymes nitrate reductase, which catalyzes the conversion of nitrate to nitrite (NO_2^-), and nitrite reductase which reduces nitrite to ammonia (NH_3) (Bidwell 1979). The reduction of nitrate can occur in the roots after uptake, or it can be transported to other organs for later reduction, such as occurs in most grasses where the nitrate is accumulated in the leaves and then reduced as required (Bidwell 1979).

2.2 Nitrogen fertiliser and yield

The majority of N fertilisers are ammonia (NH_3) -based (Figure 2.1). Ammonia is produced by the Haber-Bosch process, which is illustrated by the following equation:

$$N_2 + 3H_2 \leftrightarrow 2NH_3 + \text{heat energy}$$

Fertiliser N dramatically increases pasture productivity and thus stocking rate, which in turn improves overall farm productivity (Buxton and Fales 1994). Nitrogen input increases pasture growth rate, which means that the desired level of production can be reached in a much faster time frame, or conversely, the pasture yield will be much higher for a given growth period (Peyraud and Astigarraga 1998).

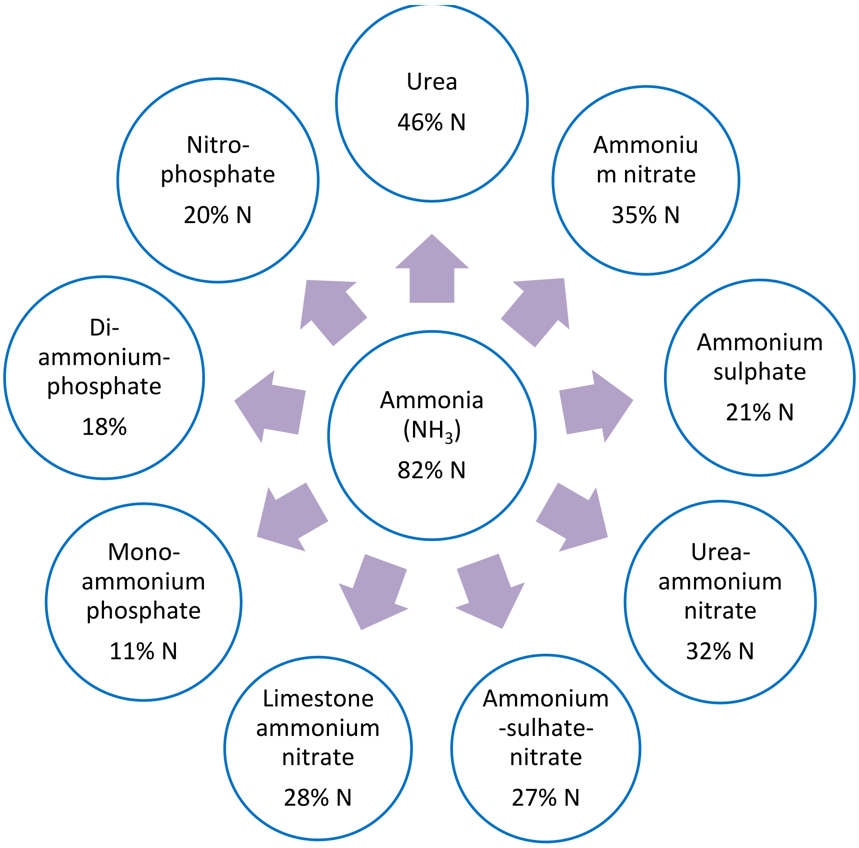

Figure 2.2: Ammonium based nitrogen fertiliser (Venter 2002)

Grasses are more responsive to high levels of fertiliser N than most other agronomic crops (Sparrow 1979). According to Frame (1992), the application of N fertiliser at a rate between 250 and 350 kg N ha^{-1} y^{-1} to grass-only swards leads to a linear increase in dry matter production of 15-25 kg per kg N applied. Further application of N at rates between 350 and 450 kg N ha^{-1} y^{-1} produced only 5 to 15 kg additional dry matter per kg N applied and at rates between 450 and 600 kg N ha^{-1} y^{-1} a turning point was reached where no further increase in DM was achieved. The economic optimum rate varies with the farming enterprise, but it will normally be below the amount of N required for maximum yield (Frame 1992).

In grass-clover pastures, the application of fertiliser N between 250 and 300 kg N ha^{-1} y^{-1} leads to a linear increase in DM production, where after fodder production increases curvilinearly as the N rate increases beyond this range. The increase in DM produced per kg N applied will eventually be lower for a grass-clover pasture (compared to a pure grass pasture) because of the eventual decline in clover percentage (Frame 1992).

Livestock performance on a 'per-head' basis is normally increased on grass-legume pastures compared to pure legume or fertilised grass pastures, but production per hectare tends to be lower (Rochon et al. 2004). Per hectare performance is normally highest on fertilised grass pasture (Rochon et al. 2004). The average dry matter production of a perennial ryegrass-white clover pasture compares favourably with a perennial ryegrass pasture receiving 200 kg N ha^{-1} y^{-1}, or conversely, a clover-ryegrass pasture produces 70 % of the dry matter production of a grass only pasture receiving 400 kg N ha^{-1} y^{-1} (Andrews et al. 2007).

2.3 Nitrogen fertiliser and quality

Pasture quality is determined by the digestibility, chemical composition and voluntary intake of a given species or species mixture (Rearte and Pieroni 2001, Rochon et al. 2004). Optimum forage quality also differs for various classes of livestock (Buxton 1996). It is believed that intake, rather than chemical composition and digestibility ultimately determine animal performance, but in reality all three these characteristics are interrelated (Meissner et al. 2000) and ultimately modified by plant maturity. These aspects are explained below.

2.3.1. Digestibility

Digestibility is primarily a function of plant anatomy and tends to decrease as a plant reaches maturity (McDonald et al. 2002). All plant tissue consist of various types of modified cells that can be classified into two groups: the cell contents, which includes most of the organic acids, crude protein, fat, soluble carbohydrates and soluble ash which is nearly 100% digestible; and the cell-wall constituents, which comprise of cellulose, hemicellulose, lignin, cutin and silica (Figure 2.3) (Minson 1990). Cell walls and the degree of lignification is the main factor determining forage digestibility (Poppi et al. 1999, McDonald et al. 2002). Forage intake and forage digestibility are closely related, because digestibility relates to the rate of particle break down and passage through the rumen. The greater the cell wall fraction, the slower the process of fermentation and disintegration in the rumen, and the lower the intake (Meissner et al. 2000).

However, where the digestibility is more than 70%, the rate of cell wall fermentation is rarely a limiting factor for intake. Factors that are more likely to play a role are pasture availability, palatability, moisture content and factors like fecal contamination (Meissner et al. 2000).

Legumes have a lower cell wall concentration (Rearte and Pieroni 2001) and their nutritive value decreases less with age (McDonald et al. 2002). Their fiber retention time is also much shorter compared to grasses and therefore they are more digestible and consumed in greater quantities (Meissner et al. 2000).

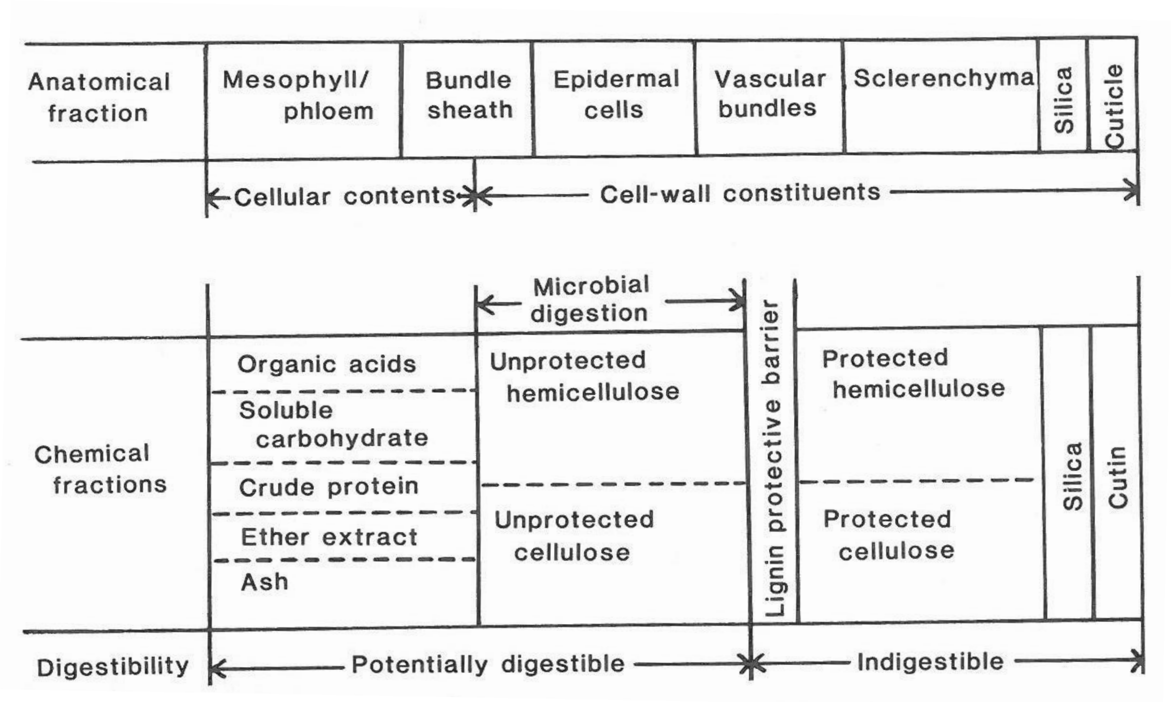

Figure 2.3: Conceptual model of the relation between plant anatomy and chemical fractions indicating areas of potential digestibility (Taken from Minson 1990)

According to Wilman (1975) and Miles and Manson (2000) nitrogen fertiliser has no effect on pasture digestibility *per se*, except in the case where forage N concentrations are too low for the growth of rumen micro-organisms, (Raymond 1969) in which case the addition of fertiliser N will improve the digestibility. Indirectly N fertilisation can also reduce pasture digestibility if it leads to a reduction in the legume fraction of a mixed sward (Raymond 1969).

2.3.2 Chemical composition

2.3.2.1 Crude Protein

Livestock protein requirements are expressed as crude protein (CP), which is defined as N content x 6.25 (NRC 2001). This is based on the assumption that all dietary protein contains 160 g N kg^{-1} DM and that all the N present is in the form of true protein (McDonald et al. 2002). This assumption is not entirely accurate as true protein in herbage accounts for approximately 80 % of the total protein and the other 20 % comprises of non-protein nitrogen (NPN) of which most is present as nitrate and amino acids (Buxton and Fales 1994, McDonald et al. 2002). Both the true protein and NPN content is linked to the physiology of the plant. Conditions that are conducive to rapid growth results in an increase in NPN and the overall N content, but levels decrease as the plant reaches maturity (McDonald et al. 2002). This is mainly due to an increase in the buildup of structural components and a change in leaf stem ratio (Blaser 1964).

Average CP content of warm and cool season grasses are 100 g kg^{-1} DM and 130 g kg^{-1} DM respectively, while legumes such as lucerne have a CP content of about 170 g kg^{-1} DM (Minson 1990, Buxton 1996). With increased application rates of N fertiliser to grasses an increase in CP can be expected. Nitrogen levels reach maximum levels 2 to 3 weeks after application, whereafter it gradually decreases as growth progresses (Jacobs et al. 1998, Peyraud and Astigarraga 1998). This initial increase in CP is characterised by the accumulation of NPN mainly in the form of NO_3^-, especially where high N rates are applied (Miles and Manson 2000). The effect of fertiliser N on forage CP content largely depends on the growth rate after application. In young, immature pasture, N stimulates rapid growth but if the interval between harvests is long enough, there will be little effect of N fertiliser on CP content. However, if the fertiliser N is applied to mature forage, the CP will be higher than when the same amount of fertiliser were applied to young, fast growing pastures (Minson 1990).

Legume CP content is generally unaffected by N fertilisation (Buxton and Fales 1994), although Labuschagne (2005) found that the leaf N content of white clover increased in response to 100 and 150 kg N ha^{-1} after a 30 day growth period. The application of N to grass-legume pastures normally have no net effect on CP content over the long term as N fertilisation leads to a reduction of the legume component (Minson 1990).

Livestock CP requirements vary from 70 to 80 g kg^{-1} for maintenance, up to 130 to 140 g kg^{-1} for high producing animals (Meissner et al. 2000), and potentially even higher for high producing dairy cows (NRC 2001). Whether the increase in CP associated with N fertilisation actually improves pasture quality depends on the requirements of the specific type of animal, and the requirement of the rumen microorganisms (van Soest 1982). The rate of microbial protein (MP) synthesis is energy dependent and therefore the amount of MP produced depends on the rate of energy release relative to amino acids and NH_3 production during protein degradation in the rumen (Minson 1990). Forages with a high CP content can often result in a protein-energy imbalance, with consequent poor animal performance (Buxton and Fales, 1994). Nitrogen is thus often wasted when applied to obtain maximum pasture yield because it is in excess of microbial requirements (van Soest 1982). In pastures containing in excess of 25 to 30 g kg^{-1} N (160 to 190 g kg^{-1} CP) there is a strong likelihood that N will be lost as NH_3 over the rumen wall (Meissner et al. 1993).

2.3.2.2 Energy

Animal performance from pastures is generally limited by an energy deficiency, rather than by limited digestible protein (Blaser 1964). The energy content of forage is correlated with the proportion of organic matter digested by the ruminant (Poppi et al. 1999). Soluble carbohydrates are a readily available source of energy for the grazing ruminant and also provide energy for the rumen microorganisms which form microbial protein from dietary NH_3 (Lambert and Litherand 2000). The fibre present in forage cell walls is an essential element for rumen health, but these cell walls also limit the feed intake due to their low digestibility. Ruminants are normally able to extract only 30 % of the energy contained in these cell walls (Buxton 1996). Typical energy values of pastures range from 8-12 MJ kg^{-1} DM and are used to varying levels of efficiency, depending on whether the energy is used for growth, maintenance or reproduction (Lambert and Litherland 2000).

Nitrogen fertilisation increases pasture CP content at the expense of the soluble carbohydrate fraction (Jones et al. 1965, van Soest 1982). This increase in protein (without a simultaneous increase in metaboliseable energy) can lead to less efficient N utilisation in the rumen (Poppi et al. 1999, Miller et al. 2001) and a slower rate of digestion which in turn will lead to a reduction in intake (Meissner et al. 1995). Nitrogen in excess of microbial requirement in the rumen will be excreted as urea in the urine of the animal (McDonald et al. 2002).

2.3.2.3 Minerals

The mineral composition of forage plants is determined by the interrelationships between factors such as growth, soil pH, fertility, fertiliser application and species. Nitrogen fertiliser may indirectly affect the mineral content of a pasture as it may modify the botanical composition (i.e. reduce the legume component), affect plant composition through nutrient dilution because of its effect on yield (or vegetative growth) or have a direct effect on the uptake of minerals from the soil (Noller and Rhykerd 1974).

Calcium (Ca) and phosphorus (P) nutrition are of particular importance due to their role in milk production and bone development in young animals. The content of these elements also vary greatly between pasture species and may even show seasonal fluctuations in uptake (Miles and Manson 2000).

Temperate grasses and legumes usually have a higher Ca and P content than their tropical counterparts, legumes having higher Ca than grasses in either case (Minson 1990). There is no consistent effect of fertiliser N on pasture Ca and P content, except for the indirect reduction in Ca and P content as application of fertiliser N may lead to a reduction in the legume component (Minson 1990). Typical Ca and P concentrations can be seen in Table 2.1.

Animal requirement for Ca and P is often expressed as a Ca:P ratio, which should be in the range of 1:1 to 2:1, depending on the type of animal (McDonald et al. 2002). Wide Ca:P ratios are implicated in a number of metabolic disorders (Buxton and Fales 1994) but according to Minson (1990) and Miles and Manson (2000) there is evidence which suggests that the importance of this ratio is exaggerated and that livestock can tolerate a wide range of Ca:P ratio's without any negative impact on growth or reproduction as long as both nutrients are present in sufficient quantities to avoid deficiency.

Table 2.1: Concentrations of Ca, P and the Ca:P ratio of several forages in 4 to 6 week old regrowth of high yielding pastures. Adapted from Miles and Manson (2000)

Species	Ca (g kg^{-1})	P (g kg^{-1})	Ca:P
Italian ryegrass	4.9	2.9	1.7
Tall Fescue	2.8	2.3	1.2
Kikuyu	2.5	3.5	0.7
White Clover	17.6	3.2	5.5
Lucerne	9.8	2.6	3.8

2.3.2.4 Dry matter content

Herbage consist mostly of water (85 to 90 %) and dry matter (including cell wall and cell content) of between 10 and 15% (Lambert and Litherland 2000). Nitrogen fertilisation stimulates vegetative growth, producing leafy material high in moisture content (Buxton and Fales 1994). A trend of decreasing DM content as N fertiliser rates increased were reported by various scientists (Noller and Rhykerd 1974, Wilman 1975, Peyraud et al. 1997). This may lead to lower animal performance due to the inability to consume enough DM (Meissner et al. 1995), although this is not always the case. Peyraud et al. (1997) found increases in DM content with reduced fertilisation, but this was not mirrored by an increased intake in dairy cows grazing low or highly fertilised perennial ryegrass.

2.3.3 Nitrogen fertiliser and intake

Dry matter intake is the most important factor governing animal performance (Poppi et al. 1999, Meissner et al. 2000) and is positively correlated with palatability and the water soluble carbohydrate (WSC) content (Reid et al. 1966; Jones and Roberts 1991). Studies suggest that high levels of fertiliser N may decrease pasture palatability. Reid and Jung (1965) and Reid et al. (1966) showed that sheep displayed a preference for tall fescue and cocksfoot grass hay fertilised with low levels of nitrogen fertiliser (56 kg ha^{-1}) and rejected hays that received medium to high levels (504 kg ha^{-1}) of fertiliser N. A reduction in WSC content associated with N fertilisation may point to the underlying mechanism for this response. This is supported by Maryland et al. (2000) who also found that cattle preferences among tall fescue cultivars were related to the total non-structural carbohydrate (TNC) (which includes WSC) content.

In contrast, in forage with a low CP content intake may be limited due to a lack of degradable protein, which limits the activity of rumen organisms that maintains rumen fill and thus reduces intake (Hoover 1986).

2.4 Nitrogen over-fertilisation

The loss of nitrogen to the environment is of increasing concern. When crop demand and N supply (via organic matter mineralisation or fertiliser application) are not synchronised N can be lost to the environment via various pathways (Crews and Peoples 2005).

In most grass pastures, only 50 to 70% of applied N is taken up by the plant and this value can decrease even further when higher rates are applied (Smil 1999; Miles and Manson, 2000). This is due to losses to the environment as gaseous emissions in the form of nitrous oxide (N_2O) and nitric oxide (NO) as a result of denitrification or due to the volatilisation of ammonia (NH_3); and through erosion and runoff in the form of nitrate (NO_3^-) (Eickhout et al. 2006), all of which can be detrimental to ecosystem and human health (Crews and Peoples 2005). The loss of N through gaseous emissions play a role in global warming, the development of acid rain and the destruction of the stratospheric ozone layer (Roy et al. 2002). Nitrate leaching can be significant in areas of high rainfall where precipitation exceeds evapotranspiration, or simply when pastures are over-irrigated (Roy et al. 2002). This leads to eutrophication and algal blooms in marine and riverine ecosystems (Matsushima et al. 2010).

The effect of excessive N can also affect feeding value. The leaves of over fertilised grasses can become dark green, soft and weak which reduces the palatability and may potentially be more susceptible to attacks by fungal pests and insects (Ohyama. 2010). These soft stemmed grasses are also prone to lodging (which will increase damage through trampling) and can be difficult to dry out if it is destined for hay (Frame 1992)

2.4.1. Nitrate toxicity

Nitrogen which is in excess of a plant's metabolic requirement is taken up and stored in the plant tissue in the form of non-protein nitrogen (NPN), of which a portion may be in the form of nitrate (Frame 1992). In the rumen, nitrate is reduced to nitrite, which in turn oxidises the ferrous iron in the haemoglobin molecule to methaemoglobin which cannot carry oxygen in the blood. This can eventually lead to the death of an animal (McDonald et al. 2002). In the short term high nitrate may also negatively affect the growth, reproduction and milk yield of livestock (Shiel et al. 1997), possibly due to the sensitivity of rumen micro-organisms for nitrite and consequent slower digestion (Meissner et al. 1995).

Plant nitrate can accumulate to levels that are toxic to livestock under conditions that restrict plant growth such as drought (Shiel et al. 1997) or mineral deficiencies (Buxton and Fales 1994). Toxicity may occur when herbage contains more than 0.7 gram nitrate kilogram^{-1} DM (McDonald et al. 2002) and concentrations above 2.5 g kg^{-1} are normally fatal (van der Merwe and Smith 1991). Nitrate seems to be less toxic when the feed contains readily digestible components such as carbohydrates, which are normally present in young plant material which would suggest that the risk of toxicity is highest when fertilised forages with a high nitrate content and low digestibility are fed (Raymond 1969, van der Merwe and Smith, 1991).

Nitrate does not accumulate to the same extent in legumes as most of the internal N is present as NH_4^+ due to fixation and thus the NO_3^- form is avoided (Nelson and Moser 1994, Fulkerson et al. 2007).

2.5 Biological Nitrogen Fixation (BNF)

Nodulated legumes can potentially be self-sufficient with regards to their N requirement (Hardarson and Atkins 2003). The process of biological nitrogen fixation (BNF) relies on a nutritionally complementary relationship between soil borne bacteria of the *Rhizobium* genus and a specific legume plant. In this symbiotic relationship the plant supplies an energy source (as a portion of the shoot photosynthate) and a favourable micro-environment and in turn the *Rhizobium* bacteria fixes atmospheric nitrogen (N_2) in the form of ammonia (NH_3), which can then be used for protein synthesis by the plant (Hardarson and Atkins 2003).

The bacteria enter the root via the root hair in a process that is initially mediated by a complex signaling process, involving root exudates, between the plant and bacteria (Mateos et al. 2011). The adhesion of the bacteria on the root hair causes the root hair to curl and form a ‘pocket’ with suitable micro-climate where the bacteria can infect the root hair cells and multiply (Mateos et al. 2011). This includes the maintenance of a low internal O_2 concentration (required by nitrogenase) which is regulated by the protein leghaemoglobin (Bijl et al. 2011).

The *Rhizobium* bacteria in the nodule contain the enzyme nitrogenase, which is responsible for catalysing the conversion of N_2 to NH_3 (Bidwell 1979, Hardarson and Atkins 2003). Adequate levels of photosynthesis are required for optimal nitrogen fixation as the process is energy intensive (Bidwell 1979). The nitrogen acquired through fixation is rapidly converted to amino acids, which use carbon skeletons supplied by respiration (Bidwell 1979).

The amount and weight of nodules as well as the degree of red pigmentation, (caused by leghaemoglobin) can give an indication of the relative level of N fixation (Hardarson and Atkins 2003).

2.6. Biological Nitrogen Fixation in mixed grass legume pastures

There are three predominant factors that govern the process of BNF in mixed grass-legume pastures: i) the soil N status, ii) competition and iii) legume persistence and production (Ledgard and Steele1992).

2.6.1 The effect of N on biological nitrogen fixation

When pasture legumes are planted in combination with grass species there is a negative feedback mechanism that limits the amount of N that can be acquired by fixation (Ledgard 2001). Biological nitrogen fixation is maximised when the amount of soil N is at a minimum because inorganic nitrogen inhibits fixation (Ledgard and

Steele 1992). High levels of inorganic N in the soil, irrespective of its origin (from mineralisation or fertiliser addition) cause the inhibition of root-hair infection, nodule growth and development and accordingly a reduction in the amount of N fixed (Ledgard and Giller 1995). Thus, the extent to which fixation occurs in the legume plant is more or less directly related to the level of available N in the soil and these two sources are essentially complementary (Hardarson and Atkins 2003). When soil N is low, legumes have a competitive advantage over grass as they are able to acquire most of their N through BNF, but as soil N increases over time the grass component will gradually increase at the expense of the legume (Ledgard and Steele 1992). With the repeated application of N fertiliser to a grass-clover sward the total herbage production will increase, but at the expense of clover production and content (Labuschagne et al. 2006). The benefit of this competition and feedback system is that it serves as a natural mechanism for the regulation of N losses to the environment (Ledgard 2001). This response differs according to species, cultivar, *Rhizobium* strain, form and amount of N, time and site of application, age of the host plant and environmental conditions (Frame and Newbould 1986). Ledgard et al. (1996) reported a 17 % reduction in white clover growth in a perennial ryegrass-white clover pasture in response to the application of 390 kg N ha^{-1} y^{-1} as well as a 58 % reduction in N fixation due to the substitution of fertiliser N.

Factors that influence photosynthate production and translocation to the nodules can also have an influence on BNF (Hardy and Silver 1976). There is a linear relationship between light intensity, nodulation and N fixation (Wu and McGechan 1999). Temperature also has a marked effect on the ability of a legume to fix N. For most temperate legumes the optimum temperature range for fixation is between 20 and 35˚C. White clover specifically requires a minimum temperature of 9˚C for fixation, while the enzyme Nitrogenase functions optimally between 13 and 26˚C (Wu and McGechan 1999). On the other hand, fixation may be reduced during summer as a result of increased mineralisation and consequent higher soil N levels due to higher temperatures (Ledgard and Steele 1992). Biological nitrogen fixation is also lower when grazing animals are present due to the increase in inorganic nitrogen in the form of urea in urine. Biological nitrogen fixation may decrease by up to 90 % in urine affected areas (Ledgard and Steele 1992).

2.6.2 Grass – legume competition

In general, legumes are weak competitors for nutrient uptake compared to grasses (Peoples et al. 1995) because grasses are taller, have a mass of fine roots and have less precise climatic and nutritional requirements (Frame and Newbould 1986). Grasses are much better competitors than legumes for the uptake of immobile nutrients such as phosphate (P), potassium (K) and sulfur (S) (Haynes 1980), while nodulated legumes need higher levels of plant available cobalt, copper molybdenum and phosphorus, the latter which is critical for the development of a successful symbiosis (Haynes 1980, Hardarson and Atkins 2003).

Grass-legume competition however, is ultimately determined by the competition for light (Haynes 1980). Grass growth is correlated with N status, and increasing N fertilisation can depress legume growth simply by increased shading and nutrient competition (Ledgard et al. 1996). According to Frame and Newbould (1986), the competition for light and nutrients is more detrimental to the growth of the clover plant than the effect of high fertiliser N on nodule activity in a grass-clover sward.

The shading of legumes limit the amount of carbohydrates that are transported to the root system which eventually leads to the death of nodule tissue and increases the rate of N transfer to the accompanying grasses (Haynes 1980). This ultimately leads to a reduction in the amount of legume in the sward which decreases as the level of N fertilisation increases (Frame and Newbould 1986).

Low growing species may suffer more from shading, but grazing management can alleviate this if frequent defoliation avoids long term shading (Haynes 1980, Ledgard et al. 1996). The canopy structure of lucerne as well as the fact that it is a bigger plant, allows for more light penetration which leads to more efficient use of light energy and may thus be more resistant to shading (Haynes 1980).

2.6.3 Legume persistence and production

The production potential of a grass-legume pasture mainly depends on the legume content and the stability of the legume – grass ratio (Botha 2002). The optimum ratio in terms of both quality and yield is a legume fraction of 30 to 50%: If the clover content increases above 50%, there will be a decline in overall yield, and should it decrease below 30% there will be a loss of quality (Botha 2002). This is in agreement with Rochon et al. (2004), who stated that for maximum animal production pastures should contain a minimum of 30% legume, although Harris et al. (1997) found that a clover content of 50 to 65% was necessary for maximum milk production. According to Frame (1992) the clover content in a mixed grass-clover pasture should be at least 20 to 40% by mid-season to benefit from BNF.

There are numerous other factors that influence the grass-legume association, any of which can have a definite influence on pasture production and the amount of N that is fixed. Any edaphic or climatic factor that restricts legume growth, especially at the seedling stage will have a negative effect on the eventual ability to fix N (Hardarson and Atkins 2003). Other conditions that play an important role are the availability of soil moisture, soil acidity, nutrition and the presence of pests and diseases (Ledgard and Steele 1992). Both drought and water logging lead to a decrease in N fixation, although deep rooted legumes may be more resistant to these conditions. Drought conditions may lead to the accumulation of soil inorganic nitrogen which can have long term effects on the ability of legumes to fix nitrogen (Ledgard and Steele 1992).

2.7 Potential of legumes to supply N

Common estimates for N fixation are between 50 and 250 kg N ha^{-1} y^{-1} (Frame 1992, Ledgard and Steele, 1992, Roy et al. 2002). Ledgard (2001) states realistic values in a mixed grass-legume pasture to be in the range of 66 to 152 kg N ha^{-1} y^{-1} but this value can decrease to approximately 50 kg N ha^{-1} y^{-1} in permanent, long-term grazed pastures due to the eventual decline in legume content. Andrews et al. (2007) reports that white clover can fix between 200 and 300 kg N ha^{-1} y^{-1} in a white clover-perennial ryegrass pasture if the clover comprises at least 50 % of the total pasture dry matter yield. Ultimately the amount will vary from site to site, depending on factors such as soil characteristics, environmental conditions, pests and disease as well as pasture and grazing management (Ledgard and Steele 1992).

2.8 Transfer of legume nitrogen to grasses

The amount of N fixed by pasture legumes vary with climate and management but up to 30% can be transferred to the companion non-legume plant (Ledgard and Giller 1995). This transfer of legume N is governed by two opposing forces:

Generally the legume increases the amount of available soil N, which is then available for uptake by the grass component, but it is generally not taken into account that the legume also competes for the uptake of this same N. Normally only the net effect of these two processes is known as it is very difficult to measure separately, but it is generally accepted that the grass benefits more compared to the companion legume (Simpson 1965). The reason for this is because grasses are stronger competitors for available soil N (due to having a more prolific root system) which can have a positive effect on BNF as the soil is effectively drained of N. This then 'forces' the legume to satisfy the N requirement by means of nitrogen fixation (Giller and Cadish 1995, Hardarson and Atkins 2003).

There are two main pathways through which legume N can be transferred in a mixed sward. The first is through the mineralisation of organic matter (which consists of both above and below ground legume fractions) and the second is via the excreta of grazing animals (Ledgard 2001, Fillery 2001). Only 5 to 25% of the N ingested by livestock is retained in the animal body, while the rest is excreted back onto the pasture as manure or urine (Frame 1992). Urinary N, after the ingestion of legume rich pastures, is the fastest way in which rhizobially fixed N is transferred back to the pasture (Frame 1992). This pathway is considered to be an inefficient method for N cycling due to the potential of large amounts being lost through leaching and volatilisation (Fillery 2001) because the N is deposited predominantly as urea and is highly concentrated (Ledgard and Giller 1995). This is accentuated by the variability in the spatial distribution of excreta (Ledgard 2001). Most of the N present in dung is in an organic form and thus not immediately available (Frame 1992).

Nitrogen cycling via mineralisation is a slower process as it is dependent on the death and decay of plant nodules, roots and surface leaf material (Ledgard and Giller 1995). This underground N transfer is accelerated when the legume plants are stressed by drought or defoliation because this stimulates the decay of nodules, which implies that there is greater N transfer after defoliation (Simpson 1965). This pathway is also the main way in which soil N is built up over the long term (Frame 1992). There are also other mechanisms of N transfer, such as root exudation and direct transfer via interconnected roots, but these make a negligible contribution to the total N cycled.

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CHAPTER 3

Materials and methods

Experimental site

The study was carried out under irrigation at the Elsenburg research farm in the Western Cape Province of South Africa, (33˚ 51'S, 18˚ 50' E; altitude 177 m) during the 2010 to 2012 production years. The climate is Mediterranean receiving an average annual rainfall of 622.7 mm per year, of which 84% occurs during winter (April - October). The mean annual summer and winter (maximum and minimum) temperatures during the experimental period were 25.7, 11.9 and 20.5, 8.8˚C respectively (ARC-ISCW, 2013) (Figure 3.1). These temperature and rainfall values compare favourably with long term (17 year) averages, except for July and August 2010 and July 2011 which received less rainfall than expected.

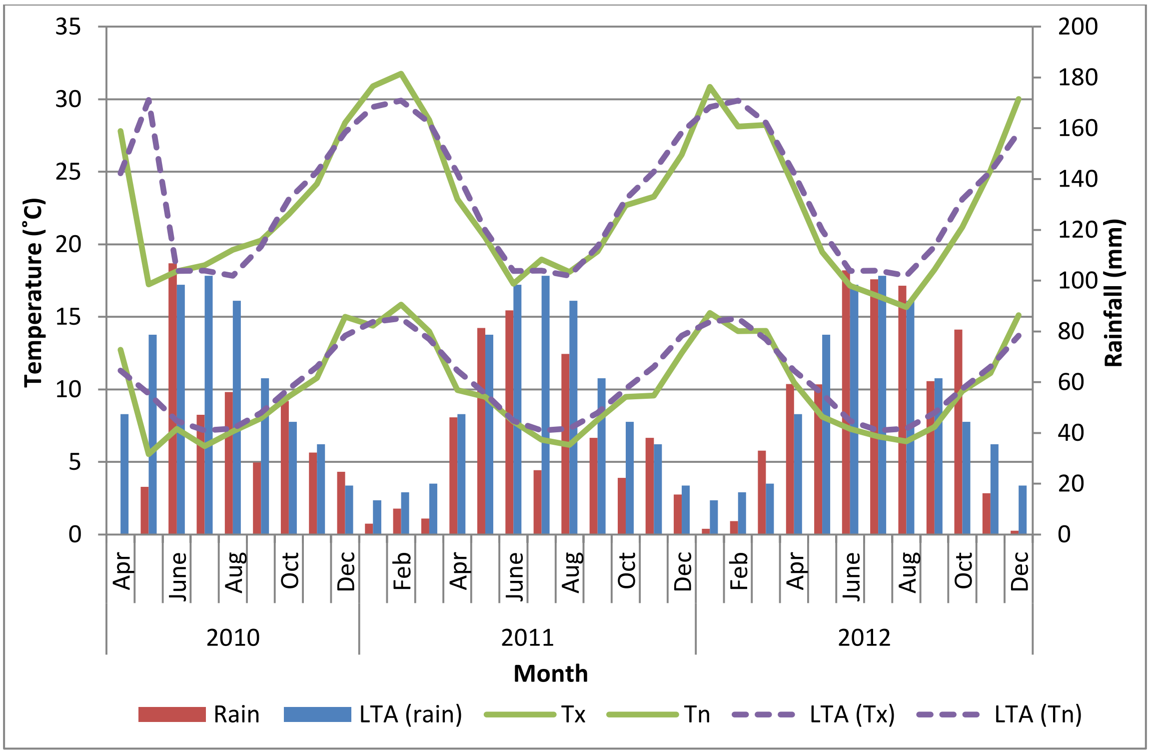

Figure 3.1: Monthly rainfall (mm) and mean minimum (Tn) and maximum (Tx) temperatures (˚C) for study the period (April 2010 to October 2012), compared to the long term averages (LTA) at Elsenburg Research Farm

The soil was classified as a sandy loam Oakleaf consisting of an Orthic A horizon overlaying a Neocutanic B horizon (Soil Classification Working Group, 1991). After site identification soil samples were taken to a depth of 200 mm in November 2009. Soil nutrient status and pH were corrected to levels recommended by Beyers (1983) (Table 3.1). Calcitic lime and single super-phosphate was surface applied and subsequently ploughed to address the deficiencies dictated by soil analyses. Selected chemical and physical soil characteristics at establishment of the study are summarised in Tables 3.1 and 3.2. Soil carbon content was relatively low due to continuous cultivation and cropping of the research area in the past. The chemical and physical composition of the soil was expected to ensure normal pasture growth and production. The clay content increased with soil profile depth (Table 3.2) which reduced the potential for N leaching.

Table 3.1: Selected chemical characteristics of the soil at the experimental site at Elsenburg Research Farm

Parameter	**Value**
pH (KCl)	6.17
Resistance (Ohms)	570.00
Calcium (cmol(+)/kg)	3.08
Magnesium (cmol(+)/kg)	1.09
Potassium (mg/kg)	126.67
Sodium (mg/kg)	77.67
Phosphorous (citric acid) (mg/kg)	37.00
Total cations (cmol(+)/kg)	4.84
Copper (mg/kg)	1.18
Zinc (mg/kg)	1.15
Manganese (mg/kg)	140.63
Boron (mg/kg)	0.35
Carbon (%)	0.80
Sulphur (mg/kg)	4.67

Table 3.2: Selected physical characteristics of the soil at the experimental site at Elsenburg

	0-100 mm	**100-200 mm**	**200-300 mm**	**300-500 mm**
Texture	Sandy loam	Sandy clay loam	Sandy clay loam	Sandy clay loam
Sand (%)	76.0	68.0	62.0	63.0
Clay (%)	18.0	24.0	32.0	30.0
Silt (%)	6.0	8.0	6.0	7.0

Pasture establishment and management

Three different pasture mixtures were established during April 2010 (Table 3.3). Composition of pasture mixtures were based on information related to the seasonal production potential of the different species (van Heerden 1986) and anticipated patterns of dry matter production to minimise fluctuations in fodder production throughout the year.

A fine, firm seedbed was prepared for the germination of pasture crops. The seed was broadcast by hand where after it was rolled with a light roller to ensure proper contact between the pasture seed and soil. Irrigation scheduling was based on tensiometer readings and irrigation was started as soon as the meter registered -25 kPa at 15 cm depth. To prevent over irrigation and leaching of applied N a second tensiometer was placed 30 cm deep and in the grass - lucerne pasture a third at 60 cm. The aim was to keep readings at -25 to -35 kPa. Twenty kilograms of N was applied after emergence to stimulate early pasture growth. Excluding N, further fertiliser applications were based on annual soil fertility recommendations (Beyers 1983).

Crop protection (weed, pest and disease control) was performed according to best practices for irrigated pastures in the Winelands sub-region of the Western Cape Province of South Africa.

Table 3.3: Species composition, cultivar and sowing density of the respective pasture mixtures established at Elsenburg Research Farm during April 2010

Pasture	Species composition	Cultivar	Seeding rate (kg ha^{-1})
Mixed grass pasture	Perennial ryegrass *(Lolium perenne)*	Bronsyn	5
	Cocksfoot (*Dactylis glomerata)*	Cambria	7
	Tall fescue (*Festuca arundinaceae)*	Au Triumph	7
Mixed grass-clover pasture	Perennial ryegrass *(Lolium perenne)*	Bronsyn	4
	Cocksfoot (*Dactylis glomerata)*	Cambria	6
	Tall fescue (*Festuca arundinaceae)*	Au Triumph	6
	White clover (*Trifolium repens)*	Haifa	3
	Red clover (*Trifolium pratense)*	Kenland	4
Mixed grass-lucerne pasture	Perennial ryegrass (*Lolium perenne)*	Bronsyn	6
	Tall fescue (*Festuca arundinaceae)*	Au Triumph	6
	Lucerne (*Medicago sativa)*	Aurora	12

Experimental design and treatments

Experimental design was a completely randomised design with five and six replicates for the grass-only and grass-legume pastures respectively. The mixed grass pasture, mixed grass clover pasture and mixed grass lucerne pasture were treated as three separate studies, including the statistical analyses procedures. The treatment design was a factorial design with two factors, seasons and nitrogen application rates. Year was added as a sub-plot factor because measurements were taken repeatedly (once annually) on the same plot. This statistical approach is consistent with procedures described by Little and Hills (1972).

An establishment period of approximately 5 months was allowed for the grass-only pasture to establish before N treatments commenced in August 2010. Data collection for the grass-legume pastures commenced in April 2011 (First cool season

N treatments on legume based pastures were applied in March 2011). Nitrogen was applied as LAN (28% N) at the beginning of each re-growth cycle, after the pasture was grazed, and washed into the soil through application of 10-15 mm irrigation. Plot dimensions were 3.75 x 3.75 m (14.06 m^2) of which 6.6 m^2 was harvested using a sickle-bar mower. After sampling the pasture was mob-grazed by sheep to an estimated height of 30 mm. If necessary the pasture was mowed to 30 mm to remove the effects of patch grazing and the material removed from the site.

Specific details of treatment application to the respective pasture mixtures are described in chapters 4-6.

Data collection

Samples from plots that received N treatments were cut with a sickle-bar mower at a height of approximately 30 mm. Sample fresh (wet) mass was determined by weighing the material on site, where after two representative sub-samples of approximately 350 grams each were collected. For the grass-only pasture, one sub-sample was used to determine dry matter production and quality and the second one to determine species composition. For the grass-legume pastures, one sub sample was used to determine dry matter production, while the other was used to determine botanical composition and then quality. The separate grass and legume fractions used to determine species composition were dried and quality analyses performed.

Samples used for quality analyses were dried and ground to pass through a 1 mm sieve and analysed for *in vitro* organic matter digestibility (IVOMD) (Tilley and Terry, 1963) and crude protein (CP). The weed fraction was separated and disregarded in the quality analyses. Dry matter content (%) was determined by calculating the difference between the fresh and wet weight of the sample, expressing the DM content as percentage, by using the following formula: DM content = (wet sample weight – dry sample weight) ÷ wet sample weight x 100. Crude protein (CP) was calculated as % N x 6.25 and herbage N yield as: herbage N yield = herbage N content x Dry matter production.

For dry matter production determination the fresh weight of the sub-samples collected was recorded, where after the samples were dried at 60°C for 72 h. The following equation used to calculate dry matter production:

Dry matter production (kg ha^{-1}) = [((Wet sample weight x sub-sample $weight_{dry}$) ÷ $sub\text{-}sample_{wet}$) x 10 000)] ÷ 6.6

Botanical composition was determined by visually separating the various grass and/or legume species. Grasses were identified using the description supplied in Tainton et al. (1990). Grass species were also grown in pots to aid identification.

After separation, the various fractions where dried for 72 h at 60˚C to determine their dry matter weight and contribution to total fodder production using the formula:

Fraction (%) = [species A ÷ (species A + species B)] x 100

Dry matter production was recorded as primary dry matter production (PDMP) which refers to the dry matter produced during the first regrowth cycle after the application of N fertiliser treatments; residual dry matter production (RDMP) which refers the amount of dry matter produced during the second regrowth cycle after fertiliser N application (no N applied before second regrowth cycle); total dry matter production (TDMP), which refers to the total dry matter produced during the first and second regrowth cycles (PDMP plus RDMP).

Statistical analyses

A Shapiro-Wilk test for normality was done before the results could be assumed reliable (Shapiro & Wilk, 1965). A Student t test with a least significant difference (LSD) at a 5% level of significance was used to compare treatment means (Ott, 1993). Statistical Analyses System (SAS) version 9.2 was used to analyse data (SAS 2008).

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CHAPTER 4

The effect of nitrogen fertiliser application on the yield, botanical composition and selected nutritive characteristics of a mixed grass pasture in the Winelands sub-region of the Western Cape Province of South Africa

4.1 Introduction

Livestock production from pasture depends on the intake of dry matter and the quality of the herbage on offer (Lambert and Litherland 2000). Pasture production in the Western Cape Province of South Africa has traditionally been based on temperate species such as perennial ryegrass (*Lolium perenne*), cocksfoot (*Dactylis glomerata*), tall fescue (*Festuca arundinaceae)*, red and white clovers (*Trifolium pratense* and *T. repens* respectively) and lucerne (*Medicago sativa*) (van Heerden and Tainton, 1989). Temperate pastures typically produce the majority of its fodder on offer during spring and autumn where after production decreases in winter (Christian 1987), a period commonly referred to as the winter 'fodder gap' (Labuschagne and Agenbag 2006). The negative effect of this seasonality can potentially be reduced by the establishment of diverse pasture mixtures, where the growth curves of various species are combined to provide a more consistent amount of fodder throughout the year (Haynes 1980, Sleugh et al. 2000). Another approach is through boosting pasture production with the application of N fertiliser. Perennial ryegrass has the potential to make a valuable contribution in this regard because it is able to grow and respond to N fertilisation at temperatures as low as 6 ˚C (Labuschagne and Agenbag 2008), which could improve fodder availability during the winter gap.

The application of N fertiliser can also affect pasture quality, especially the crude protein (CP) and dry matter (DM) content (Poppi et al. 1999). Livestock protein requirements vary according to the type of animal and level of production, but fertilised forages often contain CP levels that are in excess of livestock requirement (van Soest 1982, Buxton and Fales 1994). Nitrogen fertiliser is thus often wasted in the pursuit of maximising yield, which can lead to poor animal performance (Eckard 1990), as well as serious negative environmental consequences such as nitrate leaching (Eickhout et al. 2006).

The aims of this study were therefore i) the development of nitrogen fertiliser application strategies to ensure optimum dry matter production during different seasons, ii) to determine the effect of fertiliser N on the botanical composition of these pastures and iii) to determine the effect of fertiliser N on selected nutritive characteristics of the fodder produced.

4.2 Materials and methods

A comprehensive overview of the site characteristics and general procedures and management is provided in Chapter 3. Specific details for the mixed grass pasture is given below.

A mixed grass pasture consisting of perennial ryegrass, cocksfoot and tall fescue was established in April 2010. Data collection commenced in September 2010. Nitrogen treatments of 0, 20, 40, 60 or 80 kg ha^{-1} were applied throughout the year (after every grazing), according to specific regrowth cycles. The experimental design was a completely randomised design with N treatments randomly replicated five times. Nitrogen was applied as LAN (28% N) at the beginning of each regrowth cycle, after the pasture was mob-grazed by sheep to a height of approximately 30 mm, and washed into the soil by light (10-15 mm) irrigation. If necessary the pasture was mowed to 30 mm to remove the effects of patch grazing and the material removed from the site. Sub-plot dimensions were 3.75 x 3.75 m (14.06 m^2) of which 6.6 m^2 was harvested using a sicklebar mower**.** Various sub-samples were collected for the determination of dry matter production, botanical composition and selected nutritive characteristics.

The regrowth cycles (grazing frequency) was determined by season: Spring (September – October) had a regrowth cycle of 24 days, winter (June – August) 35 day frequency and the rest of the year (November – May) 28 days. To avoid carry-over effects, especially at the higher treatment levels, a specific plot received an N treatment only once during a calendar year. To avoid total N deficiency, plots not receiving a treatment during a specific cycle received a base N level of 30 kg N ha^{-1}.

Dry matter production for the various cutting cycles was combined and represented as seasons (spring, summer, autumn and winter) for Year 1 (September 2010 –

August 2011) and Year 2 (September 2011 – August 2012) respectively. Season allocation was as follows: Spring: September – November; Summer: December – February; Autumn: March – May and Winter: June – August. Disruptions in irrigation water availability as a result of cable theft occurred during both years covered by the study, and most certainly influenced dry matter production. Quality analyses were done during the following regrowth cycles: Year 1: Spring (September), early summer (November), early autumn (March), mid-autumn (April), winter (June/July). Year 2: Spring (September), early summer (November), late summer (February), mid-autumn (April), late autumn (May), winter (June/July).

Experimental design and treatments

Experimental design was a completely randomised design with five replicates. The treatment design was a factorial design with two factors, seasons (autumn, winter, spring and summer) and nitrogen application rates. Year was added as a sub-plot factor because measurements were taken repeatedly (once annually) on the same plot. This statistical approach is consistent with procedures described by Little and Hills (1972).

4.3 Results and discussion

4.3.1 Dry matter production

4.3.1.1 Primary dry matter production (PDMP)

Primary dry matter production (kg DM ha^{-1} day^{-1}) as influenced by the treatment combinations is presented in Table 4.1. A tendency of increased PDMP as fertiliser N rate increased was observed in both years covered by the study. Productivity (kg DM ha day^{-1}) tended to be the highest in spring and summer at high N application rates (60 – 80 kg N ha^{-1}) followed by a sharp decrease towards autumn and winter. The PDMP during the second year after establishment was slightly lower than in the first year after establishment over all seasons.

During both years a significant interaction ($P < 0.05$) between N application rate and season of application was recorded. At zero N application no differences in

PDMP between summer, autumn and winter were found, but differences became more pronounced as N level was increased. At 80 kg ha^{-1} there was a significant difference in PDMP between all seasons, with the highest production in summer (Year 1) and Spring (Year 2). Greatest differences in PDMP occurred at the higher N application rates.

Based on these findings, preliminary recommendations for the application of fertiliser N to a mixed grass pasture would be 60 – 80 kg N ha^{-1} during spring and summer and 40 kg N ha^{-1} in autumn and winter. These values are relatively high due to the low soil carbon content of the experimental site. Therefore 60 kg N ha^{-1} in spring and summer will be more appropriate for farms in the region. The value of 60 kg N ha^{-1} is also supported by Eckard (1998) who state that 90 % of the maximum biological yield will normally be achieved with the single application of 60 kg N ha^{-1} after one regrowth period.

Table 4.1: Seasonal primary dry matter yield response (kg DM ha^{-1} day^{-1}) of a mixed grass pasture in response to fertiliser N rate (kg N ha^{-1}) during Year 1 and 2 after establishment at Elsenburg Research Farm

			Year 1			
			Nitrogen (kg ha^{-1})			
Season	**0**	**20**	**40**	**60**	**80**	**Mean (S)**
Spring	28.12 ^{efg}	36.80 ^{ed}	54.40 ^{bc}	62.54 ^{bc}	64.37 ^{b}	**49.25**
Summer	16.29 ^{fghij}	29.02 ^{efg}	49.02 ^{cd}	58.46 ^{bc}	80.61 ^{a}	**46.44**
Autumn	7.25 ^{ij}	8.85 ^{hij}	18.44 ^{fghi}	22.33 ^{fgh}	29.96 ^{ef}	**18.00**
Winter	3.40 ^{j}	4.71 ^{ij}	7.47 ^{ij}	11.15 ^{hij}	14.62 ^{ghij}	**8.72**
Mean (N)	**16.31**	**20.68**	**33.68**	**39.34**	**47.34**	
			Year 2			
Spring	26.17 ^{bcde}	34.14 ^{b}	57.64 ^{a}	59.61 ^{a}	67.34 ^{a}	**48.84**
Summer	7.63 ^{g}	26.68 ^{bcd}	21.14 ^{cdef}	32.24 ^{bc}	29.76 ^{bc}	**23.43**
Autumn	3.53 ^{g}	8.59 ^{fg}	11.95 ^{fg}	13.54 ^{efg}	14.33 ^{defg}	**10.88**
Winter	3.92 ^{g}	6.39 ^{g}	6.48 ^{g}	5.63 ^{g}	9.61 ^{fg}	**6.40**
Mean (N)	**12.46**	**19.79**	**27.67**	**29.73**	**33.63**	

LSD (0.05) compared N treatments and season of N application within year. Means followed by the same letter are not significantly different (P > 0.05).

4.3.1.2 Residual dry matter production (RDMP)

The effect of fertiliser N rate and season of application on RDMP (kg DM ha^{-1} day^{-1}) is summarised in Table 4.2.

In Year 1 RDMP was affected by both N rate ($P < 0.05$) and season of application ($P < 0.05$). The RDMP tended to increase as N rate increased although not significantly so between the 40, 60 and 80 kg N ha^{-1} treatments. Highest RDMP was recorded in summer and winter.

During the second year RDMP was mainly influenced by season of application ($P < 0.05$) and not by N application rate, although a non-significant increase in RDMP in response to N did occur. Winter and spring produced the highest RDMP of 30.55 and 42.57 kg DM ha day^{-1} respectively.

Table 4.2: The effect of season of N application and fertiliser N application rate (kg N ha^{-1}) on residual dry matter production (kg DM ha^{-1} day^{-1}) of a mixed grass pasture during Year 1 and 2 after establishment at Elsenburg Research Farm

			Year 1			
			Nitrogen (kg ha^{-1})			
Season	**0**	**20**	**40**	**60**	**80**	**Mean (S)**
Spring	*	*	*	*	*	*
Summer	20.8	19.61	29.57	30.04	28.59	**26.12** [a]
Autumn	5.75	6.9	9.75	8.78	8.62	**8.05** [b]
Winter	20.51	18.19	21.16	29.09	33.59	**24.51** [a]
Mean (N)	**17.11** [b]	**14.90** [b]	**20.16** [ab]	**22.64** [a]	**23.59** [a]	
			Year 2			
Spring	37.42	40.62	45.54	40.77	48.49	**42.57** [a]
Summer	12.14	17.09	10.9	17.69	14.5	**14.46** [c]
Autumn	7.16	7.29	8.23	7.37	11.38	**8.29** [c]
Winter	22.56	26.89	40.31	28.46	34.54	**30.55** [b]
Mean (N)	**19.82**	**22.97**	**26.24**	**23.57**	**27.23**	

*RDM data not available

LSD (0.05) compared N treatments and season of N application within year. Means followed by the same letter are not significantly different ($P > 0.05$).

RDMP tended to be higher than PDMP in winter for both years (Figure 4.1). The reason for this observation could be attributed to the fact that winter RDMP is the regrowth measured on average 35 days later than that for PDMP and may therefore include considerable amounts of fodder produced well into spring, with consequent higher growth rates. The increase in temperature associated with the onset of spring could have contributed to increased mineralisation of soil N (Kirschbaum 1995). Alternatively the high winter RDMP could be caused by residual inorganic soil N being present due to reduced plant uptake in winter, which was then utilised during spring. This latter scenario is not ideal as it increases the risk of N being lost through leaching due to the high rainfall during winter in the Western Cape. These results thus confirm the potential for N to be carried over to the second regrowth cycle after N application during certain times of the year. This was also confirmed in a study by Labuschagne et al. (2006) where the application of 150 kg N ha^{-1} lead to N leaching below the root zone of a perennial ryegrass – white clover pasture over the winter period. Losses of N will also result in lower nitrogen use efficiency (NUE) of the pasture crop, a situation detrimental to cost efficient fodder production.

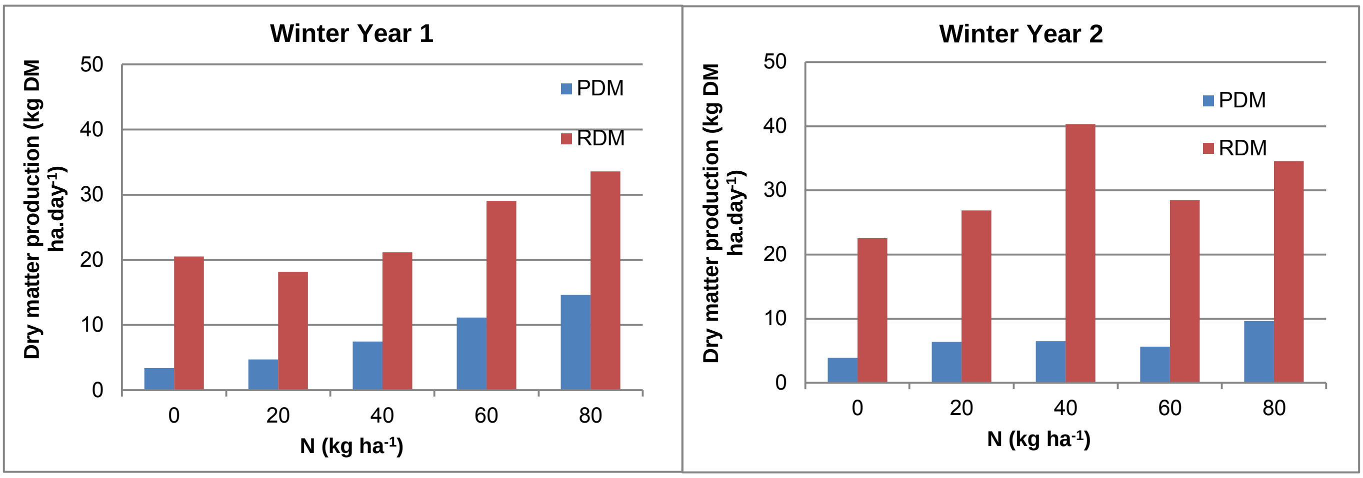

Figure 4.1: Primary (PDMP) and residual (RDMP) dry matter production of a mixed grass pasture in response to different fertiliser N rates (kg ha^{-1}) at Elsenburg Research Farm during Year 1 and 2 after establishment

4.3.1.3 Total dry matter production (TDMP)

The effect of fertiliser N on TDMP (kg DM ha^{-1} day^{-1}) showed a similar trend to that reported for PDMP and RDMP, namely an overall increase in TDMP as the rate of N increased (Table 4.3). During both years a significant interaction between season of N application and N rate ($P < 0.05$) was recorded, although the interaction cannot be defined clearly. There was a significant increase in TDMP with the increase in N application from 60 to 80 kg N ha^{-1} in summer of both years, but not in other seasons.

Table 4.3: The effect of season of N application and fertiliser N application rate (kg N ha^{-1}) on total dry matter production (kg DM ha^{-1} day^{-1}) of a mixed grass pasture at Elsenburg Research Farm during Year 1 and 2

	Year 1 Nitrogen (kg ha^{-1})					
Season	**0**	**20**	**40**	**60**	**80**	**Mean (S)**
Spring	*	*	*	*	*	*
Summer	18.12 ^{cdef}	22.2 ^{cd}	38.98 ^{b}	46.21^{b}	57.51 ^{a}	**36.60**
Autumn	5.81 ^{g}	7.14 ^{g}	14.09 ^{defg}	17.23 ^{cdef}	19.29 ^{cde}	**12.71**
Winter	10.26 ^{fg}	11.45 ^{fge}	13.94 ^{defg}	20.12 ^{cd}	24.10 ^{c}	**15.97**
Mean (N)	**11.39**	**13.59**	**22.34**	**27.85**	**33.63**	
			Year 2			
Spring	31.79 ^{cd}	37.38 ^{c}	51.76 ^{ab}	49.1 ^{b}	57.91 ^{a}	**45.89**
Summer	9.88 ^{ghi}	20.55 ^{ef}	16.02 ^{efgh}	19.28 ^{ef}	22.13 ^{e}	**17.57**
Autumn	6.46 ^{i}	7.66 ^{hi}	10.09 ^{ghi}	12.16 ^{fghi}	15.91 ^{efgh}	**10.46**
Winter	13.04 ^{fghi}	16.32 ^{efg}	10.32 ^{ghi}	18.09 ^{efg}	23.50 ^{de}	**16.26**
Mean (N)	**15.30**	**20.48**	**22.05**	**24.66**	**29.86**	

***** *Data not available*

LSD (0.05) compared N treatments and season of N application within year. Means followed by the same letter are not significantly different ($P > 0.05$).

4.3.2 Botanical Composition

The effect of season of N application and N application rate on the species composition of a pure grass pasture at Elsenburg Research Farm during the years covered by the study are summarised in Figures 4.2 and 4.3. Due to the small contribution of tall fescue in the mixture, and the difficulty of differentiating between mature ryegrass and tall fescue it was decided to combine the two species and represent it as perennial ryegrass + tall fescue (PRTF).

During Year 1 (Figure 4.2) the cocksfoot (CF) and the perennial ryegrass + tall fescue (PRTF) percentage was significantly ($P < 0.0001$) influenced by season of N application and not by N application rate. There was a distinct dominance of CF during the warmer months, while the PRTF component dominated during the winter-spring period. Cocksfoot contributed the highest percentage to the pasture on offer in summer (63.37 %) and autumn (51.21 %) and the PRTF component contributed highest percentages in winter (82.88 %) and spring (78.74 %).

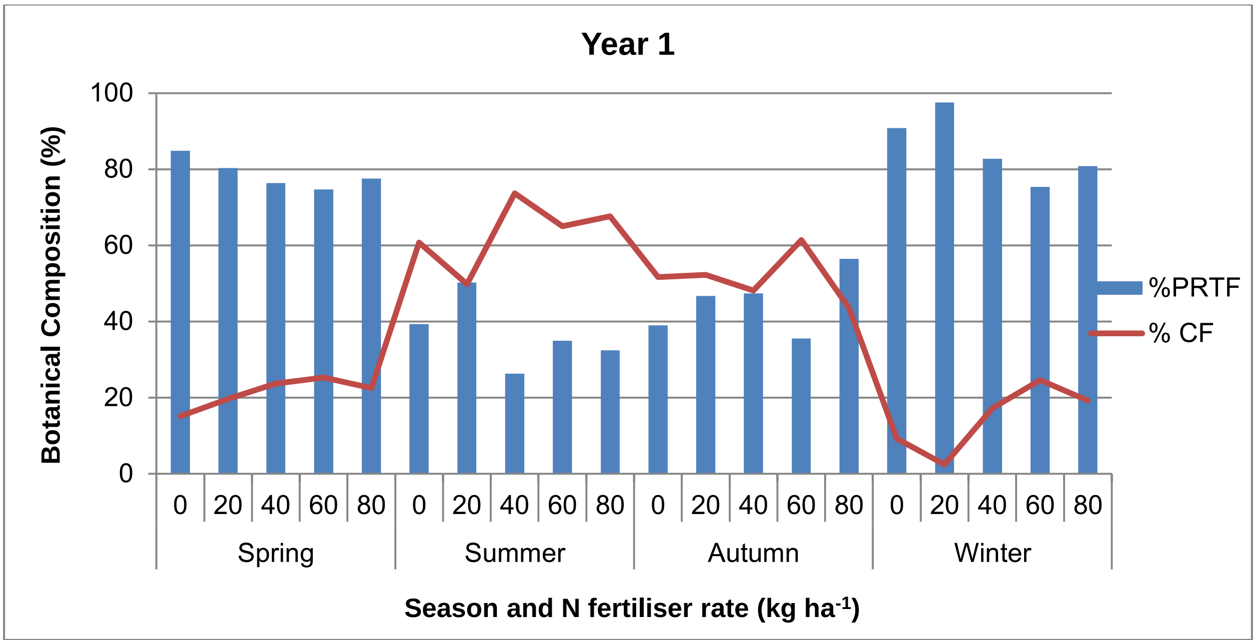

Figure 4.2: Effect of fertiliser N (kg ha^{-1}) and season of N application on the percentage of cocksfoot (CF) and perennial ryegrass + tall fescue (PRTF) in a pure grass pasture during Year 1 after establishment at Elsenburg Research Farm

Similar to Year 1, species composition was determined by season of N application of application and not by N fertiliser application rate during Year 2 (Figure 4.3). There was a slight shift in the seasonality of production of the various species compared to year one. The highest CF content was recorded in summer (89.34 %) and spring (59.36 %), while the PRTF species dominated during winter (60.65 %)

and autumn (47.43 %) although autumn production did not differ significantly from spring production (40.64 %).

This seasonal distribution of dry matter is typical of this pasture mixture (van Heerden 1986). Cocksfoot is known to be less winter-hardy (Coulman 1987) and more resistant to summer heat and mild drought conditions (Stevens and Hickey 2000) relative to perennial ryegrass. The small contribution of of tall fescue in the mixture could have been a result of the competitiveness of perennial ryegrass during the seedling phase (Charlton 1991). Unfortunately seedling survival rates were not recorded after emergence and future studies to investigate reasons for tall fescue failure in these mixed pastures could be valuable.

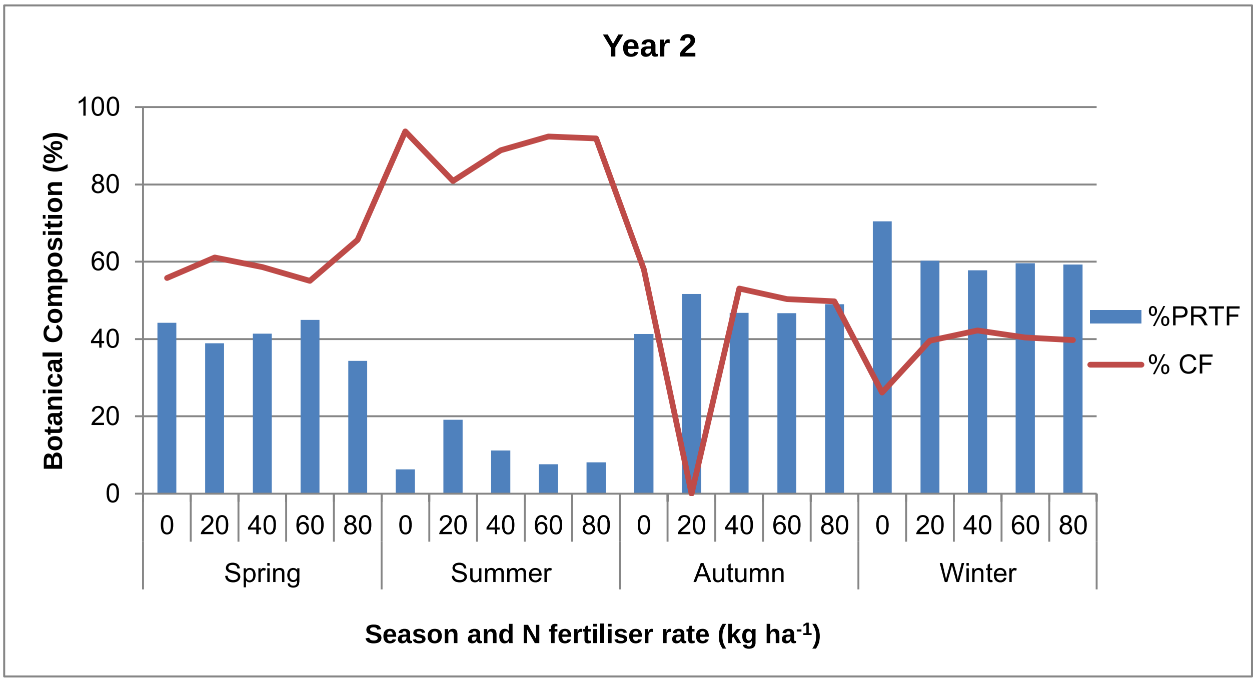

Figure 4.3: Effect of fertiliser N (kg ha^{-1}) and season of N application on the cocksfoot (CF) and perennial ryegrass + tall fescue (PRTF) content in a grass only pasture during Year 2 at Elsenburg Research Farm

4.3.3 Nutritive Characteristics

4.3.3.1 Pasture Dry Matter (DM) content

During year one, both fertiliser N ($P < 0.0001$) and season of N application of application ($P < 0.0001$) affected pasture DM content (Table 4.4). Pasture DM content decreased as the rate of N increased from 0 to 80 kg N ha^{-1}. This is in agreement with results reported by Peyraud et al. (1997). With regard to the effect of season of N application, highest DM was recorded in summer (25.68 %) and lowest in autumn (20.85 %).

Table 4.4: The effect of N fertiliser rate (kg N ha^{-1}) on the dry matter content (%) of a pure grass pasture at Elsenburg Research Farm during Year 1

	Year 1 Nitrogen (kg ha^{-1})					
Season	**0**	**20**	**40**	**60**	**80**	**Mean (S)**
Spring	25.53	23.37	20.81	21.54	20.54	**22.36 ^{b}**
Summer	30.63	27.36	24.43	23.54	22.00	**25.68 ^{a}**
Autumn	24.60	21.64	20.70	19.55	19.14	**20.85 ^{c}**
Winter	24.26	23.20	21.01	20.60	19.88	**21.65 ^{bc}**
Mean (N)	**26.31 ^{a}**	**23.74 ^{b}**	**21.55 ^{c}**	**21.06 ^{cd}**	**20.28 ^{d}**	

LSD (0.05) compared N treatments and season of N application within year. Means followed by the same letter are not significantly different (P > 0.05).

During Year 2 (Table 4.5) a significant interaction (P < 0.0097) between N application and season of N application was recorded. The interaction was the result of pasture response in winter to fertiliser N, which remained nearly constant, while all other seasons of application showed a decrease as the level of fertiliser N increased. If DM content drops too low as a result of N fertilisation, animal performance may suffer due to the inability to consume enough DM (nutrients) (Meissner et al. 1995). The DM content recorded in this study fall within the expected range for perennial ryegrass (20-25 % DM) and Cocksfoot (25-35 % DM in summer) (Meissner et al. 2000).

Table 4.5: The effect of varying rates of fertiliser N (kg N ha^{-1}) on the dry matter content (%) of a pure grass pasture at Elsenburg Reseach Farm during Year 2

	Year 2 Nitrogen (kg ha^{-1})					
Season	**0**	**20**	**40**	**60**	**80**	**Mean (S)**
Spring	25.43 ^{b}	24.6 ^{bcd}	21.44 ^{fghji}	21.02 ^{ghij}	19.5 ^{j}	**22.50**
Summer	30.61 ^{a}	25.15 ^{bc}	25.06 ^{bc}	23.28 ^{cdef}	22.83 ^{defg}	**25.39**

Autumn	23.86 bcde	22.15 efgh	20.19 hji	19.4 j	19.53 j	**20.80**
Winter	21.95 efghi	21.29 fghij	20.99 ghij	21.16 fghij	19.95 ij	**20.98**
Mean (N)	**25.80**	**23.34**	**21.73**	**21.01**	**20.30**	

LSD (0.05) compared N treatments and season of N application within year. Means followed by the same letter are not significantly different (P > 0.05).

4.3.3.2 Crude Protein (CP)

Crude protein content (%) as influenced by treatment combinations is presented in Tables 4.6 and 4.7.

There was a significant interaction ($P < 0.05$) between N application rate and season of N application during Year 1 (Table 4.6). Highest CP content was recorded during the cooler months (mid-autumn and winter) at high N rates (60 and 80 kg ha^{-1}), while lowest CP contents occurred during the warmer months at low N application rates (0 and 20 kg N ha^{-1}), but with some exceptions during early autumn. The interaction recorded was the result of the spring and early autumn response to N, which remained nearly constant over all levels of N, unlike other seasons which showed an increase as N rate increased.

Table 4.6: The effect of varying rates of fertiliser N (kg N ha^{-1}) on the crude protein (%) content of a mixed grass pasture at Elsenburg Research Farm during Year 1

	Year 1					
	Nitrogen (kg ha^{-1})					
Season	**0**	**20**	**40**	**60**	**80**	**Mean (S)**
Spring	13.34 lmn	13.09 mn	16.13 jklm	15.05 klm	16.16 jklm	**14.81**
Early summer	11.13 n	13.54 lm	13.18 mn	15.04 klm	16.67 ijkl	**13.91**
Early autumn	19.66 efgh	18.65 fghij	17.87 ghijk	18.96 fghij	20.07 efgh	**19.04**
Mid-autumn	16.33 ijklm	21.39 cdef	24.69 abc	26.44 ab	27.75 a	**23.93**
Winter	17.01 hijk	18.35 fghijk	21.14 defg	22.50 cde	23.67 bcd	**20.68**
Mean (N)	**15.44**	**17.00**	**18.60**	**19.60**	**20.87**	

LSD (0.05) compared N treatments and season of N application within year. Means followed by the same letter are not significantly different (P > 0.05).

During Year 2 (Table 4.7) CP content was affected by both N rate and season of N application ($P < 0.0001$). The CP content increased as the N rate increased, with the highest CP content (22.89 %) associated with the application of 60 and 80 kg N ha^{-1}. The highest CP content (26.77 and 24.24 %) was recorded during late autumn and winter respectively.

It has been shown that perennial ryegrass is able to respond to N fertiliser at temperatures as low as 6 ˚C (Labuschagne and Agenbag 2008), although DM production in winter is typically low which could account for the high CP content. Eckard (1990) indicated that CP content above 20 to 22% could have adverse health effects on grazing livestock. This author also reported no yield benefit in applying fertiliser N when CP levels were between 20 and 22% and that doing so would be considered wasteful fertiliser use.

During both years of this study, pasture CP content exceeded the abovementioned threshold during the autumn - winter period at N rates between 40 and 80 kg ha^{-1}. During Year 2, spring CP also exceeded this value. It is therefore doubtful whether the application of N levels higher than 40 kg ha^{-1} is justifiable during the cooler seasons.

Table 4.7: The effect of varying rates of fertiliser N on the crude protein (%) content of a mixed grass pasture at Elsenburg Research Farm during Year 2

	Year 2 Nitrogen (kg ha^{-1})					
Season	**0**	**20**	**40**	**60**	**80**	**Mean (S)**
Spring	17.98	19.56	21.96	24.21	23.92	**21.56 ^{c}**
Early summer	11.87	12.50	13.97	15.75	15.10	**13.84 ^{e}**
Late summer	14.47	16.66	17.16	20.13	18.61	**17.44 ^{d}**
Mid-autumn	19.07	20.32	21.79	23.89	24.54	**22.25 ^{c}**
Late autumn	22.08	23.36	27.73	29.07	28.80	**26.77 ^{a}**
Winter	20.51	23.45	24.15	26.52	26.39	**24.24 ^{b}**
Mean (N)	**16.98 ^{d}**	**19.22 ^{c}**	**21.13 ^{b}**	**23.23 ^{a}**	**22.89 ^{a}**	

LSD (0.05) compared N treatments and season of N application within year. Means followed by the same letter are not significantly different ($P > 0.05$).

4.3.3.3 In Vitro Organic Matter Digestibility (IVOMD)

During Year 1 there was a significant interaction ($P < 0.05$) between season of N application and N fertiliser application rate on IVOMD (Table 4.8). The interaction recorded was the result of i) a sharp increase in IVOMD with application of 20 N during mid-autumn (but no further response as N was increased) and ii) a sharp decrease in IVOMD as N was increased from 0 to 20 and 40 during early autumn. Interactions were not observed in the other seasons included in the study.

Table 4.8: The effect of varying rates of fertiliser N on the IVOMD (%) of a pure grass pasture at Elsenburg Research Farm during Year 1

	Year 1 Nitrogen (kg ha^{-1})					
Season	**0**	**20**	**40**	**60**	**80**	**Mean (S)**
Spring	85.08 ^{ab}	83.22 ^{abcd}	83.06 ^{abcd}	82.57 ^{abcde}	82.99 ^{abcd}	**83.39**
Early summer	73.5 ^{gf}	75.27 ^{def}	74.94 ^{efg}	79.89 ^{abcdef}	74.21 ^{gf}	**75.56**
Early autumn	83.23 ^{abcd}	78.18 ^{cdef}	73.38 ^{gf}	75.78 ^{cdef}	76.34 ^{cdef}	**77.38**
Mid-autumn	67.13 ^{g}	84.99 ^{ab}	85.75 ^{ab}	85.56 ^{ab}	86.56 ^{a}	**83.29**
Winter	83.63 ^{abc}	86.37 ^{a}	85.14 ^{ab}	86.68 ^{a}	86.98 ^{a}	**85.85**
Mean (N)	**79.32**	**81.61**	**80.46**	**82.10**	**81.41**	

LSD (0.05) compared N treatments and season of N application within year. Means followed by the same letter are not significantly different (P > 0.05).

During Year 2 IVOMD was only influenced by season of N application (P < 0.0001) and not by fertiliser N rate (Table 4.9), with the highest IVOMD recorded during winter (90.62 %). Digestibility tends to decrease as plants reach maturity (McDonald et al. 2002) which explains why autumn – winter IVOMD was generally higher than that recorded in summer and to a lesser extent in spring. Perennial ryegrass is known to have relatively low digestibility in summer (Smith et al. 1998).

During both years IVOMD was >70 % (except for 67.13 % in mid-autumn in Year 1) and therefore it can be assumed that the rate of particle breakdown in the rumen will be rapid with consequent high intakes (Meissner et al. 2000).

Table 4.9: The effect of varying rates of fertiliser N on the IVOMD (%) of a mixed grass pasture at Elsenburg Research Farm during Year 2

Year 2

Nitrogen (kg ha^{-1})

Season	0	20	40	60	80	Mean (S)
Spring	81.34	81.06	82.29	81.67	81.66	**81.60** [bc]
Early summer	75.81	77.32	76.40	62.60	75.75	**73 58** [d]
Late summer	75.63	79.38	80.03	80.64	79.14	**78.95** [c]
Mid-autumn	80.20	81.50	82.73	84.94	83.62	**82.75** [b]
Late autumn	82.48	81.35	87.16	87.15	85.79	**84 99** [b]
Winter	88.96	90.30	90.93	91.06	91.77	**90.62** [a]
Mean (N)	**80.63**	**81.60**	**83.26**	**81.33**	**82.95**	

LSD (0.05) compared N treatments and season of N application within year. Means followed by the same letter are not significantly different (P > 0.05).

4.3.3.4 Herbage N yield:

During both years herbage N yield (kg N ha^{-1}) was affected by season of N application and N fertilisation application rate ($P < 0.0001$) (Table 4.10). Herbage N yield increased as the rate of N increased, with the highest N yield of 41.49 kg N ha^{-1} and 33.07 kg N ha^{-1} during year 1 and 2 respectively produced with the application of 80 kg N ha^{-1}.

The highest herbage N yield was associated with the warmer months of spring and early summer, while the lowest was achieved during autumn and winter in both years. This is most likely due to the improved root activity and N uptake at warmer temperatures (Labuschagne 2005).

Table 4.10: The effect of season of N application season of N application of N application and varying rates of fertiliser N on the herbage N yield (kg N ha^{-1}) of a mixed grass pasture at Elsenburg during Year 1 and 2

Year 1

			Nitrogen (kg ha^{-1})			
Season	**0**	**20**	**40**	**60**	**80**	**Mean (S)**
Spring	17.50	19.48	30.31	40.99	40.08	**30.18** [b]
Early summer	19.66	34.83	37.54	59.61	62.29	**42.79** [a]
Early autumn	13.76	14.44	29.19	40.23	50.94	**29.71** [bc]
Mid-autumn	3.44	7.33	20.32	32.70	37.99	**21.81** [c]
Winter	1.67	4.85	9.45	12.33	16.13	**9.51** [d]

Mean (N)	**12.63 [c]**	**16.19 [c]**	**25.36 [b]**	**37.15 [a]**	**41.49 [a]**	
			Year 2			
Spring	22.88	39.12	48.59	59.45	72.79	**49.21 [a]**
Early summer	13.76	14.22	36.24	45.74	48.80	**31.75 [b]**
Late summer	2.17	5.30	6.34	16.73	18.16	**9.93 [d]**
Mid-autumn	2.25	12.60	14.52	19.85	28.01	**16.78 [c]**
Late autumn	2.84	4.83	6.98	13.34	15.99	**9.63 [d]**
Winter	3.28	7.59	6.34	9.39	14.70	**8.20 [d]**
Mean (N)	**8.29 [d]**	**14.55 [c]**	**19.82 [c]**	**26.31 [b]**	**33.07 [a]**	

LSD (0.05) compared N treatments and season of N application within year. Means followed by the same letter are not significantly different (P > 0.05).

4.4 Conclusion

Pasture response to N fertilisation and season of N application was characterised by a strong interaction over both years. Highest pasture productivity (in terms of PDMP, RDMP and TDMP) was achieved with the application of high N rates (60 – 80 kg N ha^{-1}) during spring and summer, while autumn and winter displayed a characteristic decrease in yield and a lower responsiveness to N application.

There was a significant increase in RDMP during the first year as the rate of N was increased and highest RDMP was recorded during winter in both years. This proved that N application rate exceeded the N absorption capacity of the pasture during the first regrowth cycle. This situation is not recommendable as the excess N will be prone to leaching and subsequent contamination of natural resources.

The species contribution was determined by season of N application and cocksfoot was dominant during the warmer months, while perennial ryegrass + tall fescue dominated during the cooler months.

Nitrogen application rate and season of N application had a significant effect on the nutritive characteristics measured in this study, which in some instances exceeded livestock requirements. During both years, autumn - winter CP levels exceeded the upper limit of 20 – 22 % CP when N rates between 40 and 80 kg ha^{-1} were applied. It is therefore doubtful whether the application of N rates of 40 kg ha^{-1} and higher is justifiable during the cooler seasons, especially when the high winter RDMP is taken into consideration.

The IVOMD remained high (>70 %) irrespective of season of N application and N application rate, although spring and summer IVOMD tended to be lower than autumn and winter over both years, which would indicate that high intakes will be achieved during all seasons, even though there was a small decrease in DM content as the rate of N increased.

Based on the findings presented here, preliminary recommendations for the application of N to pure grass pastures in the Winelands sub-region would be 40 kg N ha^{-1} during autumn, winter and spring and 60 kg N ha^{-1} during summer when growth rates are highest. Application of these N rates will also ensure fodder of high quality to be produced during the different seasons.

4.5 References

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CHAPTER 5

The effect of strategic nitrogen fertiliser application on the yield, botanical composition and selected nutritive characteristics of a mixed grass-clover pasture in the Winelands sub-region of the Western Cape

5.1 Introduction

Grass-legume pastures have traditionally formed the backbone of livestock production systems in the Western Cape (van Heerden and Tainton 1989). Legume based pastures have the potential of supplying high quality grazing for livestock (Peoples and Baldock 2001), with the additional advantage of reducing the requirement for nitrogen (N) based fertilisers, due to their ability to fix atmospheric N (Wu and McGechan 1999). This can lead to considerable savings in the amount of N fertiliser required, as well as a reduction of the negative environmental effects associated with high nitrogen fertiliser use (Hardarson 1993).

One of the main constraints of legume-based pastures is their low winter production, also referred to as the winter fodder gap (Labuschagne and Agenbag 2006). One of the ways to address this issue is by the strategic application of fertiliser N. This entails the application of small amounts of fertiliser N during the cooler months of the year, when the legume component is essentially dormant, but the accompanying grass is still reactive to N (Eckard and Franks 1998; McKenzie et al. 1999; Labuschagne et al. 2006a). Therefore the majority of the mineral N available during the summer months can be ascribed to the presence of the legume component, while winter production is more dependent on fertiliser N.

Research results regarding fertilisation norms for pastures in the Winelands sub-region of the Western Cape Province in South Africa are not freely available as research was mainly focused on the dairying regions in the southern Cape region. The aims of this study were therefore i) to develop N fertiliser application strategies to ensure optimum dry matter (DM) production of pastures during different seasons, ii) to determine the effect of fertiliser N on the botanical composition of these pastures and iii) to determine the effect of fertiliser N on selected nutritive characteristics of the fodder produced.

5.2 Materials and methods

A comprehensive overview of the site characteristics and general procedures and management is provided in Chapter 3. Specific details for the grass-clover pasture are given below.

A grass-clover pasture consisting of white clover (*Trifolium repens*), red clover (*T. pratense*), perennial ryegrass (*Lolium perenne*), cocksfoot (*Dactylis glomerata*) and tall fescue (*Festuca arundinaceae*) was established in April 2010. Data collection commenced in April 2011. Nitrogen treatments of 0, 20, 40 and 60 kg ha^{-1} where applied at the beginning of each regrowth cycle as LAN (28% N) during the autumn-spring period, after the pasture was mob-grazed by sheep to a height of approximately 30 mm. Nitrogen was washed into the soil with light (10-15 mm) irrigation after application. If necessary the pasture was mowed to 30 mm to remove effects of patch grazing and the material removed from site. A fixed regrowth cycle of 35 days was applied. Sub-plot dimensions were 3.75 x 3.75 m (14.06 m^2) of which 6.6 m^2 was harvested using a sicklebar mower. Various sub-samples were collected for the determination of DM production, botanical composition and selected nutritive characteristics.

The N treatments were divided into two management strategies. One set of plots received a once-off application of N during the cool season (every 35 days but on a different set of plots), while a second set of plots received N after each regrowth cycle (every 35 days both on the same set of plots) during the cool season. Cutting cycles for the grass-legume pasture were as follows: autumn (April/May), early winter (May/June), late winter (July/August) and early spring (August/September). For the consecutive N application strategy a 5th harvest of mid-spring (September - October) was included.

Experimental design and treatments

Experimental design was a completely randomised design with six replicates. The treatment design was a factorial design with two factors, seasons (autumn, early winter, late winter and early spring) and nitrogen application rates. Year was added as a sub-plot factor because measurements were taken repeatedly (once annually) on the same plot. This statistical approach is consistent with procedures described by Little and Hills (1972).

5.3 Results and discussion

5.3.1 Dry matter production

5.3.1.1 Once-off N application strategy

i) Primary dry matter production (PDMP)

The effect of fertiliser N and season of N application on PDMP for the first and second year of the study are summarised in Table 5.1.

Table 5.1: The effect of season of N application and once-off fertiliser application rate (kg N ha^{-1}) on the primary dry matter production (PDMP) (kg DM ha^{-1} day^{-1}) of a grass-clover pasture during Year 1 and 2 at Elsenburg Research Farm

		Year 1			
		Nitrogen (kg ha^{-1})			
Season	**0**	**20**	**40**	**60**	**Mean (S)**
Autumn	28.17	31.78	37.99	39.68	**34.40** ^{b**}
Early winter	8.57	12.9	13.47	14.41	**12.34** ^{d}
Late winter	20.24	20.53	20.7	20.39	**20.40** ^{c}
Early spring	35.86	36.45	38.24	41.36	**37.98** ^{a}
Mean (N)	**23.21** ^{c}	**25.35** ^{bc}	**27.60** ^{ab}	**28.96** ^{a}	
		Year 2			
Autumn	7.58	9.71	10.8	14.26	**10.59** ^{bc}
Early winter	5.14	5.48	7.03	4.81	**6.66** ^{c}
Late winter	12.04	18.17	17.26	16.86	**16.08** ^{b}
Early spring	39.31	45.69	42.83	58.88	**46.68** ^{a}
Mean (N)	**16.02**	**19.76**	**19.48**	**24.76**	

**Means (in bold) in the same column or row followed by the same superscripted letter are not significantly different at P = 0.05

During the first year both season of application and N application rate influenced ($P < 0.001$) PDMP. An increase in PDMP as the N rate increased was noted, with the maximum mean PDMP of 28.96 kg ha day^{-1} being produced in response to application of 60 kg N ha^{-1}.

The highest PDMP was produced in early spring (37.98 kg DM ha day^{-1}) and the lowest in early winter (12.34 kg DM ha day^{-1}). There was a drastic decrease in productivity from autumn to early winter, even at high N rates. This is similar to results obtained by Labuschagne et al. (2006a) and highlights the important influence that temperature has on pasture productivity and responsiveness to fertiliser N.

During Year 2, differences in PDMP could mainly be attributed to the effect of season of N application ($P < 0.0001$) and not to fertiliser N application rate, although there was a non-significant increase in PDMP as the rate of N was increased. Highest mean PDMP was recorded during early spring (46.68 kg DM ha day^{-1}), which could be attributed to an increase in mean temperature associated with the onset of spring.

ii) Residual dry matter production (RDMP)

The effect of the once-off application of fertiliser N on the RDMP of a grass-clover pasture is summarised in Table 5.2.

During both years the RDMP was affected by season of N application ($P < 0.0001$) but not by N fertiliser application rate. The RDMP increased as the seasons progressed from autumn to early spring, with the highest mean RDMP recorded in early spring in Year 1 and early spring and late winter in Year 2. This can probably be attributed to the fact that RDMP is herbage harvested 35 days after the first cut, and therefore consists largely of material harvested well into spring when mean temperatures were higher. Alternatively this can also occur when pasture production is limited by environmental conditions, which leaves the applied N prone to leaching. This latter scenario is likely, considering the low response of PDMP to applied N.

Table 5.2: The effect of season of application and the once-off application of fertiliser N on the residual dry matter production (RDMP) (kg DM ha^{-1} day^{-1}) of a grass-clover pasture during Year 1 and Year 2 at Elsenburg Research Farm

Year 1

		Nitrogen (kg ha^{-1})			
Season	**0**	**20**	**40**	**60**	**Mean (S)**

Autumn	7.18 *	6.58	8.74	11.29	**8.45 [d] ****
Early winter	16.85	16.4	15.26	19.13	**16.91 [c]**
Late winter	29.14	30.93	28.12	29.21	**29.35 [b]**
Early spring	35.06	34.97	32.98	36.75	**34.94 [a]**
Mean (N)	**22.06**	**22.22**	**21.27**	**24.1**	
			Year 2		
Autumn	1.26	1.83	2.56	2.57	**2.05 [b]**
Early winter	NA	NA	NA	NA	NA
Late winter	34.73	39.25	37.87	37.44	**37.32 [a]**
Early spring	38.59	47.67	40.41	49.80	**44.12 [a]**
Mean (N)	**29.58**	**35.13**	**31.82**	**35.41**	

NA: Data not available

*Means followed by the same letter are not significantly different at P = 0.05

**Means (bold) in the same column or row followed by the same letter are not significantly different at P = 0.05

iii) Total dry matter production (TDM)

The effect of season of N application and the once-off application of fertiliser N on TDMP of a grass-clover pasture is shown in Table 5.3.

During both years, TDMP was primarily affected by season of N application and not by N application rate ($P < 0.0001$). Mean TDMP was highest during early spring in both years (36.46 kg ha day^{-1} and 45.40 kg ha day^{-1} in Year 1 and 2 respectively). During both years, lowest productivity was recorded during autumn (Year 1) and early winter (Year 2). This highlights the importance of temperature on the productivity of grass-clover pastures.

Table 5.3: The effect of season of application and the once-off application of fertiliser N on the total dry matter production (TDM) (kg DM ha^{-1} day^{-1}) of a grass-clover pasture during Year 1 and 2 at Elsenburg Research Farm

			Year 1		
			Nitrogen (kg ha^{-1})		
Season	**0**	**20**	**40**	**60**	**Mean (S)**
Autumn	17.68 *	19.18	23.36	25.48	**21.42 [c] ****
Early winter	12.71	14.65	14.37	16.77	**14.63 [d]**

Late winter	24.7	25.59	24.41	24.8	**24.87** [b]
Early spring	35.46	35.71	35.61	39.06	**36.46** [a]
Mean (N)	**22.64**	**23.78**	**24.44**	**26.53**	
			Year 2		
Autumn	4.1	5.31	6.04	7.77	**5.81** [c]
Early winter	2.57	2.74	3.51	4.5	**3.30** [c]
Late winter	23.29	28.71	27.56 c	27.15	**26.70** [b]
Early spring	38.95	46.68	41.62	54.34	**45.40** [a]
Mean (N)	**17.25**	**20.86**	**19.68**	**23.44**	

**Means (bold) in the same column or row followed by the same letter are not significantly different at P = 0.05

5.3.1.2 Consecutive N application strategy during the cool seasons

i) Primary dry matter production (PDMP)

Results for the consecutive application of N is summarised in Table 5.4.

During Year 1, season of N application had a significant (P < 0.0001) effect on PDMP. Highest mean PDMP were recorded during autumn, early and mid-spring (30.77 kg DM ha^{-1}, 48.89 kg DM ha day-1 and 39.94 kg DM ha day^{-1} respectively).

Table 5.4: The effect of season of N application and the consecutive application of fertiliser N (kg N ha^{-1}) on the primary dry matter production (PDMP) (kg DM ha^{-1} day^{-1}) of a grass-clover pasture during Year 1 and 2 at Elsenburg Research Farm

			Year 1		
			Nitrogen (kg ha^{-1})		
Season	**0**	**20**	**40**	**60**	**Mean (S)**
Autumn	25.7 *	33.27	31.77	32.34	**30.77** [ab] **
Early winter	7.06	9.29	10.33	10.28	**9.24** [c]
Late winter	16.97	16.6	20.94	23.81	**19.58** [bc]
Early spring	32.23	31.26	34.56	40.64	**48.89** [a]
Mid-spring	33.65	38.23	42.79	45 08	**39.94** [a]
Mean (N)	**23.12**	**25.73**	**39.45**	**30.43**	
			Year 2		
Autumn	4.96	7.63	14.15	13.51	**10.06** [bc]
Early winter	1.55	2.53	4.71	5.71	**3.54** [c]

Late winter	11.72	10.53	14.92	16.88	**13.51** [b]
Early spring	34.16	42.73	51.74	53.41	**45.51** [a]
Mean (N)	**13.10** [b]	**15.85** [ab]	**21.80** [a]	**22.66** [a]	

**Means (bold) in the same column or row followed by the same letter are not significantly different at P = 0.05

During Year 2 both fertiliser N application rate (P < 0.05) and season of application (P < 0.0001) significantly influenced PDMP. Mean PDMP increased as N application rate was increased, with the highest PDM produced with the application of 20, 40 and 60 kg N ha^{-1}. The highest mean PDMP occurred during early spring (45.51 kg DM ha day^{-1}) which can again be attributed to the effect of increasing temperatures associated with the onset of spring.

5.3.2 Botanical Composition

5.3.2.1 Once-off N application strategy

The effect of fertiliser N application rate and season of application on the botanical composition of a mixed grass-clover pasture is illustrated in Figures 5.1 and 5.2 as well as Table 5.5a.

During the first harvest in the autumn of Year 1 (Figure 5.1) the pasture consisted predominantly of clover (approximately 75%). The application of fertiliser N had a significant (P < 0.0001) effect on the relative contribution of both grass and clover fractions in the autumn – spring period (Figure 5.1). Maximum grass content was associated with the application of high N rates (40 - 60 kg N ha^{-1}), while the opposite was true for clover, i.e. maximum clover content occurred at low N rates (0 - 20 kg N ha^{-1}) and decreased as the rate of N increased. Similar results are reported by Labuschagne et al. (2006b). The pasture remained clover dominant throughout the first year of the study, with the lowest clover content of 58.61 % occurring in early winter with the application of 60 kg N ha^{-1}. This is still well above the recommended minimum of 20 - 40% reported by Frame (1992) for optimum N fixation and fodder quality in spring.

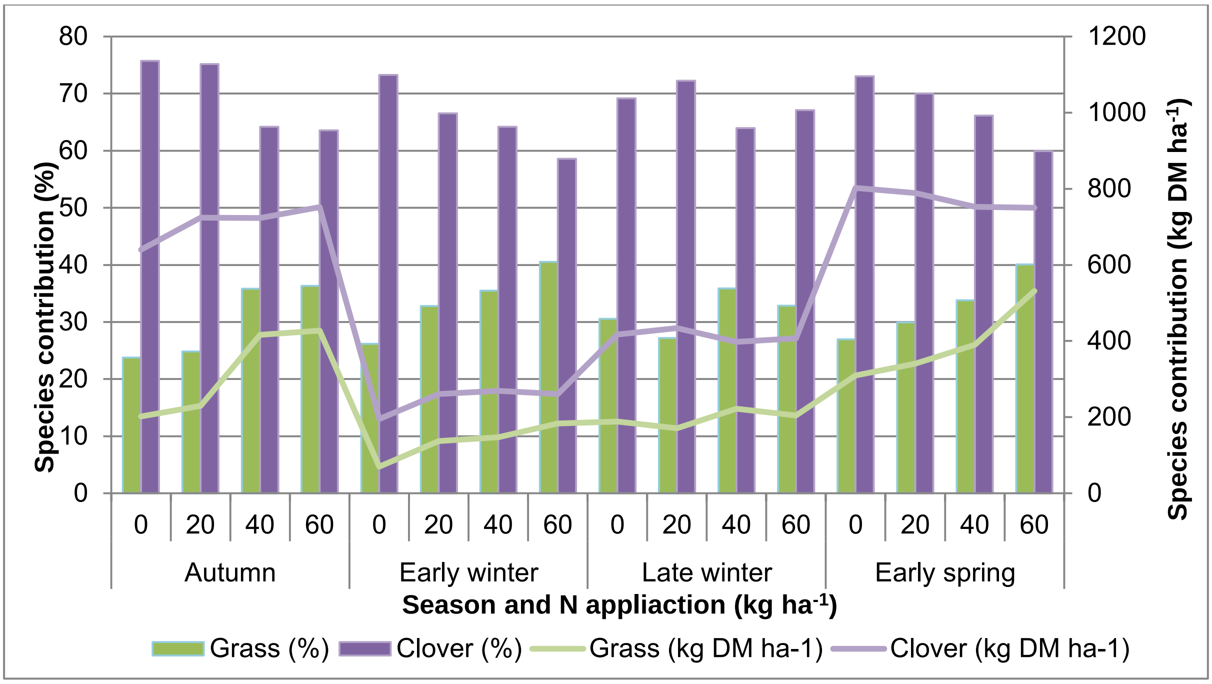

Figure 5.1: The effect of season of application and the once-off application of fertiliser N on the botanical composition of a grass-clover pasture during Year 1 at Elsenburg Research Farm

During Year 2 (Figure 5.2) of the study, the pasture composition was more in balance compared to Year 1 with a clover content of approximately 58% during autumn when N treatments resumed for Year 2. Contrary to Year 1, season of application had a significant effect ($P < 0.0001$) on both the grass and clover fractions. Highest grass content where recorded during early winter (59.28%) and early spring (59.81%), while the highest clover content occurred during autumn (48.64%) and late winter (53.32%) (Table 5.5 a). A drastic reduction in grass content from early-winter to late winter was observed, with the grass content reaching its lowest level of 41.89% during this period. The high clover and low grass content during late winter was contrary to what was expected, as perennial ryegrass is known to be more cold - tolerant (Frame 1992) than legumes which is one of the reasons for its inclusion in pasture mixtures.

Unlike Year 1, the pasture was grass dominant during early-winter and early spring, which coincided with the lowest clover content of 33.74% and 39.80% respectively. The minimum clover content remained at acceptable levels (≥ 30 %) for high animal production and N fixation (Frame 1992, Botha 2002).

Fertiliser application rate influenced (P < 0.0001) grass content. There was an increase in grass content with application of N, the highest grass percentage of 58.19% recorded with the application 60 kg N ha^{-1}, but similar percentages being obtained with the application of 20 and 40 kg N ha^{-1}. Clover content was not influenced (P < 0.0825) by N fertiliser application rate in the second year.

The actual clover content (kg DM ha^{-1}) was less influenced by N fertilisation compared to clover percentage in the fodder on offer, especially in autumn and early winter. A similar trend was reported by Labuschagne et al. (2006b). The apparent reduction in clover content (%) is thus likely due to the stimulating effect of N on the accompanying grass component and not due to the direct negative effect of N on legume growth (Frame and Newbould 1986, Ledgard et al. 1996). This is supported by McKenzie et al. (2002) who found that N fertilisation increased ryegrass tiller densities in a perennial ryegrass-white clover pasture, although the effect was modified by grazing management.

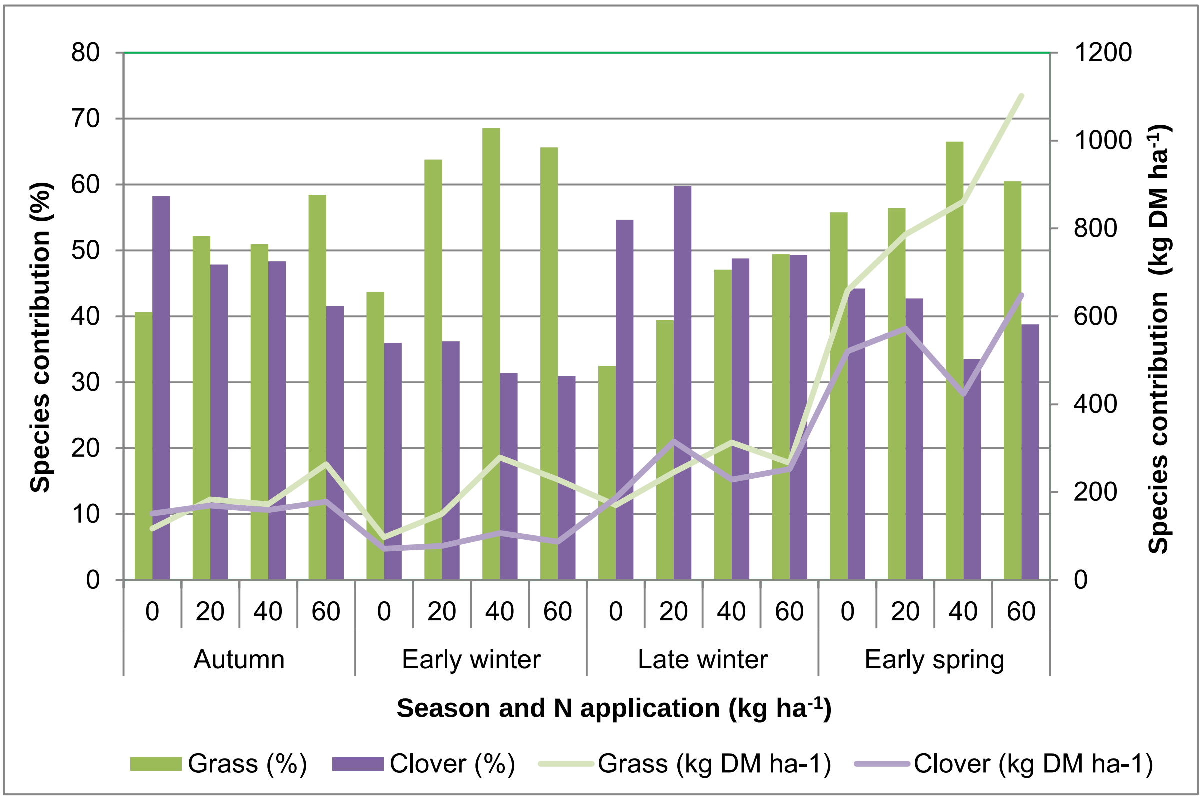

Figure 5.2: The effect of season of application and the once-off application of fertiliser N on the botanical composition of a grass-clover pasture during Year 2 at Elsenburg Research Farm

Table 5.5a: The effect of season of application and the once-off application of fertiliser N on the botanical composition of a grass-clover pasture during Year 1 and 2 at Elsenburg Research Farm

	Year 1									
	% Contribution									
N (kg ha^{-1})	0		20		40		60		Mean (S)	
Season	***Grass***	***Clover***	***Grass***	***Clover***	***Grass***	***Clover***	***Grass***	***Clover***	***Grass***	***Clover***
Autumn	23.77	75.73	24.81	75.19	35.83	64.17	36.32	63.57	**30.18 ****	**69.67**
Early winter	26.18	73.26	32.77	66.52	35.47	64.19	40.54	58.61	**33.74**	**65.65**
Late winter	30.54	69.18	27.17	72.29	35.86	63.95	32.86	67.1	**31.61**	**68.13**
Early spring	26.97	73.03	29.96	70.04	33.82	66.18	40.06	59.94	**32.65**	**67.35**
Mean (N)	**26.86 b**	**72.80 a**	**28.68 b**	**71.01 a**	**35.31 a**	**64.56 b**	**37.44 a**	**62.31 b**		
	Year 2									
Autumn	40.68	58.27	52.16	47.84	50.99	48.36	58.46	41.55	**50.95 b**	**48.64 a**
Early winter	43.73	35.97	63.78	36.22	68.61	31.39	65.63	30.93	**59.28 a**	**33.74 b**
Late winter	32.49	54.64	39.42	59.75	47.08	48.8	49.44	49.34	**41.89 c**	**53.32 a**
Early spring	55.79	44.21	56 47	42.72	66.52	33.48	60.47	38.77	**59.81 a**	**39.80 b**
Mean (N)	**43.26 b**	**48.38**	**51.97 a**	**47.57**	**57.31 a**	**41.46**	**58.19 a**	**40.55**		

******Means (bold) in the same column or row followed by the same letter are not significantly different at P = 0.05

5.3.2.2 Consecutive N application strategy

Results for the consecutive application strategy of fertiliser N are illustrated in Figures 5.3 and 5.4 as well as Table 5.5 b. Similar trends as was recorded for once-off N application (Figures 5.1 and 5.2) were observed for the consecutive treatments.

During Year 1 (Figure 5.3), both season of application ($P < 0.0001$) and N application rate ($P < 0.0001$) had a significant influence on the species composition of the pasture (Table 5.5 b). The grass content (%) increased with the application of high N rates (40 - 60 kg N ha^{-1}), while the clover content increased (%) with low N rates (0 - 20 kg N ha^{-1}). The pasture was clover dominant (67.88%) at the start of harvesting during autumn, but the contribution steadily decreased through winter and reached a minimum level of 38.50% during mid-spring. In contrast the grass fraction started off at the minimum of 31.25% in autumn and steadily increased through winter, reaching maximum levels of 61.50% in mid-spring. The stimulating effect of fertiliser N on the grass component is well-known (McKenzie et al. 2002, Labuschagne et al. 2006b) During mid-spring there was also a change in composition, from clover-dominance to grass-dominance, although the clover content remained above the recommended minimum of approximately 30% throughout the harvesting period.

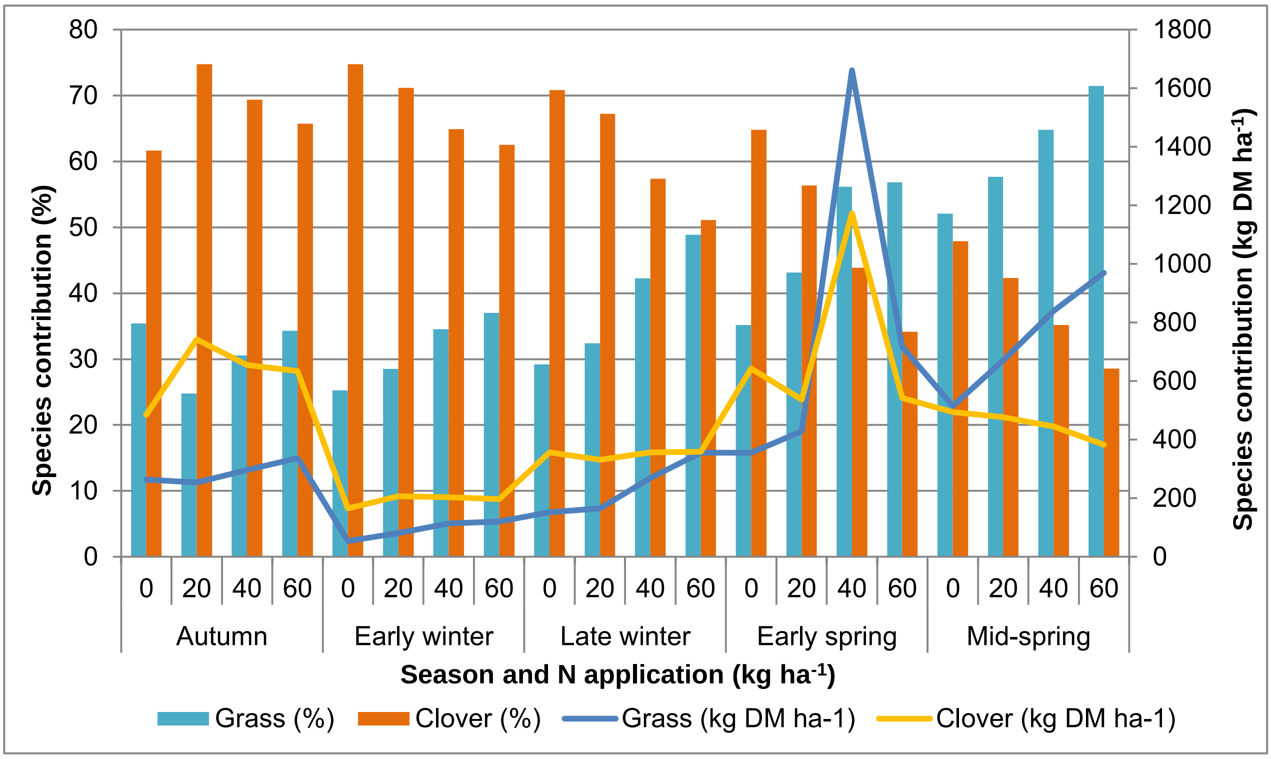

Figure 5.3: The effect of season of application and the consecutive application of fertiliser N on the botanical composition of a grass-clover pasture during Year 1 at Elsenburg Research Farm

Results recorded in Year 2 (Figure 5.4) are similar to those for Year 1. Both N application rate ($P < 0.0001$) and season of application ($P < 0.0001$) had an effect on clover and grass fractions (Table 5.5 b). Grass content increased with increasing rates of N application, while clover content tended to increase as N fertilisation rate was decreased from 60 to 0 kg N ha^{-1}. Unlike year one, the pasture was grass dominant for most of the autumn-spring period during Year 2. This decrease in clover content from Year 1 to 2 can be a result of the N cycled as urea from grazing animals (Nevens and Rehuel 2003). Highest mean grass content was recorded in early winter (66.07%) and early spring (70.79%), while highest mean clover content was recorded during autumn (45.60%) and late winter (47.31%).

Early spring coincided with a drastic increase in grass productivity (kg DM ha^{-1}), while clover productivity remained static. This leads to increased competition from the grass components and poor biological N fixation rates in the summer months (Frame and Newbould 1986, Ledgard et al. 1996).

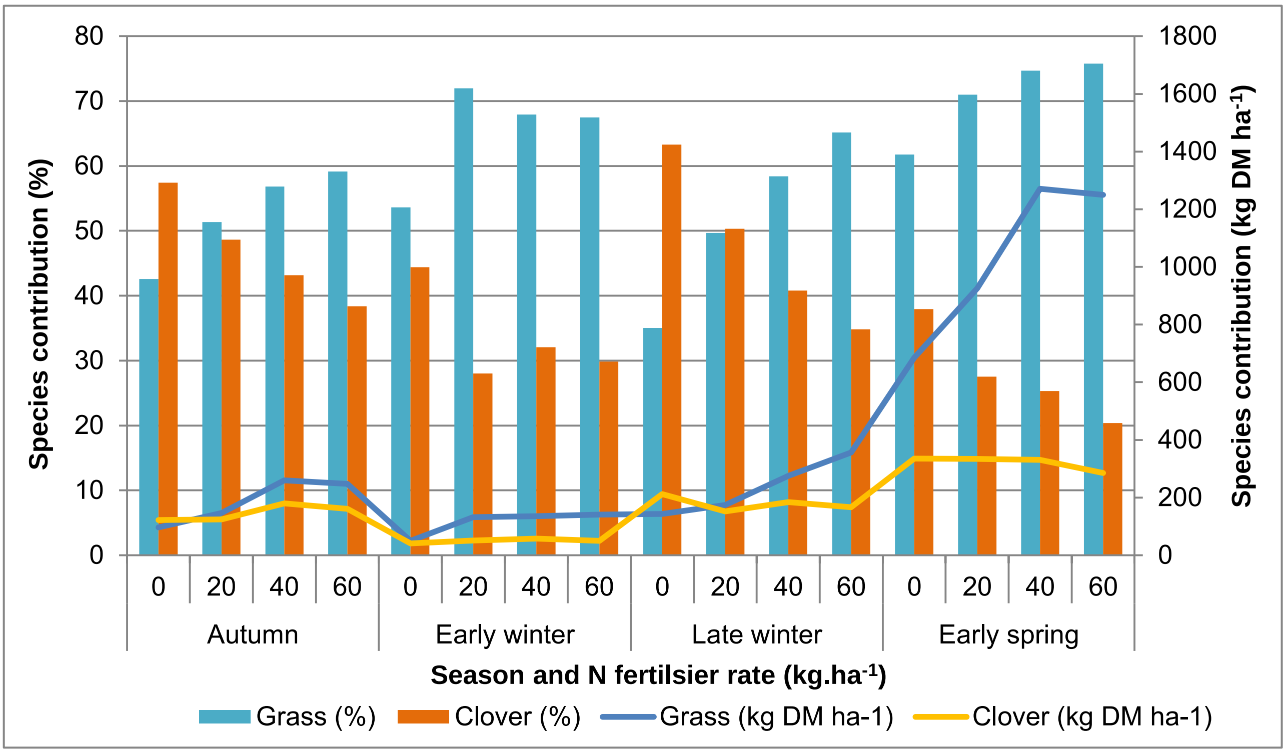

Figure 5.4: The effect of season of application and the consecutive application of fertiliser N on the botanical composition of a grass-clover pasture during Year 2 at Elsenburg Research Farm

Table 5.5b: The effect of season of application and the consecutive application of fertiliser N on the botanical composition of a grass-clover pasture during Year 1 and 2at Elsenburg Research Farm

Year 1 % Contribution										
N (kg ha^{-1})	**0**		**20**		**40**		**60**		**Mean (S)**	
Season	***Grass***	***Clover***	***Grass***	***Clover***	***Grass***	***Clover***	***Grass***	***Clover***	***Grass***	***Clover***
Autumn	35.42	61.65	24.78	74.78	30.54	69.37	34.28	65.72	**31.25 [d] ****	**67.88 [a]**
Early winter	25.25	74.75	28.53	71.19	34.56	64.9	37.02	62.53	**31.34 [d]**	**68.34 [a]**
Late winter	29.19	70.81	32.43	67.26	42.26	57.37	48.88	51.12	**38.19 [c]**	**61.64 [b]**
Early winter	35.18	64.82	43.14	56.37	56.14	43.87	56.85	34.14	**47.83 [b]**	**52.05 [c]**
Mid-spring	52.1	47.9	57.68	42.32	64.8	35.2	71.44	28.56	***61.50 [a]***	**38.50 [d]**
Mean (N)	**35.43 [b]**	**63.99 [a]**	**37.31 [b]**	**62.38 [a]**	**45.66 [a]**	**54.14 [b]**	**49.7 [a]**	**50.21 [b]**		
Year 2										
Autumn	42.58	57.42	51.37	48.63	56.84	43.17	59.16	38.4	**53.76 [b]**	**45.60 [a]**
Early winter	53.6	44.42	71.99	28.02	67.92	32.09	67.51	29.86	**66.07 [a]**	**32.72 [b]**
Late winter	35.02	63.31	49.66	50.34	58.41	40.78	65.18	34.82	**52.07 [b]**	**47.31 [a]**
Early spring	61.74	37.94	70.97	27.53	74.69	25.31	75.77	20.37	**70.79 [a]**	**27.79 [d]**
Mean (N)	**47.97 [c]**	**51.10 [a]**	**59.19 [b]**	**40.34 [b]**	**64.14 [ab]**	**35.63 [bc]**	**67.22 [a]**	**30.60 [c]**		

******Means (bold) in the same column or row followed by the same letter are not significantly different at P = 0.05

5.3.3 Nutritive Characteristics

5.3.3.1 Pasture Dry Matter (DM) content

During Year 1, both fertiliser N (P < 0.0024) and season of N application (P < 0.0001) affected pasture DM content (Table 5.6). Mean DM content decreased as the rate of N increased from 0 to 60 kg N ha^{-1}. It is well known that the application of fertiliser N can lead to a decrease in pasture DM content (Noller and Rhykerd 1974, Wilman 1975; Peyraud et al. 1997, Labuschagne et al. 2006c). The highest DM was recorded in early winter (16.45%).

During Year 2, DM content was only affected by season of application (P < 0.0001), where the highest mean DM was recorded in early spring (20.02%), and the lowest in early winter (16.96%) and late winter (17.64%). Expected DM content for grass-clover pastures falls within the range of 17 – 25% DM, depending on the season and the relative contribution of the grass and clover fractions (Meissner et al. 2000). The values reported in this study fall within this range (although not exceeding 20% DM) and are similar to that reported by Labuschagne et al (2006c).

Table 5.6: The effect of season of N application and the once-off application of fertiliser N (kg N ha^{-1}) on the dry matter content (%) of a grass-clover pasture at Elsenburg Research Farm during Year 1 and 2

		Year 1			
		Nitrogen (kg ha^{-1})			
Season	**0**	**20**	**40**	**60**	**Mean (S)**
Autumn	15.63	15.17	15.34	15.3	**15.36 ^{b} ****
Early winter	16.93	16.47	16.38	16.06	**16.45 ^{a}**
Late winter	15.7	15.73	15.37	14.81	**15.40 ^{b}**
Early spring	15.95	15.11	14.69	14.28	**15.00 ^{b}**
Mean (N)	**16.05 ^{a}**	**15.62 ^{ab}**	**15.44 ^{bc}**	**15.10 ^{c}**	
		Year 2			
Autumn	19.65	18.71	18.26	17.56	**18.55 ^{b}**
Early winter	17.59	17.55	16.29	16.94	**16.96 ^{c}**
Late winter	17.28	17.33	17.9	17.53	**17.64 ^{bc}**
Early spring	20.51	19.91	20.23	19.44	**20.02 ^{a}**
Mean (N)	**18.76**	**18.38**	**18.17**	**17.87**	

**Means (bold) in the same column or row followed by the same letter are not significantly different at P = 0.05

5.3.3.2 Crude Protein (CP)

The CP content of the respective pasture species are shown in Table 5.7.

Pasture CP was adequate for high animal production as all fractions had CP contents >20% over all seasons and N levels, which meets the requirements of even high producing animals (NRC 2001).

i) Grass CP content

During Year 1, grass CP was affected by both fertiliser N rate ($P < 0.0001$) and the season of application ($P < 0.0001$). Crude protein content increased as N rates were increased. The highest mean annual CP content (30.79 and 31.70%) was recorded with the application of 40 and 60 kg N ha^{-1} respectively (Table 5.7). Similar trends were reported by Labuschagne et al. (2006c) and Eckard (1994). Highest mean seasonal grass CP contents (31.24 and 31.14%) were recorded during early- and late winter.

Results for Year 2 were similar to year 1, with the highest mean annual CP levels recorded with applications of 40 and 60 kg N ha^{-1}.The CP content during Year 2 was slightly lower than recorded during Year 1. The highest seasonal mean CP content was found during autumn and early winter and the lowest during early spring. Mean seasonal CP averages for temperate grasses range from 21% to 31% (depending on the season), but can be as high as 35% (Fulkerson et al. 2007). The CP values recorded in this study were within this range.

i) Clover CP content

In Year 1 the clover CP content ranged between 31.84 and 36.79 % over all treatment combinations (Table 5.7). This is higher than that noted by Labuschagne et al. (2006c) in a similar study, as well as averages listed by Fulkerson et al (2007). Mean clover CP content was influenced by season of N application ($P < 0.0001$) and not by N application rate during Year 1, although a non-significant increase in CP in response to N application rate was observed. This is in contrast to Labuschagne et al. (2006c) who found a significant decrease in clover CP content as the rate of fertiliser N was increased from 0 – 150 kg N ha^{-1}, and Eckard (1994) who reported a linear increase in clover N % with increasing rates of N fertiliser in a grass-clover pasture. The low response of clover CP to applied N can be attributed to the effect of cold temperatures on the uptake of N in legumes (Labuschagne 2005). During Year 1, the highest CP was recorded in early- and late winter.

In Year 2, N application caused a significant increase in mean clover CP content ($P<0.05$). The highest CP content occurred with the application of 60 kg N ha^{-1}. Season of application also had an effect on mean CP content, with the highest CP content recorded in autumn and early winter.

Table 5.7: The effect of season of N application and the once-off application of fertiliser N (kg N ha^{-1}) on the crude protein content (%) of a grass-clover pasture at Elsenburg during Year 1 and 2

Year 1
CP (%)

N (kg ha^{-1})	**0**		**20**		**40**		**60**		**Mean (S)**	
Season	**Grass**	**Clover**	**Grass**	**Clover**	**Grass**	**Clover**	**Grass**	**Clover**	**Grass**	**Clover**
Autumn	29.14	36.67	28.86	32.64	29.13	31.84	31.51	33.06	**29.67 [b]**	**33.55 [b]**
Early winter	29.24	35.97	31.36	36.07	32.53	36.37	31.84	36.79	**31.24 [a]**	**36.30 [a]**
Late winter	28.89	35.18	30.19	34.56	32.17	35.27	33.32	35.97	**31.14 [a]**	**35.11 [a]**
Early spring	26.68	32.38	28.70	32.72	29.02	33.17	30.15	32.79	**28.62 [b]**	**32.75 [b]**
Mean (N)	**28.49 [c]**	**33.05**	**29.78 [b]**	**33.99**	**30.79 [ab]**	**34.20**	**31.70 [a]**	**34.51**		
Autumn	27.33	35.41	28.60	34.10	29.92	36.12	31.10	34.18	**29.35 [a]**	**34.97 [a]**
Early winter	26.19	34.16	27.04	34.42	29.36	34.71	30.12	34.91	**28.18 [ab]**	**34.55 [a]**
Late winter	26.89	30.78	27.31	31.98	26.34	33.65	28.19	31.87	**27.22 [b]**	**32.00 [b]**
Early spring	21.30	29.77	22.60	30.92	25.07	31.46	24.80	31.41	**23.29 [c]**	**30.89 [b]**
Mean (N)	**25.91 [c]**	**32.56 [b]**	**26.81 [bc]**	**32.85 [ab]**	**28.09 [ab]**	**33.07 [ab]**	**28.92 [a]**	**34.32 [a]**		

**Means (bold) in the same column or row followed by the same letter are not significantly different at P = 0.05

ii) Total CP (TCP)

The TCP content ((% grass x % CP $_{grass}$) + (% clover x % CP_{clover})) was high, in many instances > 30% (Table 5.8). Meissner et al. (2000) reported the CP range of a grass-clover pasture as between 13.5 and 18.4 % depending on the season and the clover content. Crude protein levels reported in this study are also higher than that noted by Labuschagne et al. (2006c).

The effect of treatment combinations on TCP was not consistent over years. During Year 1, TCP was only affected by season of N application ($P < 0.0001$) with the highest TCP recorded in early – and late-winter. During year two, TCP was affected by season of N application ($P < 0.0001$) and N application rate ($P < 0.0291$), and the highest mean TCP of 28.72 and 28.77% was recorded with the application of 40 and 60 kg N ha^{-1}.

Dietary energy is considered the primary limitation for high animal production, and not CP content (Buxton and Fales 1994, Poppi and McLennan 1995, Lambert and Litherland 2000). According to a study by Meissner et al. (1993) rumen NH_3 concentration (and thus N losses) increased significantly above pasture N contents of 2.5 – 3% (15.6 – 18.75 %CP), principally due to an inadequate energy supply for the synthesis of microbial protein. Similarly Eckard (1990) reports adverse health effects in sheep grazing annual ryegrass pastures with CP levels between 20 and 22 %, and stated that there was little yield benefit in applying fertiliser levels when pasture CP is within this range. The inclusion of legumes in pastures is widely known to increase animal production, presumably as a result of their high CP content. Poppi and McLennan (1995) argued that, although legumes do increase protein intake, that protein does not reach the small intestine due to a relatively lower intake of digestible energy. The beneficial effect of legumes on animal production is thus mainly attributable to their ability to stimulate intake. This is supported by results of Cruickshank et al. (1992) who found that lambs grazing on legumes grew significantly faster than lambs grazing grasses, which was associated with increased intake (and increased flow of non-ammonia N to the small intestine) from legume pastures, but also coupled with significant losses of dietary N across the rumen.

Table 5.8: The effect of season of N application and the once-off application of fertiliser N (kg N ha^{-1}) on the total crude protein content (%) of a grass-clover pasture at Elsenburg Research Farm during Year 1 and 2

		Year 1			
		Nitrogen (kg ha^{-1})			
Season	**0**	**20**	**40**	**60**	**Mean (S)**
Autumn	34.62	31.76	30.72	32.47	**32.39** ^{b}
Early winter	34.00	34.27	34.88	34.39	**34.39** ^{a}
Late winter	33.25	33.08	34.08	34.69	**33.77** ^{a}
Early spring	30.83	31.50	31.72	31.79	**31.45** ^{b}
Mean (N)	**33.18**	**32.65**	**32.90**	**33.34**	
		Year 2			
Autumn	31.71	31.37	32.67	31.96	**31.96** ^{a}
Early winter	16.05	19.43	21.84	22.44	**19.94** ^{c}
Late winter	25.73	29.61	28.92	29.60	**28.44** ^{b}
Early spring	24.80	26.05	26.69	27.04	**26.10** ^{b}
Mean (N)	**25.62** ^{b}	**27.70** ^{ab}	**28.72** ^{a}	**28.77** ^{a}	

**Means (bold) in the same column or row followed by the same letter are not significantly different at P = 0.05

5.3.3.3 In Vitro Organic Matter Digestibility (IVOMD)

The IVOMD for the separate grass and legume fractions are summarised in Table 5.9.

The IVOMD of both the grass and clover fractions was influenced by season of N application ($P < 0.0001$) and not by N application rate. The IVOMD for the grass and clover fractions was the lowest during early spring in Year 1 and autumn in Year 2. Similar trends were reported by Labuschagne et al. (2006c).

Table 5.9: The effect of season of N application and the once-off application of fertiliser N (kg N ha^{-1}) on the *in vitro* organic matter digestibility (%) of a grass-clover pasture at Elsenburg Research Farm during Year 1 and 2

	Year 1									
	IVOMD (%)									
N (kg ha^{-1})	**0**		**20**		**40**		**60**		**Mean (S)**	
Season	***Grass***	***Clover***	***Grass***	***Clover***	***Grass***	***Clover***	***Grass***	***Clover***	***Grass***	***Clover***
Autumn	86.52	86.03	84.57	86.27	88.40	87.28	86.84	85.65	**86.06** [a]	**86.27** [a]
Early winter	86.08	83.12	86.11	81.30	85.98	81.87	87.14	82.12	**86.33** [a]	**82.10** [b]
Late winter	84.76	84.38	82.76	83.50	86.57	85.32	86.81	85.15	**85.22** [a]	**84.58** [ab]
Early spring	79.73	77.85	80.81	75.54	81.03	78.82	80.59	76.57	**80.52** [b]	**77.12** [c]
Mean (N)	**83.82**	**82.55**	**83.42**	**81.65**	**84.91**	**83.16**	**85.05**	**82.23**		
	Year 2									
Autumn	82.09	82.30	86.78	81.86	86.68	82.59	84.98	81.51	**85.20** [c]	**82.06** [c]
Early winter	89.01	87.48	91.23	89.27	90.37	87.89	90.60	89.49	**90.30** [a]	**88.53** [a]
Late winter	86.59	89.72	88.46	89.34	87.90	89.59	88.41	90.8	**87.89** [b]	**89.88** [a]
Early spring	83.97	83.74	82.92	82.15	83.04	84.28	82.48	84.14	**83.12** [d]	**83.58** [b]
Mean (N)	**85.14**	**86.09**	**87.47**	**85.86**	**87.30**	**85.88**	**86.64**	**86.37**		

******Means (bold) in the same column or row followed by the same letter are not significantly different at P = 0.05

5.4 Conclusion

Pasture productivity was more often determined by season of N application than by the rate of fertiliser N application. The highest PDMP was associated with the application of 40 and 60 kg N ha^{-1}. There was a drastic decrease in PDMP from autumn to early winter and the highest PDMP occurred in spring – concurrent with increasing mean temperatures. These trends were observed in both the once-off and consecutive application of fertiliser N.

The RDMP was unaffected by fertiliser N. The TDMP was only affected by season, with highest TDMP recorded in spring in both years.

The effect of season and N application (once-off) on the botanical composition was inconsistent over the two years of the study. During the first year the pasture consisted predominantly of clover (approximately 75%). Maximum grass content (%) was associated with the application of high N rates (40 - 60 kg N ha^{-1}), while maximum clover content occurred at low N rates (0 - 20 kg N ha^{-1}) and decreased as the rate of N increased.

The actual clover content (kg DM ha^{-1}) was less influenced by N fertilisation compared to clover % in the fodder on offer.

During the second year season of N application had a significant effect on both the grass and clover fractions and a drastic reduction in grass content from mid-winter to late winter was observed. The minimum clover content remained at acceptable levels (≥ 30%) for high animal production and N fixation throughout both years of the study. The consecutive application of N displayed a similar trend, and even though there was a reduction in legume percentage with repeated N application, the clover fraction remained at acceptable levels.

With the once-off and consecutive applications of fertiliser N the actual clover content (kg ha^{-1}) decreased much less than clover percentage, proving that N application stimulated grass growth, leaving the clover component almost unaffected.

Nitrogen application rate and season had a significant effect on the nutritive characteristics measured in this study, which in some instances exceeded livestock requirements.

Pasture DM levels remained within the expected range, although there was a slight decrease in DM content in response to increased N fertiliser rates in the first year.

The TCP content was high (exceeding 30% in the first year), although this was due to the effect of season and not as a result of N fertilisation. The TCP was lower in the second year, and increased as the rate of applied N increased.

The IVOMD remained high (>70%) for both the grass and clover fractions, and was determined by season of N application and not N application rate. The IVOMD was at a minimum in spring for both grass and clover fractions in year one and in autumn (clover) and spring (grass) in year two.

Overall, pasture productivity and nutritive characteristics were a result of seasonal effects and not a result of N application (with a few exceptions). Nevertheless, based on the strong response of PDMP in autumn and late winter/early spring it could be beneficial to apply 40 kg N ha^{-1} during this period – provided that the clover content is sufficient. Although N application appeared not to have a negative effect on the actual clover content, the fact that the pasture changed from clover dominance in the first year, to near grass dominance in the second year could in part be attributed to the competitiveness of the grass component as a result of the stimulating effect of N in the first year.

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Chapter 6

The effect of nitrogen fertiliser application on the yield, botanical composition and selected nutritive characteristics of a mixed grass-lucerne pasture in the Winelands sub-region of the Western Cape

6.1 Introduction

Grass-legume pastures have traditionally formed the backbone of livestock production systems in the Western Cape (van Heerden and Tainton 1989). Legume based pastures have the potential of supplying high quality grazing for livestock (Peoples and Baldock 2001), with the additional advantage of reducing the requirement for nitrogen based fertilisers, due to their ability to fix atmospheric N (Wu and McGechan 1999). This can lead to considerable savings in the amount of N fertiliser required, as well as a reduction of the negative environmental effects associated with high fertiliser use (Hardarson 1993). Lucerne (*Medicago sativa*) in particular is widely regarded as a superior pasture crop due to its high dry matter production potential and its high nutritive value (McKenzie et al. 1990, Dickinson et al. 1993) and is also known as a nitrate scavenger, reducing the potential for nitrate pollution (Cherney et al. 1994) due to its deep root system. A further benefit of lucerne based pastures is that they require less irrigation than traditional grass-clover pastures (Durand and van Heerden no date).

One disadvantage is that the dry matter production of lucerne (in a grass-lucerne pasture) is typically low during the cooler months of the year, even for the winter-active cultlivars (Botha 1998). One of the ways to address this issue is by the strategic application of N fertiliser. This entails the application of small amounts of N fertiliser during the cooler months of the year, when the legume component is essentially dormant, but the accompanying grass is still reactive to N (Eckard and Franks 1998, McKenzie et al. 1999, Labuschagne et al. 2006a). Therefore the majority of the mineral N available during the summer months can be ascribed to the presence of the legume component, while winter production is more dependent on N fertiliser.

Research results regarding fertilisation norms for pastures in the Winelands sub-region of the Western Cape Province of South Africa are not freely available as research was mainly focused on the dairying regions in the southern Cape. The aims of this study were therefore i) to develop N fertiliser application strategies to ensure optimum dry matter production during different seasons ii) to determine the effect of N fertiliser on the botanical composition of these pastures and iii) to determine the effect of N fertiliser on selected nutritive characteristics of the fodder produced.

6.2 Materials and methods

A comprehensive overview of the site characteristics and general procedures and management is provided in Chapter 3. Details for the grass-lucerne pasture is given below.

A grass-lucerne pasture consisting of perennial ryegrass (*Lolium perenne*), tall fescue (*Festuca arundinaceae*) and lucerne was established in April 2010. Data collection commenced in April 2011. Nitrogen treatments of 0, 20, 40 and 60 kg N $^{-1}$ where applied at the beginning of each regrowth cycle as LAN (28% N) during the autumn-spring period, after the pasture was mob-grazed by sheep to a height of approximately 30 mm. Nitrogen was washed into the soil with a light (10-15 mm) irrigation after application. If necessary the pasture was mowed to 30 mm to remove effects of patch grazing and the material removed from site. A fixed regrowth cycle of 35 days was applied. .Sub-plot dimensions were 3.75 x 3.75 m (14.06 m^2) of which 6.6 m^2 was harvested using a sicklebar mower**.** Various sub-samples were collected for the determination of dry matter production, botanical composition and selected nutritive characteristics.

The N treatments were divided into two management strategies. One set of plots received a once-off application of N during the cool season (every 35 days but on a different set of plots), while a second set of plots received N after each regrowth cycle (every 35 days both on the same set of plots) during the cool season. The cutting cycles for the grass-lucerne pasture were: autumn (April/May), early winter (June), mid-winter (July/Aug) and early spring (September). For the consecutive N application strategy a 5th harvest of mid-spring (September - October) was included.

Experimental design and treatments

Experimental design was a completely randomised design with six replicates. The treatment design was a factorial design with two factors, seasons (autumn, early winter, mid- winter and early spring) and nitrogen application rates. Year was added as a sub-plot factor because measurements were taken repeatedly (once annually) on the same plot. This statistical approach is consistent with procedures described by Little and Hills (1972).

6.3 Results and Discussion

6.3.1 Dry matter production

6.3.1.1 Once-off N application strategy

i) *Primary dry matter production (PDMP)*

The effect of fertiliser N rate and season of N application on the PDMP of the grass-lucerne pasture is presented in Table 6.1. During both years, PDMP was affected by season of application and not by N application rate. During Year 1 the highest mean PDMP was recorded in autumn (48.98 kg DM ha^{-1} day^{-1}) and early spring (44.87 kg DM ha^{-1} day^{-1}), and the lowest PDMP in winter ($P < 0.001$).

Year 2 showed a similar trend with the highest mean PDMP occurring in early spring (54.37 kg DM ha^{-1} day^{-1}) and the lowest in early and mid-winter (13.64 and 16.05 kg DM ha^{-1} day^{-1} respectively) ($P < 0.0001$).

Table 6.1: The effect of season of N application and N rate (kg N ha^{-1}) of a once-off application on the primary dry matter production (PDMP) (kg DM ha^{-1} day^{-1}) of a grass-lucerne pasture during Year 1 and 2 at Elsenburg Research Farm

	Year 1				
	Nitrogen (kg ha^{-1})				
Season	**0**	**20**	**40**	**60**	**Mean (S)**
Autumn	45.73	50.78	47.71	51.71	**48.98 ^{a**}**
Early-winter	27.33	29.54	29.79	30.04	**29.17 ^{b}**
Mid-winter	23.54	28.34	27.35	31.32	**27.61 ^{b}**
Early spring	42.36	48.62	44.45	52.75	**44.87 ^{a}**

Mean (N)	**34.74**	**37.19**	**37.33**	**41.46**	
			Year 2		
Autumn	23.82	23.55	23.48	28.63	**24.92** [b]
Early winter	11.04	12.1	14.34	16.65	**13.64** [c]
Mid-winter	12.56	15.68	16.2	19.76	**16.05** [bc]
Early spring	47.89	53.13	49.95	66.51	**54.37** [a]
Mean (N)	**24.41**	**26.12**	**25.99**	**32.89**	

**Means (bold) in the same column or row followed by the same letter are not significantly different at P = 0.05

ii) Residual dry matter production (RDMP)

The effect of the once-off application of fertiliser N on the RDMP production is summarised in Table 6.2. Results were mainly affected by season of application (P < 0.0001) and not by fertiliser N application rate. During both years the lowest RDMP was produced during autumn and steadily increased toward early spring.

This low responsiveness of RDMP to applied N can occur under circumstances where all of the applied N is absorbed during the first regrowth cycle, or if pasture productivity is limited due to environmental factors which exposes the applied N to leaching. The latter scenario is likely, considering the winter dominant rainfall pattern in the western part of the Western Cape as well as the low response of PDMP to N. The highest RDMP was recorded in early spring which can be ascribed to the fact that RDMP was herbage harvested 35 days after the first cut (70 days after fertiliser N application), and therefore consists largely of material harvested in spring when mean temperatures were higher.

Table 6.2: The effect of season of N application and N rate (kg N ha^{-1}) of a once-off application on the residual dry matter production (RDMP) (kg DM ha^{-1} day^{-1}) of a grass-lucerne pasture during Year 1 and 2 at Elsenburg Research Farm

			Year 1		
			Nitrogen (kg ha^{-1})		
Season	**0**	**20**	**40**	**60**	**Mean (S)**
Autumn	20.58	21.35	21.09	19.45	**20.62** [d **]
Early winter	27.67	26.76	26.02	27.95	**27.10** [c]
Mid-winter	35.53	36.13	27.55	34.5	**33.43** [b]

Early spring	51.11	53.61	51.58	57.81	**53.53** [a]
Mean (N)	**37.20**	**34.46**	**31.56**	**34.93**	
			Year 2		
Autumn	8.98	9.03	8.72	9.29	**9.00** [c]
Early winter	NA	NA	NA	NA	NA
Mid-winter	39.95	46.69	45.28	44.66	**44.15** [b]
Early spring	76.43	75.96	74.47	77.4	**76.07** [a]
Mean (N)	**48.35**	**50.87**	**49.65**	**50.68**	

NA: data not available

**Means (bold) in the same column or row followed by the same letter are not significantly different at P = 0.05

iii) Total dry matter production (TDMP)

The effect of season of N application and fertiliser N rate on the TDMP of a grass-lucerne pasture is shown in Table 6.3. Similar to PDMP and RDMP, differences in productivity was mainly due to the effect of season of N application ($P < 0.0001$), and not N application rate. During both years the highest ($P = 0.05$) TDMP was recorded in early spring (48.26 and 65.22 kg ha day^{-1} for Year 1 and 2 respectively). The lowest TDMP occurred during early- and mid-winter for Year 1 (28.14 and 29.94 kg ha day^{-1} respectively) and in autumn for Year 2 (14.19 kg ha day^{-1}).

Table 6.3: The effect of season of N application and N rate (kg N ha^{-1}) of a once-off application on the total dry matter production (TDMP) (kg DM ha^{-1} day^{-1}) of a grass-lucerne pasture during Year 1 and 2 at Elsenburg Research Farm

			Year 1		
			Nitrogen (kg ha^{-1})		
Season	**0**	**20**	**40**	**60**	**Mean (S)**
Autumn	33.16	36.07	34.40	35.58	**34.80** [b] **
Early winter	27.50	28.15	27.91	28.99	**28.14** [c]
Mid-winter	29.53	29.87	27.45	32.91	**29.94** [c]
Early spring	46.73	43.00	48.03	55.28	**48.26** [a]
Mean (N)	**34.23**	**34.28**	**34.45**	**38.19**	
			Year 2		
Autumn	12.17	14.03	13.92	16.64	**14.19** [c]
Early winter	NA	NA	NA	NA	NA
Mid-winter	26.26	31.19	30.74	32.21	**30.10** [b]
Early spring	62.16	64.55	62.21	71.95	**65.22** [a]

Mean (N)	**33.53**	**36.59**	**35.62**	**40.27**

NA: Data not available

**Means (bold) in the same column or row followed by the same letter are not significantly different at P = 0.05

6.3.1.2 Consecutive N application strategy during the cool seasons

i) Primary dry matter production (PDMP)

With the consecutive application of N, both fertiliser N rate and season of N application had an effect (P < 0.0001) on PDMP during both years of the study (Table 6.4). There was an increase in mean PDMP as the rate of N applied increased, with the highest mean PDMP recorded with the application of 40 and 60 kg N ha^{-1}.

During both years, the highest mean PDMP was associated with mid – and early spring (62.03 and 59.51 kg DM ha^{-1} day^{-1} for Year 1 and Year 2 respectively), while the lowest mean PDMP was recorded in early winter in Year 1 (23.85 kg DM ha^{-1} day^{-1}) and mid – and late winter in Year 2 (10.81 and 17.80 kg DM ha^{-1} day^{-1} respectively).

Table 6.4: The effect of season of N application and N rate (kg N ha^{-1}) of consecutive N applications on the primary dry matter production (PDMP) (kg DM ha^{-1} day^{-1}) of a grass-lucerne pasture during Year 1 and 2 at Elsenburg Research Farm

		Year 1 Nitrogen (kg ha^{-1})			
Season	**0**	**20**	**40**	**60**	**Mean (S)**
Autumn	42.7	45.05	52.83	50.12	**47.67** ^{b} **
Early winter	20.96	21.62	26.38	26.44	**23.85** ^{e}
Mid-winter	23.53	28.4	32.28	32.16	**29.09** ^{d}
Early spring	40.52	39.46	44.41	45.15	**41.21** ^{c}
Mid-spring	47.12	56.51	72.9	73.51	**62.03** ^{a}
Mean (N)	**34.03** ^{b}	**38.21** ^{b}	**45.76** ^{a}	**44.51** ^{a}	
		Year 2			
Autumn	17.16	24.43	30.75	25.93	**24.56** ^{b}
Early winter	6.42	9.56	13.13	14.15	**10.81** ^{c}

Mid-winter	14.27	16.01	21.59	18.75	**17.80** ^{bc}
Early spring	38.71	50.07	71.87	77.37	**59.51** ^{a}
Mean (N)	**19.35** ^{c}	**25.02** ^{bc}	**34.33** ^{a}	**34.05** ^{ab}	

******Means (bold) in the same column or row followed by the same letter are not significantly different at P = 0.05

6.3.2 Botanical Composition

6.3.2.1 Once-off N application

The effect of the once-off application of N on species composition is summarised in Figures 6.1, 6.2 (Year 1 and 2 respectively) and Table 6.5.

During the first year the pasture was legume dominant comprising of approximately 70-80% lucerne. The effect of season and N application was inconsistent over the two years of the study. In Year 1 only the clover fraction was affected by N application rate ($P < 0.0045$). Clover content steadily decreased as the rate of N increased, with the lowest clover fraction of 31.26 and 25.89% occurring with the application of 40 and 60 kg N ha^{-1} respectively (Figure 6.2, Table 6.5). A similar pattern was observed with the application of N to the grass-clover pasture (Chapter 5) as well as by Labuschagne et al. (2006b).

All species were influenced by season of application ($P < 0.0001$). Grass and clover displayed similar patterns, namely the lowest contribution to total sward productivity occurred during autumn, where after it steadily increased throughout winter and reached maximum levels in early spring. For lucerne, the opposite was recorded, i.e. the highest lucerne content was present in autumn (77.61%), followed by a steady decrease throughout winter with lowest levels occurring in early spring (6.22%). Pasture composition varied between lucerne dominance during the autumn-early winter period and clover dominance in mid-winter to early spring. The low grass and clover productivity in autumn was probably due to strong competition from lucerne (Hill 1991)

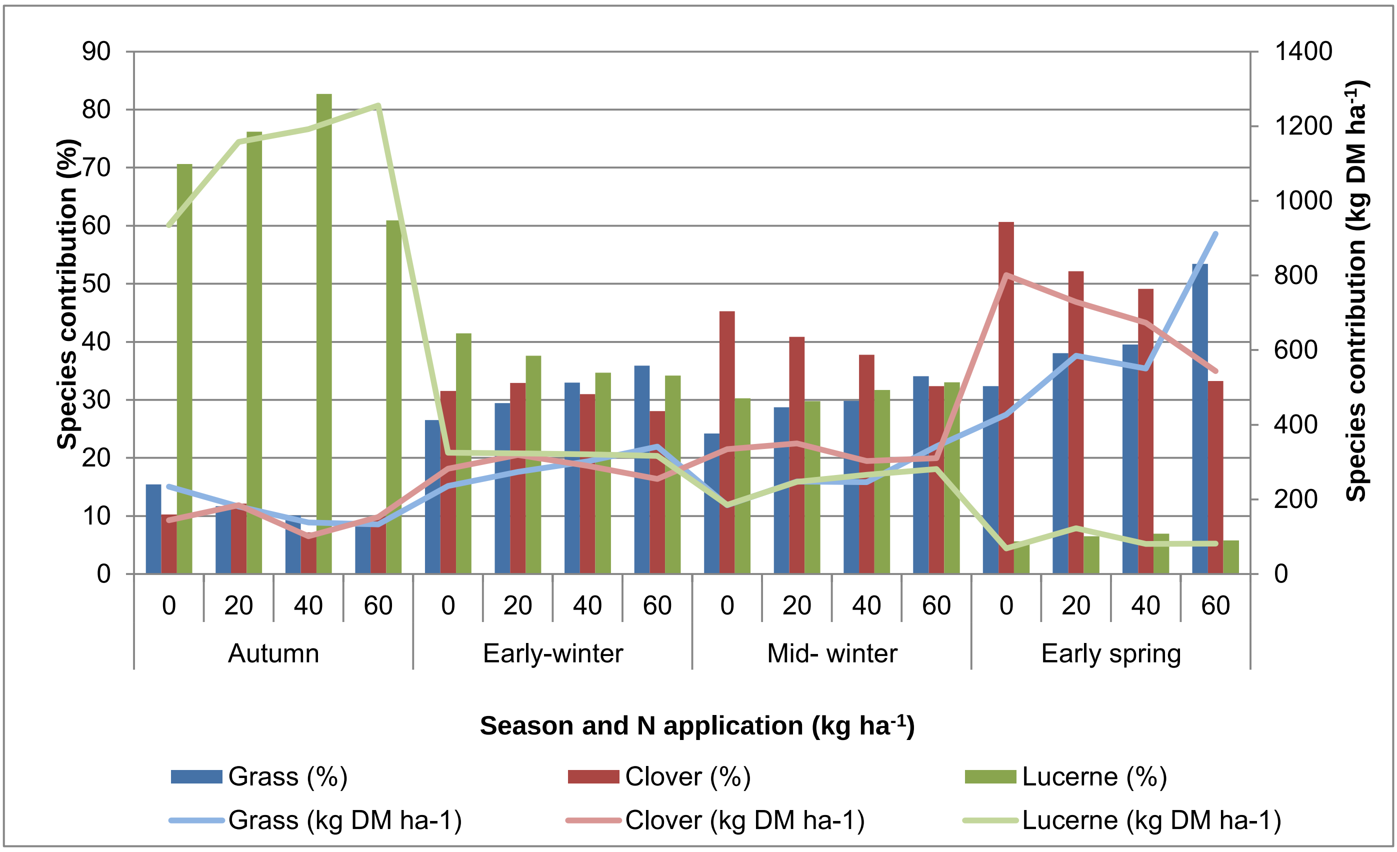

Figure 6.1: The effect of season of N application and N rate (kg N ha^{-1}) of a once-off application on the botanical composition (%) of a grass-lucerne pasture during Year 1 at Elsenburg Research Farm

During the second year (Figure 6.2, Table 6.5), all species were affected by season of application ($P < 0.0001$) and not by fertiliser N rate (Table 6.5). Highest grass content occurred in early winter and early spring (62.51 % and 59.02 % respectively) and the lowest during mid-winter (41.11 %). Lucerne productivity was lower in autumn compared to Year 1 (39.78 %) and also decreased less during winter. The lowest lucerne content was recorded in early winter (20.61 %) and early spring (26.66%). Similar to Year 1, the clover content was at its lowest level in autumn (9.05 %), but increased to maximum levels in mid-winter (21.01 %), where after contribution decreased to 14.09 % in early spring. Except for mid-winter at 40 kg N ha^{-1}, the grass component was dominant throughout Year 2 (Figure 6.2).

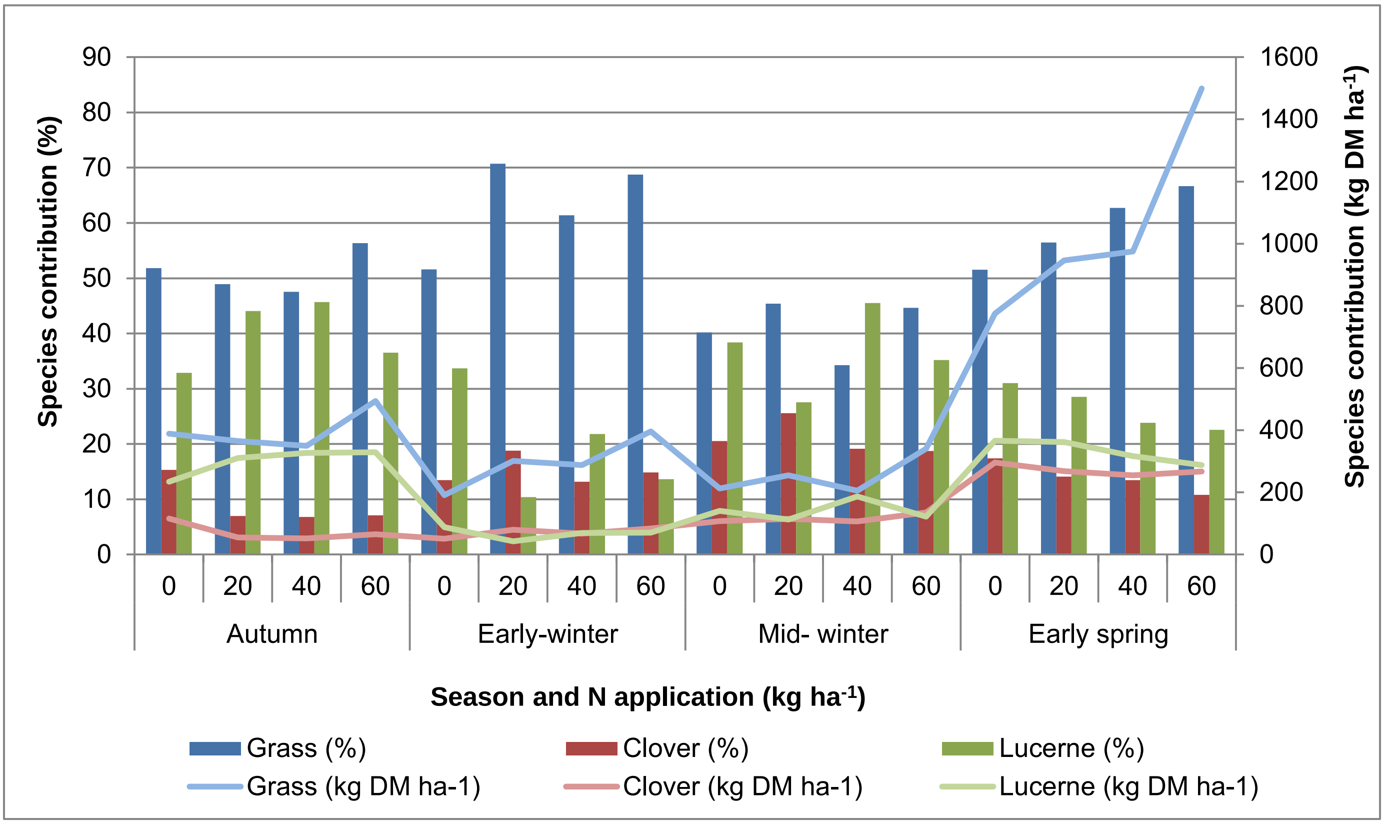

Figure 6.2: The effect of season of N application and N rate (kg ha^{-1}) of a once-off application on the botanical composition (%) of a grass-lucerne pasture during Year 2 at Elsenburg Research Farm

Table 6.5: Botanical composition (%) of a mixed grass-lucerne pasture in response to season of N application and N rate (kg ha^{-1}) of a once-off application during Year 1 and 2 at Elsenburg Reasearch Farm

Year 1

% Contribution

N (kg ha^{-1})	0			20			40			60			Mean (S)		
Season	***Grass***	***Lucerne***	***Clover***	***Grass***	***Lucerne***	***Clover***	***Grass***	***Lucerne***	***Clover***	***Grass***	***Lucerne***	***Clover***	***Grass***	***Lucerne***	***Clover***
Autumn	15.45	70.62	10.24	11.67	76.20	12.13	10.12	82.71	7.17	8.56	60.91	9.92	**11.45** [c]	**77.61** [a]	**9.86** [d **]
Early winter	26.54	41.45	31.52	29.47	37.63	32.91	32.96	34.70	30.99	35.90	34.20	28.06	**31.22** [b]	**36.99** [b]	**30.87** [c]
Mid-winter	24.23	30.26	45.24	28.71	29.79	40.83	29.83	31.71	37.75	34.08	33.04	32.34	**29.23** [b]	**31.26** [b]	**38.96** [b]
Early spring	32.38	5.63	60.67	38.02	6.50	52.17	39.53	6.94	49.13	53.45	5.81	33.23	**40.85** [a]	**6.22** [c]	**48.80** [a]
Mean (N)	**24.65**	**36.99**	**36.92** [a]	**26.89**	**37.87**	**34.23** [a]	**28.11**	**39.02**	**31.26** [ab]	**33.00**	**38.49**	**25.89** [b]			
							Year 2								
Autumn	51.81	32.87	15.32	48.96	44.06	6.99	47.53	45.67	6.80	56.36	36.53	7.11	**51.17** [b]	**39.78** [a]	**9.05** [c]
Early winter	51.58	33.70	13.49	70.71	10.38	18.79	61.39	21.83	13.17	68.75	13.64	14.88	**62.51** [a]	**20.61** [b]	**14.92** [b]
Mid-winter	40.16	38.36	20.51	45.42	27.56	25.58	34.27	45.51	19.14	44.63	35.21	18.73	**41.11** [c]	**36.59** [a]	**21.01** [a]
Early spring	51.54	31.03	17.43	56.44	28.53	14.12	62.71	23.83	13.45	66.65	22.55	10.80	**59.02** [a]	**26.66** [b]	**14.09** [b]
Mean (N)	**49.15**	**33.80**	**16.52**	**54.67**	**28.38**	**16.26**	**51.48**	**34.21**	**13.14**	**58.31**	**27.79**	**12.88**			

**Means (bold) in the same column or row followed by the same letter are not significantly different at P = 0.05

6.3.2.2 Consecutive application

The effect of consecutive applications of fertiliser N on the botanical composition of a grass-lucerne pasture is presented in Figures 6.3, 6.4 and Table 6.6 respectively.

During year one (Figure 6.3, Table 6.5), the application of fertiliser N had a significant effect on the clover ($P < 0.0003$) and grass content (%) ($P < 0.0023$). Grass content increased as the application rate of N increased, with the highest contribution of 39.36% and 40.95% occurring in response to application of 40 and 60 kg N ha^{-1} respectively. This stimulating effect of N on the grass component in a grass-lucerne pasture was also reported by Berdahl et al. (2001). Clover displayed the opposite response, with clover content steadily decreasing as the rate of N increased, reaching minimum levels of 18.72 and 21.52% in response 40 and 60 kg N respectively, similar to the trend observed with the once-off application. The actual clover content (kg DM ha^{-1}) followed a similar trend to the relative clover content (%) although the actual clover content did not show such a drastic decrease in response to N in late winter as suggested by the relative clover percentage observation. This indicates that the apparent reduction in clover content (%) was probably due to the stimulating effect of N on the grass-component (McKenzie et al. 2002).

Season of application affected all pasture species ($P < 0.0001$) (Table 6.6). The highest lucerne content occurred during autumn, where after its contribution decreased with decreasing temperatures through winter, reaching minimum levels from mid-winter to early spring. The grass fraction displayed the opposite trend, with lowest levels of 12.89% occurring in autumn, which increased throughout winter and into spring, with highest levels of 59.30% occurring in mid-spring. The reactivity of perennial ryegrass at temperatures as low as 5˚C is well known (Frame 1992, Labuschagne and Agenbag 2008) and probably accounts for its high productivity during the winter period.

Clover production fluctuated less over the winter period, with low levels of approximately 15% occurring in autumn, early winter and mid-spring and higher levels of approximately 37% in the mid-winter to early-spring period.

The pasture was lucerne dominant during autumn and early-winter and shifted to clover-grass dominance in mid-winter and then to grass dominance throughout early and mid-spring.

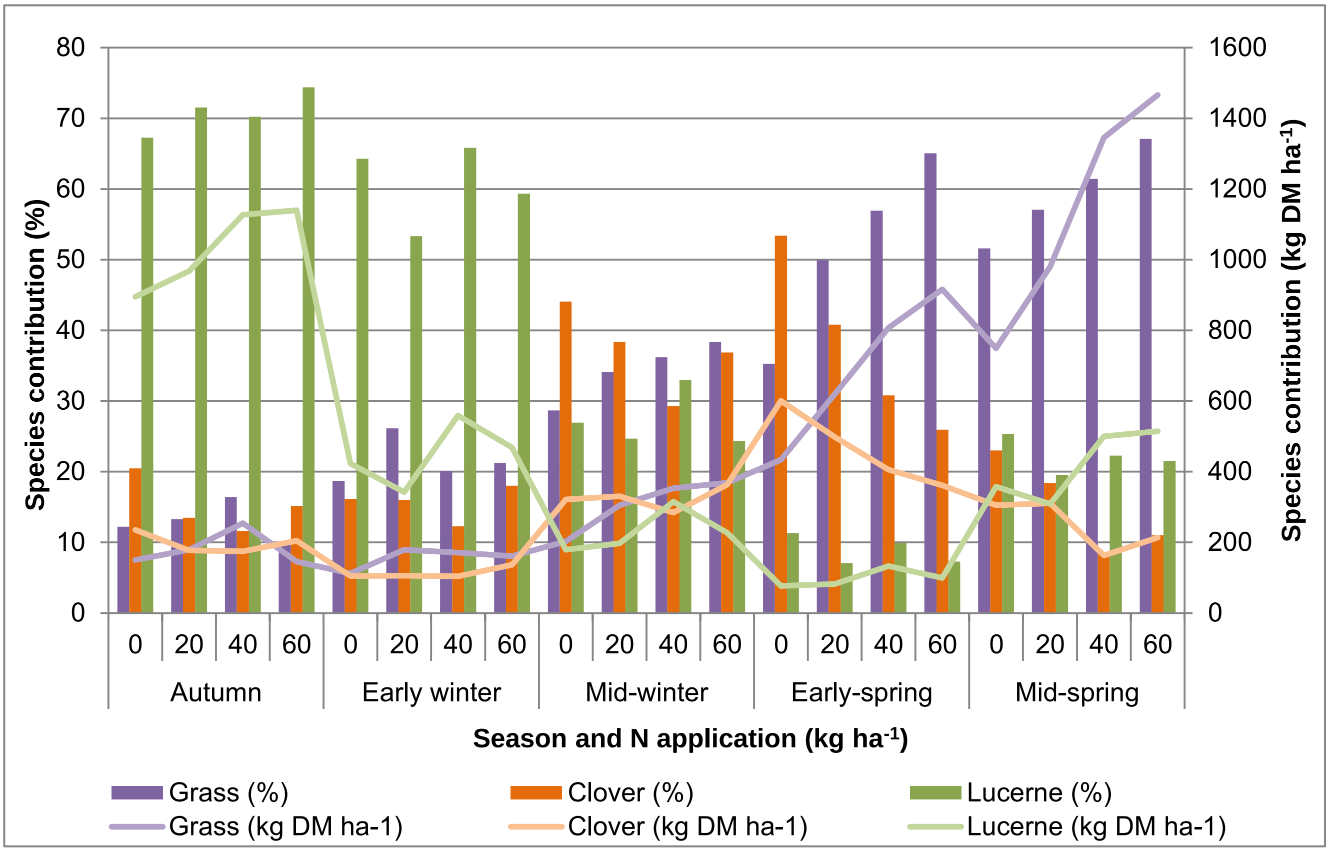

Figure 6.3: The effect of season of N application and N rate (kg N ha^{-1}) of consecutive applications on the botanical composition (%) of a grass-lucerne pasture during Year 1 at Elsenburg Research Farm

Overall the results for Year 2 (Figure 6.4) are very similar to Year 1. The N application had a significant effect ($P < 0.01$) on the grass and clover percentage of the pasture. The grass content increased as the rate of N increased from 0 – 60 kg N ha^{-1}, with highest percentage of 62.63% and 68.45% occurring with the application of 40 and 60 kg N ha^{-1}. The clover content decreased with increasing rates of N, with the lowest clover content of 7.35 % and 5.72% occurring with the application of 40 and 60 kg N ha^{-1}. The actual clover content (kg DM ha^{-1}) in early and mid-winter, and early spring remained relatively stable in response to N, which indicates that N doesn't necessarily have a negative effect on clover *per se*, but rather increases the competitive ability of the grass component.

Season of N application also had an effect on all species in the pasture mixture (P < 0.0001). The lucerne content was highest in autumn, and decreased over the winter period, reaching minimum levels of 27.04%, 23.91% and 21.26% from early winter to early spring.

Grass response to season was opposite to that of lucerne, with autumn and mid-winter producing the lowest grass content of 50.93% and 58.56% respectively while early winter and early spring produced the highest, namely 62.54% and 71.03%. Similar to year one, the clover content was highest in mid-winter, with lower levels occurring over all other seasons, with the minimum contribution of 5.94%, 7.69% and 7.00% in autumn, early winter and early spring respectively. In general the pasture was grass dominant during the second year.

The pasture changed from lucerne dominance in Year 1, to grass dominance in Year 2. This is likely due to the management strategy which adversely affected the regrowth physiological processes of lucerne. Factors which influence lucerne persistence include the maintenance of root carbohydrate reserves and crown buds and shoots (Lodge 1991). Based on these requirements the recommended resting interval for lucerne is 35 days, and the grazing height 50 mm (Lodge 1991). In this study the grazing cycle was based on the requirements of the grass component and was fixed at 24 and 28 days in spring and summer respectively. The grazing height was also approximately 30 mm. These factors could thus have contributed to the decline in the lucerne content from Year 1 to Year 2.

Overall the legume content remained at acceptable levels for high animal production with both management strategies.

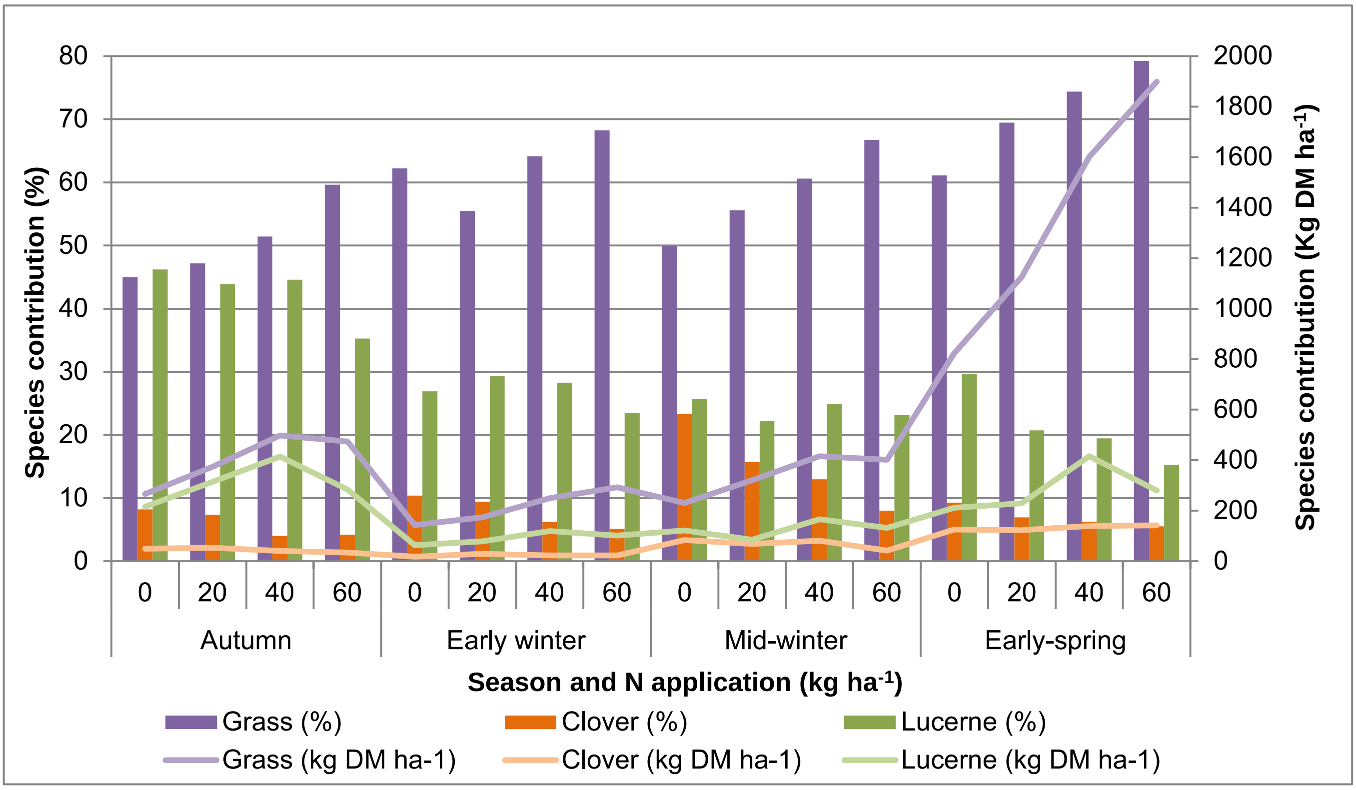

Figure 6.4: The effect of season of N application and fertiliser N rate (kg ha^{-1}) of consecutive applications on the botanical composition (%) of a grass-lucern pasture during the Year 2 at Elsenburg Research Farm

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Table 6.6: Botanical composition (%) of a mixed grass-lucerne pasture in response to season of N application and N rate (kg ha^{-1}) to consecutive applications during Year 1 and 2 at Elsenburg Research Farm

N (kg ha^{-1})	0			20			40			60			Mean (S)		
	Year 1														
	% Contribution														
Season	***Grass***	***Lucerne***	***Clover***	***Grass***	***Lucerne***	***Clover***	***Grass***	***Lucerne***	***Clover***	***Grass***	***Lucerne***	***Clover***	***Grass***	***Lucerne***	***Clover***
Autumn	12.24	67.29	20.46	13.24	71.52	13.51	16.38	70.22	11.61	9.72	74.37	15.16	**12.89** e	**70.85** a	**15.18** b **
Early winter	18.71	64.3	16.14	26.14	53.3	16.03	20.11	65.82	12.28	21.25	59.34	18.04	**21.55** d	**60.69** b	**15.63** b
Mid-winter	28.68	26.96	44.06	34.12	24.68	38.35	36.19	32.99	29.24	38.35	24.3	36.85	**34.33** c	**27.23** c	**37.12** a
Early spring	35.27	11.3	53.43	49.94	7.04	40.82	56.95	9.91	30.82	65.05	7.26	25.96	**51.80** b	**8.88** c	**37 76** a
Mid-spring	51.59	25.31	23	57.08	19.57	18.36	61.42	22.29	8.6	67.09	21.53	11.01	**59.30** a	**22.17** c	**15.24** b
Mean (N)	**29.66** b	**38.16**	**31.95** a	**36.45** a	**34.6**	**25.74** b	**38.84** a	**39.36**	**18.72** c	**40.95** a	**36.6**	**21.52** bc			
	Year 2														
Autumn	45.01	46.23	8.22	47.18	43.9	7.34	51.43	44.58	4.00	59.61	35.25	4.2	**50.93** c	**42.49** a	**5.94** b
Early winter	62.22	26.93	10.39	55.5	29.36	9.44	64.14	28.27	6.24	68.25	23.53	5.13	**62.54** ab	**27.04** b	**7.69** b
Mid-winter	49.91	25.67	23.34	55.59	22.27	15.7	60.59	24.87	12.96	66.72	23.14	8.02	**58.56** bc	**23.91** b	**14.64** a
Early spring	61.09	29.62	9.3	69.45	20.74	6.93	74.35	19.44	6.22	79.22	15.24	5.55	**71.03** a	**21.26** b	**7.00** b
Mean (N)	**54.56** b	**32.64**	**12.44** a	**56.93** b	**29.07**	**9.85** ab	**62.63** ab	**29.29**	**7.35** bc	**68.45** a	**24.29**	**5.72** c			

**Means (bold) in the same column or row followed by the same letter are not significantly different at P = 0.05

6.3.3 Nutritive Characteristics

6.3.3.1 Pasture Dry Matter (DM) content

During both years, DM content was determined by season of application ($P < 0.0097$ and $P < 0.0002$) (Table 6.7). However, the effect was not consistent over both years covered by the study. During Year 1 only the early spring DM content differed marginally from the other seasons, with a slight decrease ($P > 0.05$) in DM being recorded. During Year 2, the lowest DM of 16.96% was recorded in mid-winter. The DM values reported here are low, as expected values fall within the range of 15 to 25 % DM depending on the season and the botanical composition of the pasture on offer (Meissner et al. 2000). Pasture DM is expected to decrease with increasing rates of N (Wilman 1975; Peyraud et al. 1997, Labuschagne et al. 2006b), but only a slight non-significant decrease was recorded in this study.

Table 6.7: The effect of season of N application and N fertiliser rate (kg ha^{-1}) on the dry matter content (%) of a grass-lucerne pasture at Elsenburg Research Farm during Year 1 and 2

			Year 1		
			Nitrogen (kg ha^{-1})		
Season	**0**	**20**	**40**	**60**	**Mean (S)**
Autumn	15.60	14.64	14.21	14.28	**14.68 ^{a**}**
Mid-winter winter	14.21	14.36	13.88	13.74	**14.05 ^{a}**
Late winter	14.84	14.72	14.09	14.00	**14.40 ^{a}**
Early spring	13.86	11.20	13.62	13.38	**13.01 ^{b}**
Mean (N)	**14.62**	**13.68**	**13.95**	**13.85**	
			Year 2		
Autumn	19.27	18.36	17.51	17.50	**18.11 ^{ab}**
Mid-winter	17.17	17.17	17.29	16.24	**16.96 ^{c}**
Late winter	17.16	17.86	17.53	17.16	**17.43 ^{bc}**
Early spring	19.36	19.02	18.70	18.81	**18.97 ^{a}**
Mean (N)	**18.24**	**18.10**	**17.75**	**17.43**	

**Means (bold) in the same column or row followed by the same letter are not significantly different at $P = 0.05$

6.3.3.2 Crude Protein (CP)

The CP content of the grass and legume fractions recorded for Year 1 and 2 is summarised in Table 6.8.

i) Grass

During Year 1, grass CP was influenced by both season of application ($P < 0.0001$) and N fertilisation rate ($P < 0.0001$). Grass CP increased with increasing rates of N with the highest CP of 27.02% and 27.70% recorded with the application of 40 and 60 kg N ha^{-1}. In Year 2 the highest CP of 26.08% was recorded with the application of 60 kg N ha^{-1}. Similar trends were reported by Labuschagne et al. (2006b) and Eckard (1994).

During both years CP steadily decreased from autumn, through winter and reached minimum levels in early spring.

Common CP averages for temperate grasses range from 21% to 31% (depending on the season), but can be as high as 35% (Fulkerson et al. 2007). The CP values recorded in this study are thus within the normal range.

ii) Lucerne + Clover (LC)

During Year 1 the LC CP content was only influenced by season of application ($P < 0.0001$). Extremely high CP contents occurred in mid-winter and late winter (37.12 and 36.53% respectively). During Year 2 CP content was significantly affected by both season of application and fertiliser N application rate ($P < 0.0001$). Mean CP increased with increasing rates of N. The highest mean CP content was recorded in autumn, mid- and late winter (although not significantly higher than values recorded in spring).

iii) Total CP (TCP)

Total CP compared favourably with CP values for respective species published by Fulkerson et al. (2007). During both years there was an increase in mean TCP as the rate of N increased, although not significantly so in Year 1. During both years TCP was also affected by season ($P < 0.0001$). In Year 1, maximum TCP was recorded in mid-winter and late winter and in Year 2 in autumn and late winter. During both years the lowest TCP was recorded in early spring.

Table 6.8: The effect of season of application and N fertiliser rate (kg N ha^{-1}) on the crude protein (CP) content (%) of a grass-lucerne pasture at Elsenburg Research Farm during Year 1 and 2

Year 1
CP (%)

N (kg ha^{-1})	**0**		**20**		**40**		**60**		**Mean (S)**	
Season	***Grass***	***Lucerne + Clover***	***Grass***	***Lucerne + Clover***	***Grass***	***Lucerne + Clover***	***Grass***	***Lucerne + Clover***	***Grass***	***Lucerne + Clover***
Autumn	23.74	29.25	26.25	30.05	28.38	29.85	28.93	29.71	**27.73** ^{ab}	**29.71** ^{c} **
Mid-winter	26.65	36.64	26.52	36.61	28.14	37.30	29.48	37.96	**27.74** ^{a}	**37.12** ^{a}
Late winter	24.93	36.27	24.93	36.59	27.35	37.02	28.10	36.27	**26.27** ^{b}	**36.53** ^{a}
Early spring	23.22	32.47	23.22	30.61	24.20	31.15	24.49	30.29	**23.63** ^{c}	**31.15** ^{b}
Mean (N)	**24.55** ^{b}	**33.76**	**24.96** ^{b}	**33.45**	**27.02** ^{a}	**34.37**	**27.70** ^{a}	**34.02**		
Autumn	23.02	31.60	26.50	33.50	25.51	33.70	26.79	34.55	**25.45** ^{a}	**33.34** ^{a}
Mid-winter	22.20	34.66	24.35	33.70	25.42	34.31	25.87	34.18	**24.44** ^{a}	**34.21** ^{a}
Late winter	23.36	33.01	23.91	33.36	24.83	35.88	26.59	35.26	**24.73** ^{a}	**34.44** ^{a}
Early spring	17.07	28.98	17.69	30.10	18.97	29.41	19.76	30.83	**17.92** ^{b}	**29.52** ^{b}
Mean (N)	**21.47** ^{c}	**32.04** ^{c}	**23.87** ^{b}	**33.00** ^{bc}	**24.30** ^{b}	**33.86** ^{b}	**26.08** ^{a}	**34.78** ^{a}		

**Means (bold) in the same column or row followed by the same letter are not significantly different at P = 0.05

Table 6.9: The effect of season of N application and N fertiliser rate (kg ha^{-1}) on the total crude protein (TCP) content (%) of a grass-lucerne pasture at Elsenburg Research Farm during Year 1 and 2

		Year 1 Nitrogen (kg ha^{-1})			
Season	**0**	**20**	**40**	**60**	**Mean (S)**
Autumn	27.28	29.57	29.71	29.72	**29.04** [b]
Mid-winter	33.19	33.63	33.79	34.17	**33.71** [a]
Late winter	33.55	32.85	33.79	33.43	**33.46** [a]
Early spring	28.97	26.82	25.60	23.62	**26.58** [c]
Mean (N)	**30.80**	**30.70**	**31.75**	**31.25**	
		Year 2			
Autumn	27.20	30.00	29.82	30.19	**29.30** [a]
Mid-winter	27.94	27.27	27.35	27.66	**27.56** [b]
Late winter	29.19	28.51	32.07	31.33	**30.32** [a]
Early spring	22.81	23.57	22.45	25.87	**23.17** [c]
Mean (N)	**26.81** [c]	**27.94** [bc]	**28.77** [ab]	**29.74** [a]	

**Means (bold) in the same column or row followed by the same letter are not significantly different at P = 0.05

6.3.3.3 In Vitro Organic Matter Digestibility (IVOMD)

The IVOMD for the separate grass and legume fractions are given in Table 6.10. Due to a number of missing values and consequent low error degrees of freedom the data presented should be interpreted with caution.

i) Grass

During Year 1 grass IVOMD was unaffected by both season and N fertilisation. The highest IVOMD of 87.67% was associated with the application of 40 and 60 kg N ha^{-1} during early winter. During the second year IVOMD was determined by season ($P < 0.0001$). The highest IVOMD of 89.17 and 90.00% was recorded in mid- and late winter respectively.

ii) Lucerne + clover (LC)

During both years LC IVOMD was affected by season ($P < 0.019$ in Year 1 and $P < 0.0001$ in Year 2 respectively). *In vitro* organic matter digestibility tended to be highest in mid-winter and late winter in Year 1 and Year 2

During both years IVOMD was higher than 70 % and therefore it can be assumed that the rate of particle breakdown in the rumen will be rapid with consequent high intakes (Meissner et al. 2000).

Table 6.10: The effect of season of application and N fertiliser rate (kg N ha^{-1}) on the *in vitro* organic matter digestibility (IVOMD) (%) of a grass-lucerne pasture at Elsenburg Research Farm during Year 1 and 2

Year 1
IVOMD (%)

N (kg ha^{-1})	0		20		40		60		Mean (S)	
Season	***Grass***	***Lucerne + Clover***	***Grass***	***Lucerne + Clover***	***Grass***	***Lucerne + Clover***	***Grass***	***Lucerne + Clover***	***Grass***	***Lucerne + Clover***
Autumn		*		*		*		*		*
Mid-winter	86.28	84.45	84.87	82.87	87.67	83.89	87.67	84.37	**86.64**	**83.90** [a] **
Late winter	85.59	84.05	84.11	84.22	86.43	84.56	86.16	84.05	**81.91**	**84.22** [a]
Early spring	83.78	*	84.89	*	84.06	79.09	85.79	*	**84.67**	**79.09** [b]
Mean (N)	**85.32**	**84.25**	**84.62**	**83.48**	**86.45**	**83.49**	**86.63**	**84.21**		
					Year 2					
Autumn	86.39	82.18	84.36	78.67	87.25	79.83	88.00	82.19	**86.50** [b]	**80.72** [b]
Mid-winter	88.41	84.64	88.76	87.32	89.49	87.52	90.00	88.14	**89.17** [a]	**86.91** [a]
Late winter	90.78	88.32	89.89	86.94	89.88	86.67	89.67	87.44	**90.00** [a]	**87.24** [a]
Early spring	85.69	80.00	87.13	81.25	85.25	80.79	90.89	81.33	**86.37** [b]	**80.62** [b]
Mean (N)	**87.60**	**83.71**	**87.41**	**83.70**	**88.12**	**83.89**	**89.27**	**85.43**		

*Data not available

**Means (bold) in the same column or row followed by the same letter are not significantly different at P = 0.05

6.4 Conclusion

Overall, pasture productivity was determined by season and not by N fertilisation for both management strategies, although a non-significant increase in PDMP and TDMP in response to increasing rates of N was noted. There was a drastic decrease in PDMP from autumn to early/mid-winter and the highest PDMP occurred in spring – concurrent with increasing mean temperatures. These trends were observed in both the once-off and consecutive application of fertiliser N.

RDMP was unaffected by fertiliser N, probably as a result of the low winter temperatures which prohibited N uptake. This increases the risk of N leaching.

In the first year the pasture consisted predominantly of lucerne. The effect of season and N application on the botanical composition was inconsistent over the two years of the study. There was a tendency that increasing rates of N reduced the clover content with the once-off and consecutive application. Lucerne was unresponsive to N over both years and with both management strategies. Season of N application, however, had a significant effect on lucerne productivity, with lucerne decreasing from autumn, through winter and reaching lowest levels in early spring.

In the first year the grass and clover content was the lowest in autumn, but increased over the winter-spring period. In the second year of the study the pasture was grass dominant and lucerne made a much smaller contribution to sward productivity. The consecutive application of fertiliser N lead to a significant increase in grass production (%), which was not noted with the once-off application. The actual grass content (kg DM ha^{-1}) displayed a similar increase in response to N from late winter to spring. Over both years and both management strategies included in the study the legume fraction remained sufficient for high animal production.

The nutritive characteristics measured in this study were more influenced by season of N application than N application. Pasture DM levels and IVOMD remained within the expected range as described by Meissner et al. 2000.

The TCP content was high (>30%), especially in the winter of Year 1, although this was due to the effect of season of N application and not as a result of N fertilisation. The TCP was lower in Year 2, and increased as the rate of applied N increased.

Due to the poor response to applied N, it is doubtful whether there will be an economic advantage to applying fertiliser N to a grass-lucerne pasture in the Winelands sub-region of the Western Cape.

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Chapter 7

Summary

Pasture production in the Western Cape has traditionally been based on temperate species such as perennial ryegrass (*Lolium perenne*), cocksfoot (*Dactylis glomerata*), tall fescue (*Festuca arundinaceae)*, red and white clovers (*Trifolium pratense* and *Trifolium repens*) and lucerne (*Medicago sativa*). Temperate pastures typically produce the majority of its fodder on offer during spring and autumn where after production decreases in winter, a period commonly referred to as the winter 'fodder gap'. The negative effect of this seasonality can potentially be reduced by the establishment of diverse pasture mixtures, where the growth curves of various species are combined to provide a more consistent amount of fodder throughout the year. Another approach is through boosting pasture production with the application of fertiliser N. Perennial ryegrass has the potential to make a valuable contribution in this regard because it is able to grow and respond to N fertilisation at temperatures as low as 6˚C, which could improve fodder availability during the winter gap.

The application of fertiliser N can also affect the nutritive value of the fodder on offer, most notably the crude protein and dry matter content. The application of high N rates in pursuit of maximum yields often lead to pastures with low DM contents and CP levels in excess of livestock requirements as well as contributing to environmental problems such as nitrate leaching.

The aims of this study were therefore to develop N nitrogen application strategies for mixed grass and grass-legume pastures in the Winelands sub-region of the Western Cape to ensure optimum dry matter production during different seasons. The effect of fertiliser N on selected nutritive characteristics and the botanical composition were also evaluated. The grass-legume pastures where subjected to two management strategies: the once-off application of N and the consecutive application of N over the autumn-early spring period.

The reaction of the mixed grass pasture to applied N was mostly characterised by an interaction between the season of N application and N application rate. The productivity of the pasture in terms of PDMP and TDMP was highest in spring and summer with the application of 60 – 80 kg N ha^{-1}, and decreased in autumn and winter. There was a strong response of winter RDMP to N, which indicated that not all applied N was utilised during the first regrowth cycle, which might present a risk of nitrate being leached below the root zone.

The botanical composition of the mixed grass pasture was determined by season of N application, and not N application rate. The tall fescue content was low over all seasons, presumably due to poor establishment and strong competition from accompanying species. During the cooler months perennial ryegrass and tall fescue was the dominant species, while in the warmer months cocksfoot was the main species. Nitrogen application also had a significant effect on the quality of the pasture, most notably the CP content. The response of the CP content was characterised by a strong interaction between season of N application and N application rate. Crude protein levels in excess of 22% were recorded in autumn and winter with the application of 40 – 80 kg N ha^{-1}. Other characteristics remained within the expected range.

The response of the grass-clover and grass-lucerne pastures in terms of productivity and nutritive characteristics were mainly determined by the season of N application, and not N application rate. Productivity tended to be highest in autumn and early spring for both the once-off and the consecutive N application strategies, emphasizing the effect of temperature on pasture growth.

The effect of season of N application and the N application rate on the botanical composition of the respective pastures were inconsistent over the two years of the study. The clover content tended to decrease in response to increasing rates of N, while the grass fraction was stimulated. Lucerne productivity decreased from autumn through winter and reached minimum levels in early spring, and was unaffected by fertiliser N rate. The legume component in both the grass-clover and grass-lucerne pastures remained above recommended levels of 20 – 40 % for optimum animal production, even when N was applied consecutively.

The nutritive characteristics measured in the study (DM content, CP, IVOMD) remained within the expected range, except the TCP content which was very high in the first year (> 30 %), although N application rate did not have a significant effect.

Based on these findings, preliminary recommendations for N fertilisation for a mixed grass pasture is 40 kg N ha^{-1} during autumn and winter and 60 kg N ha^{-1} in spring and summer. Based on the poor response of the grass-legume pastures to applied N it is doubtful whether fertilisation will lead to an economical advantage, but low rates of approximately 40 kg N ha^{-1} could be beneficial in a grass-clover pasture during autumn and late winter/early spring based on the relatively strong response of PDMP to N during this period.