NITROGEN MANAGEMENT STRATEGIES ON PERENNIAL RYEGRASS-WHITE CLOVER PASTURES IN THE WESTERN CAPE PROVINCE

by

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DECLARATION

I, the undersigned, hereby declare that the work contained in this dissertation is my own original work and I have not previously in its entirely or in part submitted it at any university for a degree.

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ABSTRACT

The response of perennial ryegrass and white clover, grown under controlled conditions, to fertiliser N rates applied under variable soil temperature (6, 12 and 18 °C), soil water potential (-10, -20, -25 and -35 kPa) and seasonal growing (June/July and October/November) conditions as well as field conditions, were evaluated. Primary- (PDM), residual- (RDM) and total dry matter (TDM) production (g pot^{-1}) were recorded over the first- and second regrowth cycles as well as the accumulative DM production over the two regrowth cycles, respectively. Leaf N content (%) was recorded at the end of first and second regrowth cycles. Tiller/stolon numbers and root dry mass (g pot^{-1}) were recorded at the end of the second regrowth cycle. Soil ammonium-N and nitrate-N (mg kg^{-1}) content was monitored after fertiliser N application.

Decreasing soil temperatures resulted in decreased TDM production in both crops. Only perennial ryegrass was influenced by fertiliser N rate, with a general increase in dry matter production as fertiliser N rate was increased. Ryegrass TDM production did not differ between the 100 and 150 kg N ha^{-1} rates but were both higher (P=0.05) if compared to the 0 and 50 kg N ha^{-1} treatments. Soil nitrate levels 31 days after application of 150 kg N ha^{-1} were still sufficient to stimulate ryegrass RDM production. The 173.8% increase in ryegrass TDM production measured at 6 °C where 150 kg N ha^{-1} was applied compared to the 0 kg N ha^{-1} treatment illustrated the ability of ryegrass to respond to fertiliser N at low soil temperatures.

Soil water potential of -20 kPa resulted in higher ryegrass PDM and TDM production compared to the -25 and -35 kPa levels. White clover PDM and TDM production were however not influenced by soil water potential or fertiliser N rate. Ryegrass TDM production increased (P=0.05) as fertiliser N rates were increased. The most favourable soil water level for both ryegrass and clover root development was found to be -35 kPa.

Perennial ryegrass and white clover PDM, RDM and TDM production were higher during the October/November season compared to the June/July season. Increased fertiliser N rates resulted in increased (P=0.05) ryegrass PDM and TDM production. White clover dry matter production was not influenced by fertiliser N rates.

In the field study the effect of 0, 50, 100 and 150 kg N ha^{-1} applied as a single application either in autumn, early winter, late winter, early spring or late spring on pasture dry matter production, clover content and selected quality parameters of a perennial ryegrass-white clover pasture were investigated. Soil nitrogen dynamics in the 0-100, 200-300 and 400-500 mm soil layers were studied for 49 days following fertiliser N application.

The effect of 50 kg N ha^{-1} on soil N dynamics was generally the same as found at the 0 kg N ha^{-1} applications and may therefore be regarded as a low risk treatment. The application of 150 kg N ha^{-1} especially in autumn and early winter showed a tendency to exceed the absorption capacity of the pasture and thereby expose fertiliser N to possible leaching and contamination of natural resources.

Increased fertiliser N rate resulted in a general increase in pasture dry matter production with the highest yields recorded where N was applied in early and late spring and the lowest in early winter. The application of 150 kg N ha^{-1} in early and late spring resulted in the highest TDM production, however, the 50 kg N ha^{-1} resulted in a more efficient conversion of N applied to additional DM produced. In contrast to DM production, the clover percentage generally decreased as fertiliser N rate was increased. The effect of season of application was inconsistent. Annual trends show that the clover percentage eventually recovered to the same levels as the 0 kg N ha^{-1} treatments. Due to the above minimum levels recorded for most mineral and quality parameters tested it is envisaged that treatment combinations as used in this study will not be at any disadvantage to pasture and animal productivity.

The study has shown that the use of fertiliser N to boost perennial ryegrass-white clover productivity and thereby minimising the negative effect of the winter gap on fodder flow management during the cool season in the Western Cape Province, may be an important management tool. Except for late spring applications, all seasons of application reduced the negative impact of the winter gap on fodder availability. It is concluded that regression lines as summarised in Tables 7.2 and 8.2 show great potential to be instrumental in developing regression models, accurately predicting the effect of fertiliser N rate on pasture performance. Other factors to be considered includes the productivity of the pasture, initial clover content, expected clover content at the end of the first regrowth cycle after fertiliser N application and the quantity of additional fodder required. Additional requirements will be to maintain a clover content of between 30 and 50% and to avoid applying high rates of fertiliser N (100 and 150 kg N ha^{-1}) in winter, as the N uptake capacity of the pasture could be exceeded and thereby increasing the risk of N leaching, resulting in environmental pollution. The N response efficiency of the pasture is also the lowest at the 150 kg N ha^{-1} rates, thereby reducing the profitability of these treatments.

UITTREKSEL

Die reaksie van meerjarige raaigras en witklawer op stikstofbemestingspeile by verskillende grondtemperature (6, 12 en 18 °C), grondwaterpotensiale (-10, -20, -25 en -35 kPa) en groeiperiodes (Junie/Julie en Oktober/November) is onder gekontroleerde toestande ge-evalueer. Die primêre- (PDM), residuele- (RDM) en totale droëmateriaalproduksie (TDM) (g pot^{-1}) is oor die eerste- en tweede hergroeisiklusse asook totale droëmateriaalproduksie oor twee siklusse, onderskeidelik, gemeet. Blaar N-inhoud (%) is aan die einde van die eerste en tweede hergroeisiklusse bepaal. Die aantal halms/stolons en worteldroëmassa (g pot^{-1}) is aan die einde van die tweede hergroeisiklus bepaal. Grond ammonium-N en nitraat-N is na toediening van stikstofbehandelings gemonitor.

Beide meerjarige raaigras en wit klawer TDM produksie het afgeneem namate grondtemperatuur gedaal het. Slegs meerjarige raaigras DM produksie is deur N bemesting beïnvloed en het toegeneem namate N peile verhoog is. Raaigras TDM produksie tussen 100 en 150 kg N ha^{-1} het nie onderling verskil nie maar was beide hoër (P=0.05) as by die 0 en 50 kg N ha^{-1} peile. Grond nitraatvlakke 31 dae na toediening van 150 kg N ha^{-1} was steeds voldoende om raaigras RDM produksie te verhoog. Die verhoging van 173.8% in raaaigras TDM produksie by 6 °C met die toediening van 150 kg N ha^{-1} indien vergelyk met die 0 kg N ha^{-1} behandeling bevestig die potensiaal van raaigras om by lae temperature op N bemesting te reageer.

'n Grondwaterpotensiaal van –20 kPa het hoër raaigras PDM en TDM produksie as by die –25 en –35 kPa tot gevolg gehad. Witklawer PDM en TDM produksie was nie deur die behandelings beïnvloed nie. 'n Toename in N peile het raaigras TDM produksie betekenisvol verhoog. Grondwaterpotensiale van –35 kPa het die hoogste wortel DM in beide raaigras en klawer tot gevolg gehad.

Beide meerjarige raaigras en witklawer PDM, RDM en TDM produksie was hoër tydens die Oktober/November seisoen as gedurende Junie/Julie. Verhoogde N peile het hoër raaigras PDM en TDM produksie tot gevolg gehad terwyl witklawer produksie nie beïnvloed was nie.

In die veldstudie is die effek van 0, 50, 100 en 150 kg N ha^{-1} as enkeltoediening gedurende herfs, vroeë winter, laat winter, vroeë lente of laat lente op die DM produksie, klawerinhoud en sekere kwaliteitsparameters van 'n meerjarige raaigras-witklawer weiding ondersoek.

Grondstikstofvlakke is oor ‘n tydperk van 49 dae na toediening van stikstof in die 0-100, 200-300 en 400-500 mm grondlae gemonitor.

Die grondstikstofvlakke gemeet met die toediening van 50 kg N ha^{-1} was gewoonlik dieselfde as by die 0 kg N ha^{-1} behandelings en word dus as ‘n lae risiko N peil beskou. Die toediening van 150 kg N ha^{-1} veral gedurende die herfs en vroeë winter mag die N opnamekapasiteit van die weiding oorskry en daardeur die toegediende N blootstel aan loging en moontlike besoedeling van natuurlike hulpbronne.

Verhoogde N peile veroorsaak ‘n verhoging in DM produksie met die hoogste DM produksie waargeneem met N toedienings gedurende die vroeë en laat lente en die laagste gedurende die vroeë winter. Die toediening van 150 kg N ha^{-1} gedurende vroeë en laat lente het die hoogste DM produksie tot gevolg gehad. Die mees effektiewe omskakeling van N toegedien tot addisionele DM geproduseer is by die 50 kg N ha^{-1} peile waargeneem. In teenstelling met DM produksie het ‘n toename in N peile ‘n afname in persentasie klawer tot gevolg gehad. Geen tendens is ten opsigte van seisoen waargeneem nie. Jaarlikse tendense toon dat die persentasie klawer gewoonlik herstel tot dieselfde vlakke as die 0 kg N ha^{-1} behandelings. Die vlakke van kwaliteits en minerale parameters was meesal hoër as die minimum voorgeskryf en mag die afleiding gemaak word dat geen nadelige effek as gevolg van die behandelingskombinasies verwag word nie.

Die studie het aangetoon dat strategiese stikstofbemesting gedurende die koeler maande in die Westelike Provinsie wel aangewend kan word om droëmateriaalproduksie te verhoog. Uitsluitend die laat lente toedienings, het alle seisoene waartydens strategiese N bemesting toegedien is sekere aspekte rakende die wintergaping suksesvol aangespreek. Die regressievergelykings in Tabelle 7.2 en 8.2 toon potensiaal om modelle te ontwikkel wat gebruik kan word om die effek van N insette op meerjarige raaigras-witklawer weidings akkuraat te voorspel. Addisionele faktore wat in ag geneem moet word sluit die produktiwiteit van die weiding, aanvangsklawer-inhoud, verwagte klawerinhoud na die eerste hergroeisiklus na toediening van N bemesting en die hoeveelheid addisionele voer benodig, in. Die verwagte klawerinhoud moet tussen 30 en 50% wees en die toediening van die hoër N peile gedurende die winter moet vermy word aangesien die N opnamekapasiteit van die weiding waarskynlik oorskry sal word wat N loging en moontlike kontaminasie van natuurlike hulpbronne tot gevolg mag hê. Die feit dat die 150 kg N ha^{-1} behandelings die laagste stilstofverbruiksdoeltreffendheids-waardes tot gevolg gehad het sal ook die winsgewendheid van die behandelings verlaag.

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ABBREVIATIONS

ACC	Absolute clover content
ADM	Annual dry matter production
C	Carbon
Ca	Calcium
CP	Crude protein
DM	Dry matter
IVOMD	In vitro organic matter digestibility
LAN	Limestone ammonium nitrate
N	Nitrogen
NH_4^+ -N	Ammonium-nitrogen
NO_3^- -N	Nitrate-nitrogen
P	Phosphorus
PCP	Primary clover percentage (first regrowth cycle)
PDM	Primary dry matter production (first regrowth cycle)
RCP	Residual clover percentage (second regrowth cycle)
RDM	Residual dry matter production (second regrowth cycle)
TDM	Total dry matter production (accumulative first + second regrowth cycle)

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******Technical layout of this thesis is in accordance with norms prescribed for publication in the South African Journal of Plant and Soil.*

Chapter 1

Introduction

Background

Cultivated pastures in the Western Cape Province of South Africa occupy about 1.02 million ha, representing about 8.59% of all areas used for agricultural purposes, including forestry and natural grazing (Anon, 1996). Both annual and perennial pastures are cultivated as pastures only systems, or in combination with small grains, canola, lupins or vegetables. Various combinations of grass only, legume only or grass-legume pastures are grown on 483 984 ha or 48.7 % of the cultivated area in the southern Cape region (Anon, 2003). Grass-clover pastures (perennial ryegrass, white- and red clover) grown under irrigation form an integral part of the dairy industry (Botha, 2003). Most of the dairy production units are small and emphasis is placed on maintaining high DM production throughout the year.

Climatic conditions in the Western Cape Province however do not favour continuous high fodder productivity throughout the year. Low temperatures causing a steep decline in dry matter production, especially during the cooler autumn and winter months. Seasonal production of perennial ryegrass-white clover pastures decreases to *ca* 500 kg DM $month^{-1}$ in June and July followed by an increase to *ca* 2000 kg DM $month^{-1}$ in October and November (Botha, 1994).

Importance of legumes in mixed pastures

The lower productivity of cultivated pastures during the cool season, the so called “winter or feed gap”, is of major concern and very difficult to address successfully. Grass-clover pastures are no exception. Growing a legume and grass as companion crops intensifies the pressure on pasture management to maintain optimum grass:clover ratios especially with management techniques aiming at bridging the winter gap. Growing grass-clover crops together is challenging but the advantages outnumber the disadvantages. Firstly, clover in the pasture improves pasture quality that results in increased milk-, beef or wool production. Eerens & Ryan (2000) reported that mixed perennial ryegrass-white clover pastures produced nearly 25% more dry matter, 40% (180 kg) more carcass weight and 25% (17 kg) more wool ha^{-1} than ryegrass pastures receiving 270 kg N ha^{-1}. Secondly, a legume clover is capable of fixing large quantities of atmospheric nitrogen and through recycling this N becomes available for absorption by the ryegrass fraction (Martin, 1960) and thereby reducing fertiliser N cost. Martin (1960), in a review article, came to the conclusion that nitrogen transfer within a grass-legume pasture can either be as a result of direct movement of nitrogenous compounds from the legume root nodules through the soil to the companion crop, decaying nodules and legume roots, trampling followed by decomposition of aerial and sub-aerial parts of the legume during grazing or senescence of nodules following changes in the root:shoot ratio of the legume crop, especially if stress related such as defoliation and covering by excreta of the grazing animals. Davidson & Robson (1990) reported that ryegrass in a grass-clover pasture consistently had higher leaf N contents than pure grass grown in monoculture. Thirdly, legumes improve seasonal distribution of the forage by being more productive later in the year than the companion grass crop (Sleugh *et al*., 2000).

Disadvantages of legumes in a pasture are the possibility of bloat in animals and the poor predictability of legume performance (Miles & Manson, 2000). To ensure that fodder of optimum quality and quantity is produced a clover content of between 30 and 50 percent must be maintained (Martin, 1960; Curll, 1982; Harris, 1994).

Factors that may affect the productivity of a perennial ryegrass-white clover pasture

Nitrogen

The availability of nitrogen in the perennial ryegrass-white clover pasture affects the competition between the ryegrass and the clover components. The competitive ability of white clover can be reduced as N supply is increased which will favour the grass and suppress clover productivity (Simpson, 1987). Legume dynamics in a temperate Australian pasture is summarised by the nitrogen driven regeneration cycle as postulated by Turkington & Harper (1979). The regeneration cycle starts with *Trifolium repens* and *Lolium perenne* grown together due to the asynchronous growth cycles and the high N requirement of *L. perenne.* The second phase sees a decline of *T. repens* as N levels rise and grass increases dominance followed by an invasion of *Alopecurus pratensis* and/or *Dactylis glomerata* at high N levels resulting in a decline in *Lolium perenne*. Phase four results in a decline in N levels followed by invasion of slower growing, less N demanding species e.g., *Anthoxanthum odoratum* and

Agrostis tenuis. Invasion by *Trifolium repens* into slow-growing grasses follows and is later joined by *Lolium perenne* completing the regeneration cycle. The detrimental effect of high levels of soil N, direct or indirect, on clover productivity and persistence is eminent from the regeneration cycle.

Management techniques aiming at bridging the winter gap are well documented (Ball, Molloy & Ross, 1978; Eckard & Franks, 1998; McKenzie *et al*., 1999). One technique is the application of fertiliser N to boost pasture productivity during a predetermined season as most farmers rely on biologically fixed N as the main source of nitrogen supply to a perennial ryegrass-white clover pasture. Relying on biologically fixed N as sole N source normally results in poor pasture growth at low temperatures (Field & Ball, 1978; Frame & Boyd, 1987).

Low soil temperature during winter and early spring limits clover-derived N availability to the pasture and will also restrict N-mineralisation. These low temperatures result in grass-clover pastures that rely on biologically fixed N as their main source of N to be often N deficient (Van Berg *et al*., 1981; Nannipieri, Ciardi & Palazzi, 1985). Pasture productivity may be limited by a lack of available soil N from late autumn to late spring due to the reduction in the rate of N-mineralisation and biological N fixation as temperatures decrease (Frame & Newbould, 1986). Clover growth and microbiological activity are low and the possibility of permanent detrimental effects of moderate levels of fertiliser N on the clover fraction very slim. This leaves the opportunity to stimulate grass growth through the application of strategic N fertilization without permanently suppressing clover growth (Frame & Newbould 1986; Stout, Weaver & Elwinger, 2001). Thus, if soil N levels are limiting and optimal N application rates for different seasons during the cool months can be determined, farmers could continue grazing later in autumn and begin grazing earlier in spring and still maintain a desirable clover content to sustain high summer and autumn production. Moller *et al*., (1996 as cited by McKenzie *et al*., 1999) reported that the use of fertiliser N could advance the attainment of a "predetermined" herbage mass by about 2 weeks. However if fertiliser N application is too high, the clover content of the sward becomes too low to provide sufficient N to the sward later in the growing season (Thomas, 1992; Caradus *et al*., 1993).

Application of fertiliser N during the cooler months to boost pasture productivity while still maximizing the utilisation of clover-derived N in the warmer months might be a viable management option. This practice can be very useful since Ball & Field (1982) reports that symbiotic N fixation rarely supplies sufficient N to achieve more than 70% of potential pasture production while Eckard (1994) suggests that more consideration should be given to the possibility that both N fertiliser and N fixation may contribute to the N nutrition of the pasture, but during different seasons of the same year. In all scenarios must the increase in herbage production from fertiliser N be weighed against the possible decline in white clover performance.

Use of nitrogen fertilisers

The application of fertiliser N usually results in an increase in dry matter production of perennial ryegrass-white clover pastures. Stout, *et al*. (2001) found that early season DM yields from grass-clover pastures could be increased by *ca* 20% with an application of about 45 kg N ha^{-1} and by starting to graze at a 15 cm pasture height, the clover fraction in the sward would be maximized. Eckard & Franks (1998) recorded yield increases of between 582 and 703 kg dry matter ha^{-1} with fertiliser N application. Nitrogen response efficiency (kg additional DM produced per kg N applied) however decreases as fertiliser N rate increases (Eckard & Franks, 1998).

It is generally agreed that white clover is at a competitive disadvantage when grown with most grass species. The grasses normally grow taller, have a larger root mass and have less critical climatic and nutritional requirements (Haynes, 1984; Frame & Newbould, 1986). It is widely accepted that the application of fertiliser N results in a decrease in clover content of grass-clover swards. The mechanisms involved are not fully understood. Davidson & Robson (1990) detected evidence that white clover plants respond positively rather than negatively to mineral N. Recent work showed that clover in mixtures are not at any disadvantage relative to grass in terms of competition for sunlight because in a well fertilised sward they raise their leaves high into the canopy and intercept more light per unit leaf area than grass and fix more carbon as a result (Davidson, Robson & Dennis, 1982; Woledge, 1988). Dennis & Woledge (1985) ascribed the decreased clover portion to the increased competition for light brought on by the stimulated growth of the grass. The reduction in clover content could therefore be ascribed to the inability of the clover plant to grow and compete with the grass fraction possibly as a result of slow N supply through biological N fixation or the inability of clover roots to increase N uptake from the soil solution.

In the light of environmental concerns regarding N losses from intensively grazed pastures Olsen & Kurtz (1982) as well as Whitehead (1995), recommended that rates of fertiliser N per application should aim to remain within the steepest portion of the response curve of the pasture to ensure efficient N use. High rates of N fertiliser, applied in a single application, favour losses by volatilization and leaching (Olsen & Kurtz, 1982) and lead to N uptake surplus to the plant's requirement for growth (Eckard, 1990). Excess N taken up by the pasture may also be potentially toxic to ruminants (Eckard, 1990).

Various studies showed that N fertiliser applied to irrigated ryegrass and ryegrass-white clover pastures during active pasture growth, will have little effect after the first harvest. Stout *et al*. (2001) found that the effect of 44.8 kg N ha^{-1} applied in early spring was short lived and largely dissipated after the first month of production while Murtagh (1975, cited Whitehead, 1970) reported that most uptake of fertiliser N occured within four weeks after fertiliser N application. Reid (1984) however, demonstrated that at the harvest immediately after that for which the N was applied, a residual effect of 30-35% of the size of the direct effect was measured. Leaving excessive free N in the soil is not advisable since the possibility of leaching out of the active root zone always exists especially under the high rainfall conditions as experienced in the Western Cape Province in winter.

The growth rate of perennial ryegrass-white clover pastures are amongst others influenced by temperature, water supply as well as daylength and will therefore be determinants in the potential response to fertiliser N application (Whitehead, 1995; Frame, 1994). The application of fertiliser N as a strategic dressing to increase pasture productivity during the cool season in the Western Cape Province will mainly be affected by the three factors listed in combination with the rate of fertiliser applied.

Temperature

Pasture dry matter production (kg DM ha^{-1}), N mineralisation and nitrification are reduced as temperature decreases in autumn. The temperature requirements of perennial ryegrass and white clover differ. Perennial ryegrass is still able to grow and therefore respond to N fertiliser at temperatures below 5 ^{o}C (Frame, 1994; Whitehead, 1995). Although it is commonly believed that white clover shoot growth is possible at 5.8 °C, Martin (1960) found that active N fixation requires a temperature of 9 °C. Munro (1970) concluded that the optimum temperature requirement for clover is within the 9 to 27 °C range with an optimum of 25 °C while Brougham, Bull & Williams (1978) found the range to be 18 to 30 °C with an optimum of 24 °C. Due to higher temperature requirements, white clover will normally start growth 2 to 3 weeks later and cease growth earlier than most ryegrass species (Williams, 1970 cited by Frame & Newbould, 1986). The fact that clover growth will be restricted at soil temperatures of approximately 9 ^{o}C, while ryegrass will still respond to fertiliser N at soil temperatures of 5 °C and even lower, opens the opportunity to stimulate ryegrass production with minimum disturbance of the companion white clover crop.

Determining the effect of temperature on strategic fertilisation over seasons is complicated by the fact that falling temperatures in autumn might be comparable to rising temperatures in spring as confirmed by Eckard & Franks (1998) who found no clear relationship between soil temperature and pasture N response, as some of the responses were measured over a period of both declining and inclining trends in soil temperatures.

Soil moisture

Continuous rain, as often experienced during winter months in the Western Cape Province, can leave the soil water at levels near field water capacity and higher for several days during the cool season. It is anticipated that high soil water levels will influence Rhizobium- and root activity.

Huang, Boyer & Vanderhoff (1975) suggest that the nitrogen fixing ability of the Rhizobia, measured by acetylene reduction, is influenced by the rate of photosynthesis and the supply of assimilates from the host plant. Frederick (1978) in a review article concluded that CO_2 enrichment studies clearly showed that the capacity of the N_2 fixing system could be increased when more photosynthate becomes available. It can therefore be assumed that plants stressed as a result of too high soil water levels will indirectly reduce the Rhizobia's ability to effectively fix atmospheric N.

High soil water content will decrease the volume occupied by the soil air and as a result gaseous exchange between the soil and atmosphere might be slowed down. This restriction in gaseous exchange can reduce plant root activity as well as absorption of plant nutrients from the soil solution (Russell, 1988). Due to contrasting results the response of perennial ryegrass and white clover to fertiliser N applied under high soil water potentials must be evaluated.

Season of production

Productivity, as a function of photosynthesis, of pastures grown under high potential conditions is normally limited by incoming solar energy, while transpiration is controlled by net radiation (Russell, 1988). Most of the net radiation is dissipated as transpiration under these conditions. Since net radiation is closely correlated with incoming radiation, Russell (1988) suggested that rates of photosynthesis and transpiration would be closely related. It could therefore be assumed that the length of exposure to incoming solar energy would influence pasture productivity.

Aim

The aim of this study was to evaluate the possible use of fertiliser N as a management tool to reduce the negative impact of low pasture productivity during the cool season on fodder flow management. This may be achieved either through reducing the duration of the winter gap by stimulating productivity later in autumn or earlier in spring or through increased dry matter production in winter. Specific objectives will firstly be to determine the individual response and production potential of perennial ryegrass and white clover to different fertiliser N levels in combination with different soil temperatures, soil water levels and different seasons of production. Secondly, to monitor the concentration of the ammonium-N and nitrate-N fractions over depth and time and thereby acquiring info that could assist in determining optimum fertiliser N rates not only to maximise N use efficiency but also minimising leaching under the set of conditions that prevailed during the years covered by the study. Thirdly, to determine optimum fertiliser N rate(s) during different seasons that will ensure increased dry matter production, maintaining acceptable clover levels and producing fodder of acceptable quality.

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SECTION 1

Glasshouse study

Growing a legume and grass successfully as a mixed pasture is challenging and needs specialized knowledge of the reaction of the individual species to external influences.

Due to difficulties in the manipulation and control of environmental influences under field conditions, a series of glasshouse studies were done to develop a better understanding of how the different companion crops in a mixed pasture will react to environmental conditions that might occur occasionally under field conditions. The knowledge obtained might contribute towards improved management with special reference to strategic fertiliser N programmes during the cool season when pasture productivity under field conditions decrease dramatically.

Three trials to evaluate the effect of soil temperature, soil water potential and production season were done and are discussed in Chapters 2, 3 and 4. The results obtained from these studies can be very important in explaining certain reactions observed in the field study. Of special importance is that the growth medium was collected from the experimental site used for the field study. It could therefore be assumed that soil factors related to the treatments be representative of what could happen under field conditions if similar conditions tested in the glasshouse are experienced under field conditions.

Chapter 2

The effect of soil temperature and fertiliser N rate on soil N dynamics and the growth of perennial ryegrass *(Lolium perenne)* and white clover *(Trifolium repens)* grown under controlled conditions.

Abstract

The effects of 0, 50, 100 and 150 kg N ha^{-1} on N-mineralisation as well as the growth and development of perennial ryegrass and white clover grown in pots at root temperatures of 6, 12 and 18 °C were investigated. Soil samples for ryegrass were collected at 2, 8, 15, 31 and 60 days and clover 7, 14, 21, 31 and 60 days after fertiliser N application and analyzed for ammonium- and nitrate-N. Dry matter production and leaf nitrogen content were recorded at 31 (primary dry matter production) and 60 days (residual dry matter production) after fertiliser N application. Root dry mass was recorded at 60 days.

The highest (P=0.05) primary ryegrass dry matter yields (PDM) were recorded at 100 kg N ha^{-1} and the lowest (P=0.05) at 0 kg N ha^{-1}. Mean residual ryegrass dry matter production (RDM) increased as fertiliser N rate increased to 150 kg N ha^{-1}. The lower soil nitrate levels from days 31 to 60 restricted residual ryegrass dry matter production at 12 °C to the same level as 6 °C possibly as a result of the lower soil N content restricting N supply to roots. Perennial ryegrass PDM and TDM yields were the lowest at 6 °C. White clover PDM, RDM and TDM were influenced (P=0.05) only by soil temperature resulting in slightly lower yields at 6 °C. Increasing fertiliser N rates increased (P=0.05) the number of ryegrass tillers per plant but did not influence clover stolon production. Leaf nitrogen content at 31 days in both ryegrass and clover were higher than the 2 to 3.2% or 3.2 to 3.6% regarded as adequate for ryegrass and white clover respectively. N recovery rates of 100% were achieved at most of the treatment combinations mainly as a result of the impermeable pots. Nitrification of ammonium increased between days 15 and 31 causing nitrate content to increase, especially at 18 and 12 °C and simultaneously decreasing ammonium-N content. Soil nitrate-N levels at 31 days were still sufficient to stimulate ryegrass RDM yields at the 150 kg N ha^{-1} rates. The study showed that perennial ryegrass could respond to fertiliser N at soil temperatures as low as 6 °C while white clover response will be less affected.

Keywords: dry matter production, fertiliser nitrogen rate, leaf nitrogen, NH_4^+-N, NO_3^--N, perennial ryegrass, root dry mass, temperature, tillers

Introduction

Herbage shortages as a result of low soil temperatures occur between autumn and spring when temperatures in the Stellenbosch district of the Western Cape Province decrease from an average monthly minimum (maximum in brackets) of 11.5 °C (23.8 °C) in April to a low of 7.2 °C (16.8 °C) in July (Anon, 2004). The application of fertiliser N to boost dry matter production of perennial ryegrass-clover pastures during the cool season generally consists of a single application dictated by herbage supply and demand. Information regarding the response of perennial ryegrass to fertiliser N applied during the cool season in the Western Cape Province is required to develop an efficient strategic N fertilisation programme.

Different rates of fertiliser N will influence crop characteristics, including plant cells (Russell, 1988), foliage:root ratios (Hatch & MacDuff, 1991), dry matter production (Eckard, 1994) and herbage quality (Hegarty, 1981; Hibbet, 1984 cited by Eckard, 1994). The characteristics of the growth medium or soil will strongly influence root activity (Russell, 1988) as well as soil N reactions and availability (Clarkson & Warner, 1979; Tinker, 1979; Miles & Manson, 2000).

The fate of the applied fertiliser is influenced by a range of potential reactions related to soil temperature such as denitrification (Tinker, 1979), immobilisation and mineralisation (Miles & Manson, 2000). Soil temperature may also indirectly influence N absorption due to changes in plant growth rate and partitioning of growth between roots and shoots and thus affecting growth-led demand for soil nitrogen uptake (Kessler, Boller & Nösberger, 1990).

Varying soil temperatures will affect the fertiliser N applied as well as the ability of the pasture to effectively respond to the applied fertiliser N. Nitrogen absorption is expected to decrease as temperatures decrease (Hatch & MacDuff, 1991), as will the absorption of nitrate relative to ammonium in perennial ryegrass (Clarkson & Warner, 1979). Clarkson and Warner (1979) stated that ammonium is more readily absorbed at lower temperatures if applied in the same concentration as nitrate but attribute the differences to changes in different parts of the cell membrane rather than soil temperature suggesting that ammonium inhibits the absorption of nitrate. These arguments lead to the final suggestion that the transition temperature (difference between soil and plant temperature) seems to account for the increased ammonium absorption rather than nitrate at low temperatures, a factor not considered in this study. Low soil temperatures will also result in reduced root activity restricting the response to fertiliser N as Hatch and MacDuff (1991) reported that mean rates of total N uptake of clover per unit shoot weight changed little between 9 and 25 °C, but decreased progressively as soil temperatures drop to below 9 °C due to a decline in uptake rates of ammonium and nitrate. This reaction will be crop specific and might open the opportunity to stimulate the productivity of one crop in a mixed pasture with minimum detrimental effects to the companion crop(s). The reduced permeability of the root membranes under low temperatures may be a contributing factor (Russell, 1988). Nodulation and symbiotic N fixation will be negatively affected as soil temperatures decrease (Russell, 1988). Hatch and MacDuff (1991) however, suggest that N_2 fixation by clover under sustained low soil ammonium and nitrate concentrations will be less sensitive to low root temperatures than are either the ammonium or nitrate uptake systems. A statement supported by results showing that the contribution of N_2 fixation decreased with increased temperature from 51% at 5 °C to 18% at 25 °C. The N_2 fixation at 5 °C will possibly not sustain moderate clover productivity as Martin (1960) found that white clover requires a temperature of 9 °C for active N fixation.

The aim of this study was to evaluate the response of perennial ryegrass and white clover to fertiliser N at different soil temperatures and to investigate the possibility to increase species productivity through increased soil-N levels at these lower soil temperatures (as is found during the cool season in the Western Cape Province). Understanding and quantifying the response of perennial ryegrass to these variables are necessary to optimise fertiliser N management under low temperature conditions.

Materials and methods

Locality

Perennial ryegrass (*Lolium perenne* cv. Ellet) and white clover (*Trifolium repens* cv. Haifa) were established separately in pots in a glasshouse under natural photoperiod and light intensity conditions at the Institute for Plant Production, Elsenburg (altitude 177m, 18^{o}50'E, 33°51’S). Day/night temperatures were regulated at 18 and 12 °C for 10 and 14 hours respectively.

Growth medium preparation

Topsoil (0-150 mm layer) from the orthic A horizon of an Oakleaf soil (Soil Classification Working Group, 1991) derived mainly from granite (Anon, 1996) was used as growth medium. To achieve a relative uniform bulk density over all pots the soil was gathered in heaps, mixed and ran through a 5 mm screen to separate the larger clods (aggregates) and crop residues from the soil used as growth medium. A composite soil sample was collected and analysed for both physical- and chemical properties (Table 2.1). Physical soil properties were determined using the hydrometer method as described by van der Watt (1966). Extractable P, K, Na, Ca and Mg were determined using the citric acid (1%) method of analysis, extractable Cu, Mn and Zn by di-ammonium EDTA and extractable B by the hot water technique. The Walkley-Black method was used to determine the organic carbon content (Non-Affiliated Soil Analysis Work Committee, 1990).

Table 2.1 Chemical analysis and selected physical properties of the soil used as growth medium at Elsenburg

Chemical properties			Physical properties		
pH (KCl)		6.3	**Clay**	**%**	14
Resistance	**Ohms**	830	**Silt**	**%**	13.1
P (citric acid)	**mg/kg**	36	**Fine Sand**	**%**	49.8
K	**cmol(+)/kg**	0.27	**Medium Sand**	**%**	15.9
Ca	**cmol(+)/kg**	2.36	**Course Sand**	**%**	7.2
Mg	**cmol(+)/kg**	1.08			
Na	**cmol(+)/kg**	0.47	**Classification**		SaLm
Total cations	**cmol(+)/kg**	4.18			
Copper	**mg/kg**	0.94			
Zinc	**mg/kg**	0.81			
Manganese	**mg/kg**	76.84			
Boron	**mg/kg**	0.47			
Carbon	**%**	0.64			
Ammonium-N	**mg/kg**	3.933			
Nitrate-N	**mg/kg**	7.507			

The variation in soil properties (organic carbon etc), between the soil used as growth medium in the different glasshouse studies can be ascribed to the fact that soil for the studies were collected in different camps adjacent to the camp where the field study (see Chapter 5) was done. The pastures grown in these camps were not the same therefore resulting in the differences as observed. Soil fertility levels were corrected through application of single superphosphate and potassium chloride to levels recommended by Beyers (1983). Copper, manganese and zinc were sufficient with boron marginally low. Foliar nutrition (N, P, K, Ca, Mg, S, B, Fe, Zn and Mo) was applied twice during the pre-treatment growth period and five days after each cut to prevent any nutrient deficiencies. The C content of 0.64 was low. An equivalent of 40 kg N ha^{-1} was applied at seeding to maintain plant growth during the pre-treatment growth period. Ammonium-N (3.93 mg kg^{-1}) and nitrate-N (7.51 mg kg^{-1}) content were determined before N treatment application. The soil was dried in stainless steel bins at 60 °C. To facilitate non-destructive soil sampling during the growth period, pots were lined with plastic bags and filled with 6.6 kg of the oven dried soil, occupying a volume of 4324.57 cm^3, resulting in a bulk density of 1.53 g cm^{-3}. Pots were watered and left for ten days to enable weed seed to germinate. After removing the weeds a template with five holes, one in the centre and one in each quarter of the pot, was used to plant the seed in a predetermined configuration at 5-10 mm depth. A few seeds were planted per hole followed by water application to fill the soil to field water capacity (0.253 mm^3 mm^{-3}). Seedlings were thinned after emergence to five plants per pot. Pots were watered daily through weighing and adding water to the predetermined weight at field water capacity. Changes in plant weight as a result of growth, on a weekly basis, and soil removal due to soil sampling were considered when pot weight after watering was calculated. Pots were randomised after watering.

Plants was allowed to grow for an accumulative photoperiod of *ca* 691 hours from planting and clipped at 50 mm height. The clippings were dried at 60 °C for *ca* 18 h upon which pots showing least variation in DM production were at random allocated to the treatment combinations. Waterbaths were filled with water and covered with a layer of fermolite that served as isolation to maintain a constant water temperature. To ensure uniform soil temperature the water level was kept to the same height as the soil in the pots. Water temperature was electronically controled at 6, 12 and 18 °C. After treatments were allocated (not applied), pots were placed in the water baths and left for 72 hours to stabilise at the temperature of the water followed by application of the N treatments.

Experimental design and treatments

The experimental design was a completely randomised design (Snedecor & Cochran, 1967) with a factorial treatment design. Factors tested were soil temperatures (6, 12 and 18 °C) and fertiliser N rate (the equivalent of 0, 50, 100 and 150 kg N ha^{-1} applied as LAN dissolved in 200 ml water). Fertiliser N rate was replicated four times. Water temperatures were recorded using MCS 486 T temperature dataloggers.

Data collection

DM production was recorded by cutting the ryegrass at 50 mm height 31 and 60 days after nitrogen treatments had been applied. Fresh weight was recorded, cuttings ovendried at 60 °C for 72 hours and dry weight noted. Dried cuttings were ground to pass through a 1mm mesh screen and analyzed for N content using the Dumas-N method (AOAC, 1970). The number of tillers at 60 days was recorded. After residual dry matter production was recorded, roots were removed by wet sieving using a 2 and 1 mm combination sieve, dried at 60 °C for 72 h and dry mass recorded.

Dry matter production was recorded as primary - (PDM) at 31 days, residual – (RDM) production from 31 to 60 days and total dry matter production (TDM which is the cumulative DM production over 60 days). Leaf N yield (g N pot^{-1}) was calculated as the product of leaf N % and dry matter produced.

Soil samples were collected at 2, 8, 15, 31 and 60 days after the N treatments were applied in ryegrass and 7, 14, 21, 31 and 60 days in clover. Soil samples were collected through pulling the plastic bag containing the soil and plants from the pot. Four sub samples per pot were taken, at the soil surface, 3 cm from top, in center (9 cm) and 3 cm from the bottom, through cutting holes in the bags at the front, back, left and right hand side of the pot and bulked as one sample. After sealing the sample-holes with 50 mm cello tape the bags were put back into the pots. To minimise any changes in ammonium and nitrate content samples were immediately dried using electric fans and stored in a freezer (Westfall, Henson & Evans, 1978) until analyzed for NH_4^--N and NO_3^--N content using the Auto Analyzer method (Bessinger, 1985). Allthough some nitrate losses could be expected (Wiltshire & Du Preez, 1994a; Wiltshire & Du Preez, 1994b), air drying of soil samples was decided on as the aim of measuring soil N content was to compare soil N levels as a result of the different treatment combinations.

Statistical procedures

Analysis of variance (ANOVA) was performed using SAS version 8.2 (SAS, 1999). The Shapiro-Wilk test was used to test for non-normality (Shapiro & Wilk, 1965). Student's t-Least Significant Difference (LSD) test was calculated at the 5% confidence level to compare treatment means (Ott, 1998).

Results and discussion

Soil ammonium- and nitrate-N content

Perennial ryegrass

Differences in NH_4^+-N and NO_3^--N content in ryegrass as a result of fertiliser N rate were found within two days after fertiliser N application (Table 2.2; Figures 2.1 & 2.2). Mean NH_4^+-N content at the 6, 12 and 18 °C treatments, two days after N applications were 15.16, 21.95 and 18.68 mg kg^{-1}, respectively (Table 2.2). A decrease in NH_4^+-N content at 12 and 18 °C between days 8 and 15 coincide with an increase in NO_3^--N content at these temperatures indicating nitrification of ammonium to nitrate (Figure 2.2). NO_3^--N content increased from 16.99 to 21.4 and 23.21 mg kg^{-1}at 6, 12 and 18 °C two days after fertiliser N application. The highest mean NO_3^--N levels at 12 and 18 °C were recorded at day 15 possibly as a result of nitrification of ammonium between days 8 to 15 (Table 2.2 & Figure 2.1). Data recorded suggest that, with the exception of NO_3^--N at 12 °C, the effect of the 150 kg N ha^{-1} treatments will last for a maximum of 31 days.

Relative low mean NH_4^+-N (13.55 mg kg^{-1}) and NO_3^--N levels (13.312 mg kg^{-1}) were recorded at 6 °C (Table 2.2). No differences in NH_4^+-N content as a result of fertiliser N were observed from days 15 to 60 after fertiliser N application. Mean NH_4^+-N content between days 31 and 60 decreased from 9.88 mg kg^{-1} to 3.22 mg kg^{-1}. The application of 150 kg N ha^{-1} resulted in significantly higher NO_3^--N values compared to 0 and 50 kg N ha^{-1} for 31 days following fertiliser N application. Mean NO_3^--N content between days 31 and 60 decreased from 14.46 mg kg^{-1} to 6.49 mg kg^{-1}. Clarkson & Warner (1979) reported that the absorption of ammonium exceeded the absorption of nitrate if perennial ryegrass root systems were exposed to soil temperatures below 14 °C. The reduction in NH_4^+-N content between day 17 and 31 without the typical increase in NO_3^--N content (as observed at 12 and 18 °C), can be ascribed to the uptake of ammonium and low mineralisation as a result of the low soil temperature.

Table 2.2 Soil ammonium-N and nitrate-N content (mg kg^{-1}) in perennial ryegrass pots as influenced by soil temperature (°C) and fertiliser N rate (kg N ha^{-1}) under controlled conditions over a 60 day period following fertiliser N application at Elsenburg

		Soil temperature (°C)								
		6 °C		12 °C		18 °C		Mean	Mean	Mean
Days after fertiliser N application	N rate (kg ha^{-1})	Ammonium-N (mg kg^{-1})	Nitrate-N (mg kg^{-1})	Ammonium-N (mg kg^{-1})	Nitrate-N (mg kg^{-1})	Ammonium-N (mg kg^{-1})	Nitrate-N (mg kg^{-1})	Ammonium-N (mg kg^{-1})	Nitrate-N (mg kg^{-1})	Total-N (mg kg^{-1})
2	**0**	1.750 b*	7.813 c	4.400 b	13.013 b	0.73 b	15.653 b	***2.293***	***12.160***	***7.227***
	50	16.800 ab	11.787 bc	15.767 b	17.720 b	19.20 ab	17.947 b	***17.256***	***15.818***	***16.537***
	100	9.267 b	15.267 b	8.907 b	19.493 b	23.73 ab	21.880 b	***13.968***	***18.880***	***16.424***
	150	32.803 a	33.093 a	58.733 a	35.360 a	31.07 a	37.370 a	***40.869***	***35.274***	***38.072***
	Mean	**15.16**	**16.99**	**21.95**	**21.4**	**18.68**	**23.21**			
8	**0**	4.551 b	7.453 c	9.20 c	12.347 b	7.200 b	10.05 b	***6.984***	***9.950***	***8.467***
	50	15.867 ab	12.920 bc	24.87 bc	20.187 b	20.533 b	39.47 a	***20.423***	***24.192***	***22.308***
	100	11.480 b	14.400 b	53.07 ab	19.933 b	61.267 a	39.56 a	***41.939***	***24.631***	***33.285***
	150	28.467 a	22.040 a	72.43 a	40.480 a	51.617 a	54.72 a	***50.838***	***39.080***	***44.959***
	Mean	**15.09**	**14.2**	**39.89**	**23.24**	**35.15**	**35.95**			
15	**0**	24.66 a	8.867 b	8.853 b	13.627 b	5.467 b	12.28 c	***12.993***	***11.591***	***12.292***
	50	12.27 a	12.507 b	12.933 b	27.840 b	10.667 ab	37.97 bc	***11.957***	***26.106***	***19.031***
	100	18.53 a	12.733 b	23.067 ab	22.387 b	13.733 ab	57.13 ab	***18.443***	***30.750***	***24.597***
	150	42.13 a	23.553 a	35.520 a	54.480 a	17.467 a	78.07 a	***31.706***	***52.034***	***41.870***
	Mean	**24.4**	**14.42**	**20.09**	**29.58**	**11.83**	**46.36**			
31	**0**	7.487 a	11.813 b	4.283 b	10.373 b	8.067 a	6.280 b	***6.612***	***9.489***	***8.051***
	50	6.933 a	11.237 b	6.400 b	10.493 b	8.417 a	10.120 b	***7.250***	***10.617***	***8.933***
	100	12.833 a	15.600 ab	8.667 b	11.508 b	12.933 a	14.350 b	***11.478***	***13.819***	***12.649***
	150	12.267 a	19.187 a	18.133 a	28.600 a	18.133 a	42.440 a	***16.178***	***30.076***	***23.127***
	Mean	**9.88**	**14.46**	**9.37**	**15.24**	**11.89**	**18.3**			
60	**0**	3.280 a	5.787 a	2.280 a	6.880 b	2.6133 a	8.387 a	***2.724***	***7.018***	***4.871***
	50	3.173 a	7.827 a	4.213 a	5.693 b	2.6533 a	9.860 a	***3.346***	***7.793***	***5.570***
	100	2.947 a	6.680 a	2.830 a	5.800 b	3.8133 a	5.280 a	***3.197***	***5.920***	***4.558***
	150	3.4667 a	5.667 a	3.707 a	12.467 a	3.6133 a	7.333 a	***3.596***	***8.489***	***6.042***
	Mean	**3.22**	**6.49**	**3.26**	**7.71**	**3.17**	**7.72**			
	Mean N fractions	***13.55***	***13.312***	***18.912***	***19.434***	***16.144***	***26.308***			
	Mean temperature	***13.431***		***19.173***		***21.226***				

**** Means in the same column at a specified day following fertiliser N application followed by the same letter are not significantly different (P=0.05)***

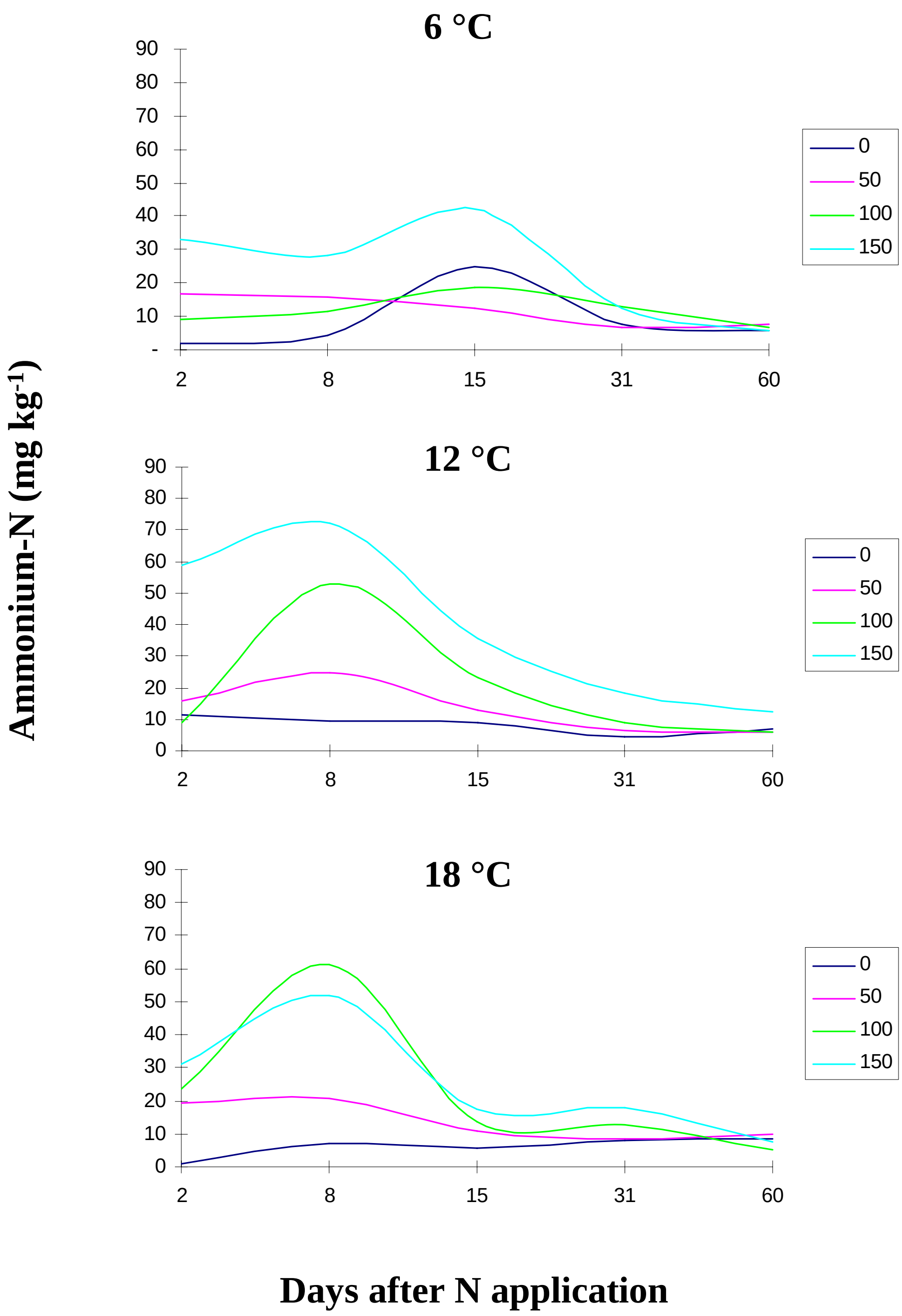

Figure 2.1 The effect of fertiliser N rate (kg N ha^{-1}) and soil temperature (°C) on soil ammonium-N content (mg kg^{-1}) in perennial ryegrass over a 60 day period following fertiliser N application under controlled conditions at Elsenburg.

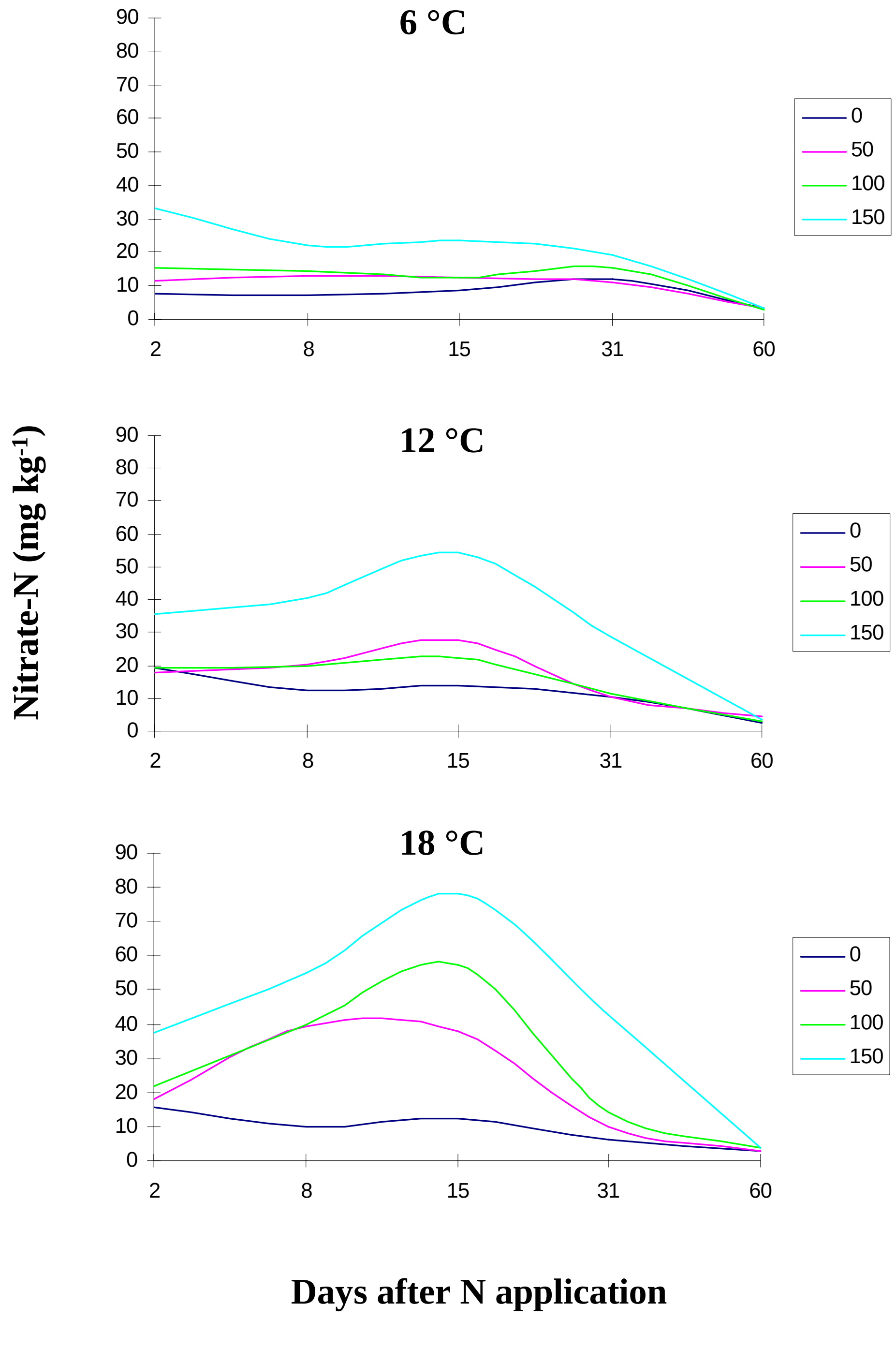

Figure 2.2 The effect of fertiliser N rate (kg N ha^{-1}) and soil temperature (°C) on soil nitrate-N content (mg kg^{-1}) in perennial ryegrass over a 60 day period following fertiliser N application under controlled conditions at Elsenburg.

Mean levels of NH_4^+-N (18.912 mg kg^{-1}) and NO_3^--N (19.434 mg kg^{-1}) at 12 °C were higher than at 6 °C (Table 2.2). The application of 150 kg N ha^{-1} resulted in significantly higher NH_4^+-N and NO_3^--N levels compared to 0 and 50 kg N ha^{-1} for 31 and 61 days following fertiliser N application respectively. The highest NH_4^+-N levels were recorded 8 days, and NO_3^--N 15 days, after fertiliser N application. The increase in NO_3^--N content recorded at 15 days, could possibly be the result of nitrification as NH_4^+-N content rapidly decreased between days 8 and 15. Mean NH_4^+-N content decreased from 9.37 mg kg^{-1} to 3.26 mg kg^{-1} and NO_3^--N from 15.24 mg kg^{-1} to 7.71 mg kg^{-1} between days 31 and 60.

The highest mean NH_4^+-N (16.144 mg kg^{-1}) and NO_3^--N (26.308 mg kg^{-1}) levels were recorded at 18 °C (Table 2.2). The application of 150 kg N ha^{-1} resulted in the significantly higher NH_4^+-N and NO_3^--N values compared to 0 and 50 kg N ha^{-1} for 15 and 31 days respectively following fertiliser N application. The highest NH_4^+-N and NO_3^--N values were recorded at the 100 and 150 kg N ha^{-1} rates 8 and 15 days after fertiliser N application respectively. The sharp increase in nitrate at 15 days is possibly the result of nitrification as NH_4^+-N content of the 100 and 150 kg N ha^{-1} treatments decreased sharply between days 8 and 15. Mean NH_4^+-N content decreased from 11.89 mg kg^{-1} to 3.17 mg kg^{-1} and NO_3^--N from 18.30 mg kg^{-1} to 7.72 mg kg^{-1} from days 31 to 60.

Data recorded suggest that the effect of the fertiliser treatments will mainly occur within the first 31 days after fertiliser N application and will generally not last beyond 60 days.

White clover

Mean NH_4^+-N content of soil from clover pots 7 days after N applications were 16.54, 8.697 and 5.947 mg kg^{-1} at the 6, 12 and 18 °C treatments respectively (Table 2.3 & Figures 2.3 & 2.4). A rapid decline in NH_4^+-N content at 6 and 12 °C were observed between 7 and 14 days after 100 kg N ha^{-1} was applied (Figure 2.3). The same response was observed at the 150 kg N ha^{-1} rate, the only difference being a decline in NH_4^+-N occurring between day 14 and 21. Results show that treatments will only affect NH_4^+-N levels at 6 and 12 °C and will last for 14 and 21 days at 100 and 150 kg N ha^{-1} respectively. In general NO_3^--N levels were the highest between 14 and 21 days following 100 and 150 kg N ha^{-1} applications (Figure 2.4). A gradual decrease in NO_3^--N levels over the 60 day monitoring period were measured at the 0 and 50 kg N ha^{-1} rates.

Relative high mean NH_4^+-N (8.463 mg kg^{-1}) and NO_3^--N levels (22.598 mg kg^{-1}) were recorded at 6 °C (Table 2.3) indicating low fertiliser N uptake under low soil temperature conditions. In contrast to ryegrass, mean NH_4^+-N content between days 31 and 60 increased from 3.7 mg kg^{-1} to 5.823 mg kg^{-1}. Mean NO_3^--N content between days 31 and 60 decreased from 12.820 mg kg^{-1} to 3.701 mg kg^{-1}, an indication that clover absorbed at least part of the fertiliser N applied.

Mean levels of NH_4^+-N (6.228 mg kg^{-1}) and NO_3^--N (17.37 mg kg^{-1}) at 12 °C were lower than at 6 °C, suggesting an increase in N uptake by white clover as temperature increases (Table 2.3). The increase in NO_3^--N content observed between 14 and 21 days after N application is possibly the result of nitrification of ammonium as NH_4^+-N content rapidly decreased between days 14 and 21. Mean NH_4^+-N content increased from 4.86 mg kg^{-1} to 4.985 mg kg^{-1} and NO_3^--N decreased from 9.825 mg kg^{-1} to 3.571 mg kg^{-1} between days 31 and 60.

The lowest mean NH_4^+-N (5.251 mg kg^{-1}) and NO_3^--N (16.358 mg kg^{-1}) levels were recorded at 18 °C (Table 2.3) suggesting increased uptake of inorganic nitrogen by clover at higher soil temperatures. No differences in NH_4^+-N were measured while the NO_3^--N levels remained relative constant between days 7 and 14 followed by a slight decrease between days 14 and 21. Mean NH_4^+-N content increased from 4.949 mg kg^{-1} to 5.910 mg kg^{-1} and NO_3^--N decreased from 12.209 mg kg^{-1} to 3.644 mg kg^{-1} from days 31 to 60.

The reduction of NH_4^+-N and NO_3^--N indicated that white clover does absorb fertiliser N from the soil solution. The higher N content at 6 °C also suggested that white clover activity was reduced which restricted the uptake of fertiliser N. Data showed that ryegrass can absorb more N compared to clover at lower temperatures and might therefore respond to fertiliser N when clover activity is low.

Table 2.3 Soil ammonium-N and nitrate-N content (mg kg^{-1}) in white clover pots as influenced by soil temperature (°C) and fertiliser N rate (kg N ha^{-1}) under controlled conditions over a 60 day period following fertiliser N application at Elsenburg

Days after fertiliser N application	N rate (kg ha^{-1})	6 °C Ammonium-N (mg kg^{-1})	6 °C Nitrate-N (mg kg^{-1})	12 °C Ammonium-N (mg kg^{-1})	12 °C Nitrate-N (mg kg^{-1})	18 °C Ammonium-N (mg kg^{-1})	18 °C Nitrate-N (mg kg^{-1})	Mean Ammonium-N (mg kg^{-1})	Mean Nitrate-N (mg kg^{-1})	Mean Total-N (mg kg^{-1})
7	**0**	3.98 a	17.200 b	3.983 a	16.150 a	4.097 a	6.623 b	***4.020***	***13.324***	***8.672***
	50	8.885 a	21.04 b	4.583 a	19.257 a	4.963 a	20.660 ab	***6.144***	***20.319***	***13.231***
	100	26.00 a	60.380 a	13.483 a	29.210 a	7.381 a	36.667 a	***15.621***	***42.086***	***28.854***
	150	27.295 a	37.745 ab	12.737 a	24.520 a	7.347 a	37.613 a	***15.793***	***33.293***	***24.543***
		16.540	**34.091**	**8.697**	**22.284**	**5.947**	**25.391**			
14	**0**	5.06 a	13.82 b	5.447 b	15.10 a	4.7067 a	6.94 a	***5.071***	***11.953***	***8.512***
	50	7.54 a	19.08 b	4.230 b	26.85 a	4.7433 a	19.90 a	***5.504***	***21.943***	***13.724***
	100	8.20a	66.38 a	6.300 b	37.14 a	4.180 a	35.65 a	***6.227***	***46.390***	***26.308***
	150	21.15 a	61.08 a	15.233 a	35.96 a	5.5633 a	39.90 a	***13.982***	***45.647***	***29.814***
		10.488	**40.090**	**7.803**	**28.763**	**4.798**	**25.598**			
21	**0**	4.220 a	10.81 a	5.1800 a	7.307 c	4.9700 a	3.863 b	***4.790***	***7.327***	***6.058***
	50	5.96 a	13.43 a	4.5433 a	15.597 bc	4.1233 a	13.913 ab	***4.876***	***14.313***	***9.594***
	100	8.1 a	41.77 a	4.6933 a	29.303 ab	3.8033 a	16.243 ab	***5.532***	***29.105***	***17.319***
	150	4.775 a	23.14 a	4.7633 a	37.423 a	5.7067 a	25.783 a	***5.082***	***28.782***	***16.932***
		5.764	**22.288**	**4.795**	**22.408**	**4.651**	**14.951**			
31	**0**	5.530 a	10.92 a	4.197 a	8.483 a	4.340 a	2.687 b	***4.689***	***7.363***	***6.026***
	50	3.320 ab	6.50 a	5.247 a	7.827 a	4.513 a	15.157 ab	***4.360***	***9.828***	***7.094***
	100	1.460 b	2.32 a	5.867 a	5.060 a	4.753 a	7.827 b	***4.027***	***5.069***	***4.548***
	150	4.490 a	31.54 a	4.127 a	17.930 a	6.190 a	23.163 a	***4.936***	***24.211***	***14.573***
		3.700	**12.820**	**4.860**	**9.825**	**4.949**	**12.209**			
60	**0**	6.495 a	2.775 a	4.600 a	2.893 a	5.177 a	2.833 a	***5.424***	***2.834***	***4.129***
	50	5.435 a	2.430 a	4.2967 a	2.783 a	6.123 a	2.807 a	***5.285***	***2.673***	***3.979***
	100	5.6600 a	5.00 a	5.5467 a	2.287 a	6.557 a	2.647 a	***5.921***	***3.311***	***4.616***
	150	5.700 a	4.600 a	5.4967 a	6.320 a	5.783 a	6.290 a	***5.660***	***5.737***	***5.698***
		5.823	**3.701**	**4.985**	**3.571**	**5.910**	**3.644**			
	Mean N fractions	***8.463***	***22.598***	***6.228***	***17.370***	***5.251***	***16.358***			
	Mean temperature	***15.530***		***11.799***		***10.805***				

**** Means in the same column at a specified day following fertiliser N application followed by the same letter are not significantly different (P=0.05)***

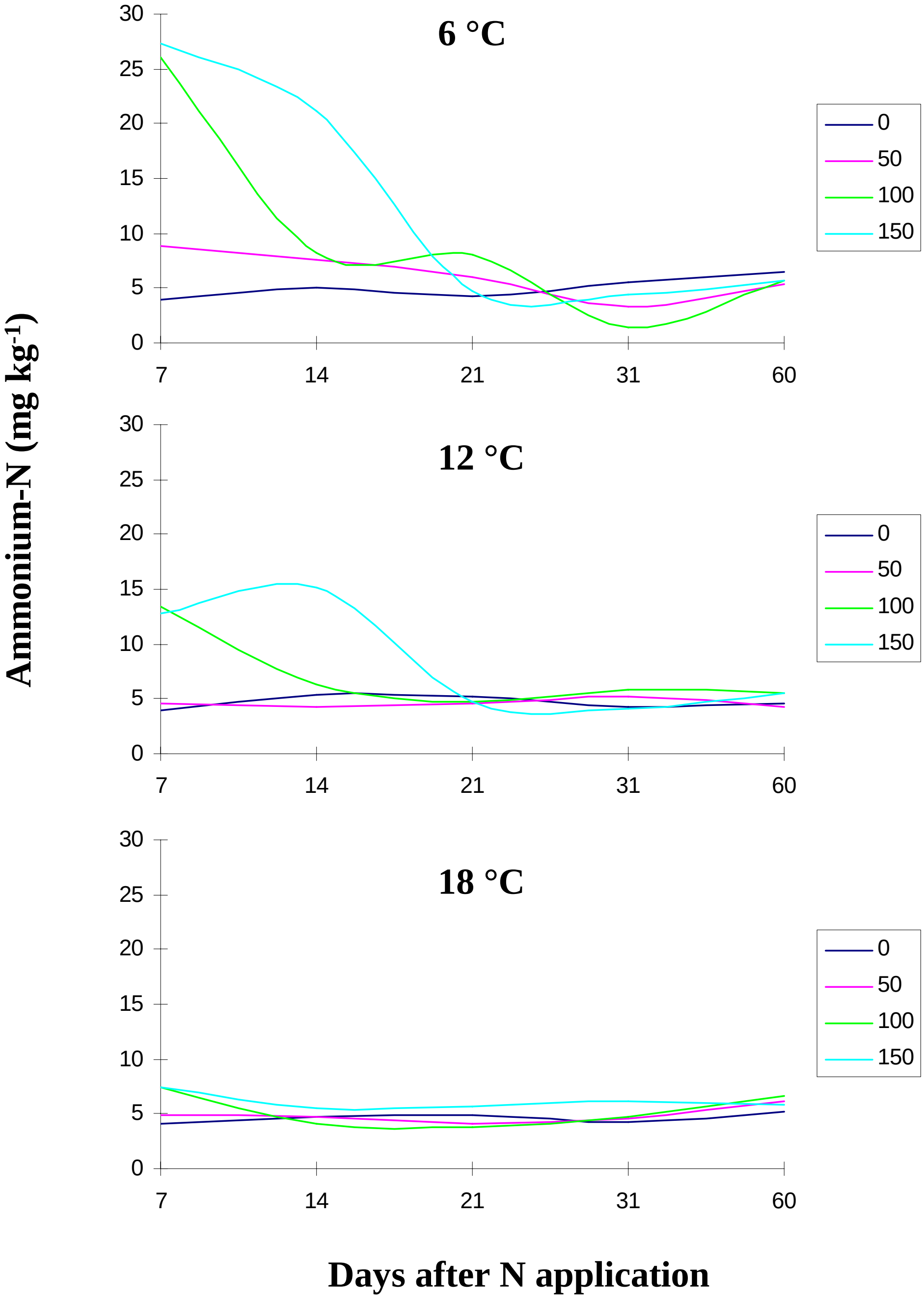

Figure 2.3 The effect of fertiliser N rate (kg N ha^{-1}) and soil temperature (°C) on soil ammonium-N content (mg kg^{-1}) in white clover over a 60 day period following fertiliser N application under controlled conditions at Elsenburg.

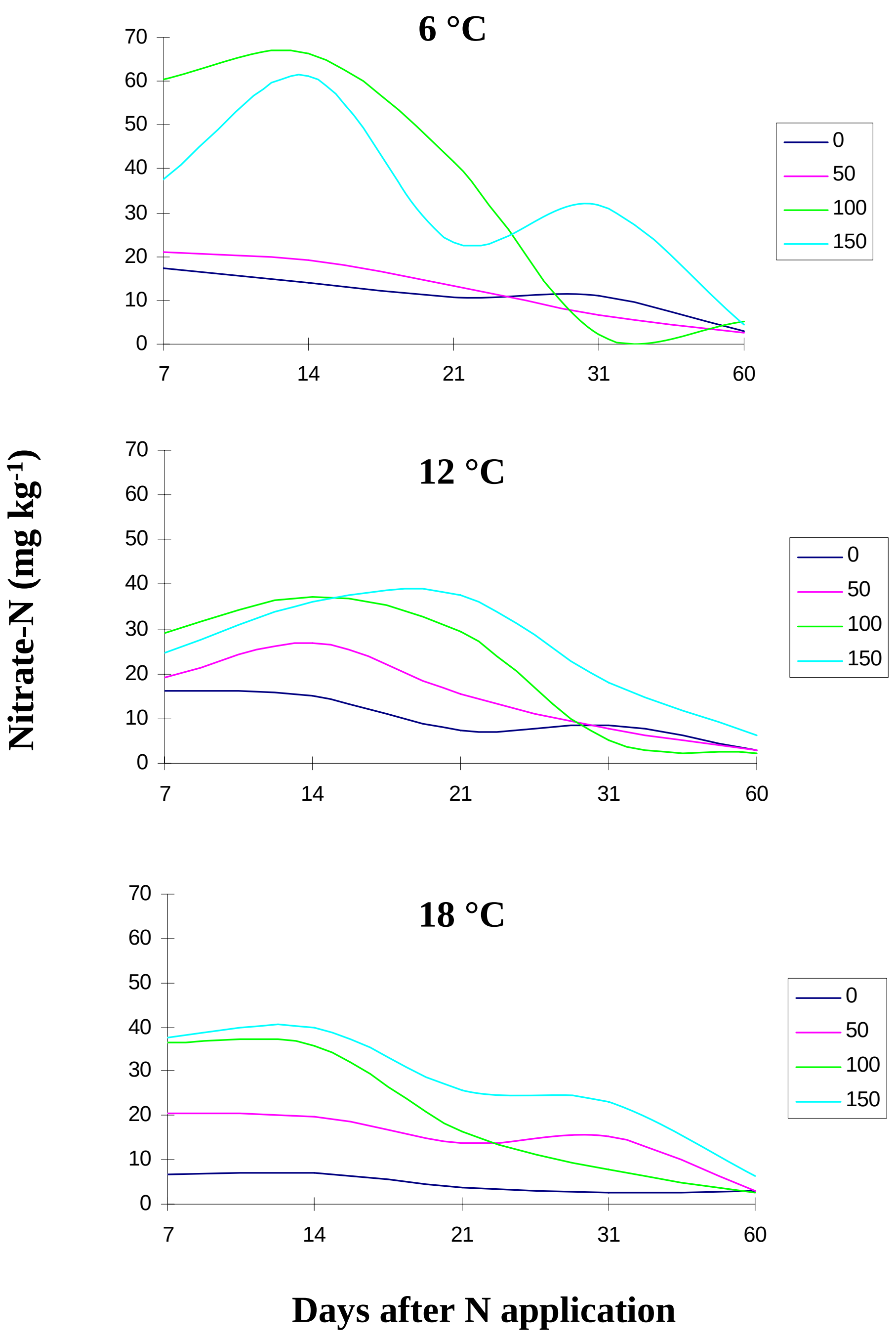

Figure 2.4 The effect of fertiliser N rate (kg N ha^{-1}) and soil temperature (°C) on soil nitrate-N content (mg kg^{-1}) in white clover over a 60 day period following fertiliser N application under controlled conditions at Elsenburg.

Number of tillers/stolons

The growth and survival of perennial ryegrass depend on the initiation and development of daughter tillers from mature parent tillers (Eckard, 1994) and white clover on stolon development and replacement (Frame & Newbould, 1986). Factors that benefit the initiation and survival of daughter tillers or stolons will influence perennial ryegrass and white clover persistency and therefore prolong the productive existence of a perennial ryegrass-white clover pasture.

Only fertiliser N rate influenced (P<0.05) the number of ryegrass tillers produced (Table 2.4). Increased fertiliser N rates resulted in an increase in the number of tillers produced. The application of 150 kg N ha^{-1} resulted in the highest number of ryegrass tillers, significantly (P=0.05) higher than 0 and 50 kg N ha^{-1}. The lowest (P=0.05) number of tillers were produced where no fertiliser N was applied. This result is in accordance with Eckard (1994) who reported increases in perennial ryegrass tuft diameter as fertiliser N rate was increased to 450 kg N ha^{-1} year^{-1}. Wolfson & Tainton (2000) stated in a review article that a high soil N level, especially nitrate, and low temperature tend to stimulate tiller development in certain grass species like *Digitaria eriantha*. The same trend was observed in the current study although the highest number of ryegrass tillers pot^{-1} produced at 6 °C was not significantly higher than at 18 °C.

The application of fertiliser N, from 50 to 150 kg N ha^{-1}, may therefore enhance tiller initiation and development, especially if temperature drops from 18 to 12 °C. Due to a weak correlation between number of tillers and dry matter production as reported by Davidson & Robson (1990) fertiliser N application should primarily aim at the increase of dry matter production and not to stimulate tiller development. The number of white clover stolon growing points was not influenced by any of the treatments applied (Table 2.5).

Weak correlations between number of ryegrass tillers, or clover stolons, and pasture DM production reported by Davidson and Robson (1990) suggested that yield is not only a function of number of shoots (tillers or stolons), but also of their size and that both may vary largely. According to Davies & Thomas (1983) and Robson & Polniaszek (1984) mechanisms that control shoot initiation are not necessarily the same as those that determine the rate of dry matter production.

Table 2.4 Number of perennial ryegrass tillers per pot 60 days after fertiliser N application as influenced by soil temperature (°C) and fertiliser N application rate (kg ha^{-1}) under controlled conditions at Elsenburg

Soil temperature	Fertiliser N rate (kg ha^{-1})				Mean (T)
	0	50	100	150	
6 °C	101.0	126.3	120.8	130.3	**119.5**
12 °C	96.3	112.8	131.0	132.0	**118.0**
18 °C	91.8	107.8	121.8	121.8	**110.8**
Mean (N)	**96.3 c***	**115.6 b**	**124.5 ab**	**128.0 a**	

LSD(0.05) NxT=NS
LSD(0.05) N mean=11.899
LSD(0.05) T mean=NS
CV = 12.38

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

Table 2.5 Number of white clover stolon growing points per pot 60 days after fertiliser N application as influenced by soil temperature (°C) and fertiliser N application rate (kg ha^{-1}) under controlled conditions at Elsenburg

Soil temperature	Fertiliser N rate (kg ha^{-1})				Mean (T)
	0	50	100	150	
6 °C	34.3	32.0	41.0	38.8	**36.5**
12 °C	41.8	39.5	38.8	34.3	**38.6**
18 °C	38.3	33.5	33.5	33.5	**34.7**
Mean (N)	**38.1**	**35.0**	**37.8**	**35.5**	

LSD(0.05) NxT=NS
LSD(0.05) N mean=NS
LSD(0.05) T mean=NS
CV = 14.37

Dry matter production

No interactions between the treatment combinations for perennial ryegrass or white clover were noted. Fertiliser N rate and soil temperature influenced ($P<0.05$) ryegrass primary dry matter production (PDM), residual dry matter production (RDM) and total dry matter production (TDM). White clover PDM, RDM and TDM was only influenced ($P<0.05$) by soil temperature.

Primary dry matter production (PDM)

Ryegrass PDM production increased as temperature increased from 6 to 18 °C with 18 and 12 °C resulting in significantly ($P=0.05$) higher PDM yields compared to 6 °C (Table 2.6).

A gradual increase in ryegrass PDM yield was found as fertiliser N rate was increased from 0 to 100 kg N ha $^{-1}$ with PDM yield measured at 100 kg N ha^{-1}, higher ($P=0.05$) than at 0 and 150 kg N ha^{-1}. The positive response of the ryegrass plants to 50 (0.722 g or 38%) and 100 kg N ha^{-1} (0.695 g or 37%) relative to 0 kg N ha^{-1} at 6 °C is indicative of the ability of the perennial ryegrass plants to respond to fertiliser N at low temperatures. Soil N levels at 150 kg N ha^{-1} possibly exceeded the absorption capacity of the ryegrass roots under the prevailing environmental conditions. The 0.438 g or 13% reduction in mean PDM production if fertiliser N rate was increased from 100 to 150 kg N ha^{-1} suggested that an oversupply of fertiliser N directly or indirectly could be the reason for the observed reduction. The relative low soil NH_4^+-N and NO_3^--N content over the two to three weeks following fertiliser N application at 6 °C (Figures 2.1 & 2.2) suggested immobilisation of fertiliser N as a possible reason for the low ryegrass PDM production recorded.

White clover PDM yield was influenced only by soil temperature with increases ($P=0.05$) in PDM production as temperature increased from 6 to 18 °C (Table 2.7).

Table 2.6 Primary, residual and total dry matter (DM) production (g pot^{-1}) of perennial ryegrass as influenced by soil temperature (°C) fertiliser N application and rate (kg ha^{-1}) under controlled conditions at Elsenburg

Primary DM Production (g pot^{-1})					
	Fertiliser N rate (kg ha^{-1})				
Soil temperature	**0**	**50**	**100**	**150**	**Mean (T)**
6 °C	1.898	2.620	2.593	2.463	**2.393 b***
12 °C	2.000	3.405	3.558	2.830	**2.948 a**
18 °C	2.305	3.015	3.675	3.218	**3.053 a**
Mean (N)	**2.068 c***	**3.013 ab**	**3.275 a**	**2.837 b**	

LSD(0.05) NxT=NS
LSD(0.05) N mean=0.373
LSD(0.05) T mean=0.323
CV = 16.10

Residual DM Production (g pot^{-1})					
	Fertiliser N rate (kg ha^{-1})				
Soil temperature	**0**	**50**	**100**	**150**	**Mean (T)**
6 °C	1.105	2.920	4.793	5.760	**3.644 b**
12 °C	1.560	2.945	5.065	5.870	**3.860 b**
18 °C	1.520	3.373	5.563	6.948	**4.351 a**
Mean (N)	**1.395 d**	**3.079 c**	**5.140 b**	**6.193 a**	

LSD(0.05) NxT=NS
LSD(0.05) N mean=0.5171
LSD(0.05) T mean=0.4478
CV = 15.80

Total DM Production (g pot^{-1})					
	Fertiliser N rate (kg ha^{-1})				
Soil temperature	**0**	**50**	**100**	**150**	**Mean (T)**
6 °C	3.003	5.540	7.385	8.223	**6.038 c**
12 °C	3.560	6.350	8.623	8.700	**6.808 b**
18 °C	3.825	6.388	9.238	10.165	**7.404 a**
Mean (N)	**3.463 c**	**6.093 b**	**8.415 a**	**9.029 a**	

LSD(0.05) NxT= NS
LSD(0.05) N mean=0.6284
LSD(0.05) T mean=0.5442
CV = 11.25

***** *Means in the same column or row at a specified dry matter production followed by the same letter are not significantly different (P=0.05)*

Table 2.7 Primary, residual and total dry matter (DM) production (g pot^{-1}) of white clover as influenced by soil temperature (°C) and fertiliser N application rate (kg ha^{-1}) under controlled conditions at Elsenburg

Primary DM Production (g pot^{-1})					
	Fertiliser N rate (kg ha^{-1})				
Soil temperature	**0**	**50**	**100**	**150**	**Mean (T)**
6 °C	1.678	2.090	1.960	2.325	**2.013 c***
12 °C	2.590	2.723	3.418	3.028	**2.939 b**
18 °C	3.553	3.825	3.750	3.780	**3.727 a**
Mean (N)	**2.607**	**2.879**	**3.043**	**3.044**	

LSD(0.05) NxT=NS
LSD(0.05) N mean=NS
LSD(0.05) T mean= 0.3261
CV = 15.72

Residual DM Production (g pot^{-1})					
	Fertiliser N rate (kg ha^{-1})				
Soil temperature	**0**	**50**	**100**	**150**	**Mean (T)**
6 °C	2.388	2.968	2.643	3.923	**2.980 b**
12 °C	3.450	3.423	4.173	4.045	**3.773 a**
18 °C	4.050	4.020	4.378	3.988	**4.109 a**
Mean (N)	**3.296**	**3.470**	**3.731**	**3.985**	

LSD(0.05) NxT=NS
LSD(0.05) N mean= NS
LSD(0.05) T mean= 0.5655
CV = 21.79

Total DM Production (g pot^{-1})					
	Fertiliser N rate (kg ha^{-1})				
Soil temperature	**0**	**50**	**100**	**150**	**Mean (T)**
6 °C	4.065	5.058	4.603	6.248	**4.993 c**
12 °C	6.040	6.145	7.590	7.073	**6.712 b**
18 °C	7.603	8.845	8.128	7.768	**7.836 a**
Mean (N)	**5.903**	**6.349**	**6.773**	**7.029**	

LSD(0.05) NxT= NS
LSD(0.05) N mean= NS
LSD(0.05) T mean= 0.7305
CV =15.64

**** Means in the same column or row at a specified dry matter production followed by the same letter are not significantly different (P=0.05)***

The results also suggest that fertiliser application rates of up to 150 kg N ha^{-1} as tested in the current study will not cause reductions in clover dry matter production. The decrease of clover percentage in perennial ryegrass-white clover pastures reported by various scientists (Eckard, 1994; McKenzie, Jacobs & Kearney, 2003) could therefore be ascribed to increased competitiveness by the stimulated ryegrass rather than suppressing of the clover plant as a result of fertiliser N application.

Residual dry matter production (RDM)

Increasing soil temperatures from 6 to18 °C resulted in increases in ryegrass RDM production although not significantly between 6 and 12 °C (Table 2.6). The restriction of ryegrass RDM production at 12 °C to the same level as at 6 °C suggested that higher soil N levels during the first regrowth cycle ensured sufficient N in the soil solution to boost PDM production despite the lower soil temperature. Higher soil N levels at lower soil temperatures may therefore compensate for the lower N absorption capacity of the ryegrass root system, reducing the negative effect of lower temperatures on dry matter production. Care must however be taken not to increase soil N levels to values exceeding the absorption capacity of the ryegrass plant under a specific set of environmental conditions. Exceeding the absorption capacity of the ryegrass will leave excess N in the soil solution. This may increase the risk of leaching, reduce N use efficiencies and increase the risk of contamination of natural resources. White clover RDM production did not differ between 12 and 18 °C, but was significantly lower at 6 °C indicating relative low white clover activity at low temperatures (Table 2.7).

Increasing fertiliser N levels from 0 to 150 kg N ha^{-1} resulted in increased (P=0.05) mean ryegrass RDM production over all N rates (Table 2.6). Mean initial soil nitrogen levels (ammonium + nitrate) of 12.65 and 23.13 mg kg^{-1} at the 100 and 150 kg N ha^{-1} treatments (Tables 2, day 31) were sufficient to increase ryegrass RDM yield by 1.865 (57%) and 3.356 g pot^{-1} (118.29%) relative to PDM yields at the same treatment combinations. Soil N levels of 8.93 mg kg^{-1} (50 kg N ha^{-1}) at the start of the second regrowth cycle increased RDM by 0.066 g pot^{-1} (2.2%) relative to PDM. RDM production of white clover was not influenced by fertiliser N rate (Table 2.7).

The increased ryegrass RDM production at 18 °C possibly resulted in increased N uptake, thereby enhancing the depletion of soil NO_3^--N as no differences in soil NO_3^--N content were recorded 60 days after 150 kg N ha^{-1} had been applied at 18 °C (Table 2.2). In contrast to this, significantly higher NO_3^--N levels were recorded at 60 days where 150 kg N ha^{-1} had been applied at 12 °C.

Total dry matter production (TDM)

Increasing fertiliser N rate from 0 to 50 and 100 kg N ha^{-1} resulted in increased (P=0.05) ryegrass TDM production (Table 2.6). The application of 150 kg N ha^{-1} resulted in the highest ryegrass TDM production although not significantly (P>0.05) higher than at 100 kg N ha^{-1}. Results supported those of Russell (1988) who came to the conclusion that water and nutrient absorption will increase as soil temperature increases, resulting in higher productivity. White clover TDM production was not influenced by fertiliser N rate, which confirmed the relative independence of clover plants to soil N supply (Table 2.7).

The application of 50, 100 and 150 kg N ha^{-1} at 6 °C resulted in a 2.537 (85%), 4.382 (146%) and 5.22 g pot^{-1} (174%) increase in ryegrass TDM production which emphasizes the success that can be achieved with fertiliser N application to cold soils planted with ryegrass. Comparative figures for clover were 0.993 (24%), 0.538 (13%) and 2.183 g pot^{-1} (54%), showing the reduced activity of white clover under low temperature conditions.

Leaf N content

Only fertilliser N rate influenced (P<0.05) ryegrass leaf N content during both the first (primary) and second (residual) regrowth cycles (Table 2.8). The application of 100 and 150 kg N ha^{-1} resulted in higher (P=0.05) ryegrass primary leaf N contents compared to the 0 kg N ha^{-1} treatments at the end of the first regrowth cycle. At the end of the second regrowth cycle (residual) the 150 kg N ha^{-1} resulted in higher (P=0.05) ryegrass leaf N contents than all other treatments with 0 kg N ha^{-1} the lowest. Results in the current study are supported by results obtained by Ehlig & Hagemann (1982) and Eckard (1994). Fertiliser N rate significantly influenced white clover leaf N content only during the first regrowth cycle with the highest (P=0.05) clover leaf N contents recorded at the 100 and 150 kg N ha^{-1} rates (Table 2.9). No differences in clover leaf N content were recorded between 0 and 50 kg N ha^{-1}.

Table 2.8 Primary and residual leaf nitrogen content (%) of perennial ryegrass as influenced by soil temperature (°C) and rate of fertiliser N application (kg ha^{-1}) under controlled conditions at Elsenburg

	N in primary DM (%)				
	Fertiliser N rate (kg ha^{-1})				
Soil temperature	**0**	**50**	**100**	**150**	**Mean (T)**
6 °C	3.061	4.548	4.917	5.213	**4.435**
12 °C	3.917	4.479	5.180	5.071	**4.662**
18 °C	3.600	4.005	5.276	5.304	**4.530**
Mean (N)	**3.525 c***	**4.375 b**	**5.124 a**	**5.186 a**	***4.542***
LSD(0.05) NxT=NS					
LSD(0.05) N mean=0.4721					
LSD(0.05) T mean=NS					
CV = 12.25					
	N in residual DM (%)				
	Fertiliser N rate (kg ha^{-1})				
Soil temperature	**0**	**50**	**100**	**150**	**Mean (T)**
6 °C	2.089	2.688	3.567	4.328	**3.168**
12 °C	2.044	2.265	2.808	4.523	**2.910**
18 °C	1.984	2.963	2.665	3.746	**2.766**
Mean (N)	**2.038 c**	**2.609 b**	**3.013 b**	**4.240 a**	***2.948***
LSD(0.05) NxT=NS					
LSD(0.05) N mean=0.483					
LSD(0.05) T mean=NS					
CV = 19.26					

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

Clover leaf N content was influenced by soil temperature in both regrowth cycles (Table 2.9) with leaf N content the lowest (P=0.05) at the 6 °C treatment for both cycles. Decreasing soil temperature from 18 to 12 °C did not affect leaf N content. The significantly lower leaf N content recorded at 6 °C furthermore emphasizes the clover's reduced activity at low temperatures. These results showed that ryegrass production can be stimulated by fertiliser N application with minimum direct effects on white clover in a perennial ryegrass-white clover pasture. The stimulation of ryegrass productivity may however induces indirect influences like competition that might influence clover performance under field conditions.

Table 2.9 Primary and residual leaf nitrogen content (%) of white clover as influenced by soil temperature (°C) and rate of fertiliser N application (kg ha^{-1}) under controlled conditions at Elsenburg

	N in primary DM (%)				
	Fertiliser N rate (kg ha^{-1})				
Soil temperature	**0**	**50**	**100**	**150**	**Mean (T)**
6 °C	4.046	4.429	4.427	4.738	**4.410 b***
12 °C	4.562	4.406	5.061	5.039	**4.767 a**
18 °C	4.68	4.316	4.941	4.908	**4.711 a**
Mean (N)	**4.429 b***	**4.384 b**	**4.810 a**	**4.895 a**	
LSD(0.05) NxT= NS					
LSD(0.05) N mean= 0.2605					
LSD(0.05) T mean= 0.2256					
CV = 6.80					
	N in residual DM (%)				
	Fertiliser N rate (kg ha^{-1})				
Soil temperature	**0**	**50**	**100**	**150**	**Mean (T)**
6 °C	4.19	3.981	3.837	4.015	**4.005 b**
12 °C	4.493	4.198	4.104	4.319	**4.279 a**
18 °C	4.511	4.297	4.511	4.53	**4.462 a**
Mean (N)	**4.400**	**4.158**	**4.151**	**4.288**	
LSD(0.05) NxT= NS					
LSD(0.05) N mean= NS					
LSD(0.05) T mean= 0.1875					
CV = 6.16					

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

All treatment combinations resulted in ryegrass leaf N concentrations of at least 2.0 to 3.2% (ryegrass) and 3.2 to 3.6% (clover), levels regarded as adequate for perennial ryegrass and white clover productivity (Reuter & Robinson, 1997). Ryegrass N levels reaching 3.5% and higher is indicative of wasteful use of fertiliser N (Eckard, 1994).

In contrast to results obtained by Eckard (1986) on Italian ryegrass, DM production in this study (Table 2.8) was stimulated in spite of ryegrass leaf N levels, during the first regrowth cycle, that were higher than the 3.5 % set as maximum above which little or no increase in DM production can be expected (Eckard, 1994).

Leaf N yield

Nitrogen removed in the ryegrass and clover cuttings (primary, residual and total) follows the same trend as measured in mean dry matter production, namely an increase as fertiliser N rate increased, mainly as a result of increased dry matter production (Tables 2.10 & 2.11).

Table 2.10 Primary, residual and total leaf nitrogen yield (g pot^{-1})* of perennial ryegrass as influenced by soil temperature (°C) and rate of fertiliser N application (kg ha^{-1}) under controlled conditions at Elsenburg

	N in primary DM (g pot^{-1})				
	Fertiliser N rate (kg ha^{-1})				
Soil temperature	**0**	**50**	**100**	**150**	**Mean (T)**
6 °C	0.059	0.119	0.127	0.128	**0.108**
12 °C	0.080	0.150	0.184	0.141	**0.139**
18 °C	0.084	0.113	0.194	0.138	**0.132**
Mean (N)	**0.074**	**0.127**	**0.168**	**0.136**	***0.126***
	N in residual DM (g pot^{-1})				
	Fertiliser N rate (kg ha^{-1})				
Soil temperature	**0**	**50**	**100**	**150**	**Mean (T)**
6 °C	0.062	0.149	0.262	0.354	**0.207**
12 °C	0.073	0.145	0.244	0.386	**0.212**
18 °C	0.076	0.147	0.245	0.427	**0.224**
Mean (N)	**0.070**	**0.147**	**0.250**	**0.389**	***0.214***
	N in total DM (g pot^{-1})				
	Fertiliser N rate (kg ha^{-1})				
Soil temperature	**0**	**50**	**100**	**150**	**Mean (T)**
6 °C	0.121	0.267	0.388	0.483	**0.315**
12 °C	0.152	0.295	0.428	0.527	**0.351**
18 °C	0.160	0.260	0.438	0.565	**0.356**
Mean (N)	**0.145**	**0.274**	**0.418**	**0.525**	***0.341***

Nitrogen yield was calculated as follows:

Nitrogen yield = DM production (g pot^{-1}) * Percentage N/100

No statistical analysis was done on this parameter as analysis were done on the components DM production and percentage N in the leaves harvested.

Table 2.11 Primary, residual ant total leaf nitrogen yield (g pot^{-1})* of white clover as influenced by soil temperature (°C) and rate of fertiliser N application (kg ha^{-1}) under controlled conditions at Elsenburg

	N in primary DM (g pot^{-1})				
	Fertiliser N (kg ha^{-1})				
Soil temperature	**0**	**50**	**100**	**150**	**Mean (T)**
6 °C	0.068	0.093	0.087	0.110	**0.089**
12 °C	0.118	0.120	0.173	0.153	**0.141**
18 °C	0.166	0.165	0.185	0.186	**0.176**
Mean (N)	**0.117**	**0.126**	**0.148**	**0.149**	***0.135***
	N in residual DM (g pot^{-1})				
	Fertiliser N (kg ha^{-1})				
Soil temperature	**0**	**50**	**100**	**150**	**Mean (T)**
6 °C	0.100	0.118	0.101	0.158	**0.119**
12 °C	0.155	0.144	0.171	0.175	**0.161**
18 °C	0.183	0.173	0.197	0.181	**0.183**
Mean (N)	**0.146**	**0.145**	**0.157**	**0.171**	***0.154***
	N in total DM (g pot^{-1})				
	Fertiliser N (kg ha^{-1})				
Soil temperature	**0**	**50**	**100**	**150**	**Mean (T)**
6 °C	0.168	0.211	0.188	0.268	**0.209**
12 °C	0.273	0.264	0.344	0.327	**0.302**
18 °C	0.349	0.338	0.383	0.366	**0.359**
Mean (N)	**0.263**	**0.271**	**0.305**	**0.320**	***0.290***

Nitrogen yield was calculated as follows:

Nitrogen yield = DM production (g pot^{-1}) * Percentage N/100

No statistical analysis was done on this parameter as analysis were done on the components DM production and percentage N in the leaves harvested.

Total leaf N yield (accumulated total over 60 days) at 6 °C with 0 kg N ha^{-1} applied were 38.8% higher in clover compared to ryegrass. However at 150 kg N ha^{-1} ryegrass N yields were 80.22% higher, clearly showing the ability of ryegrass to respond to high N applications relatively to clover at low temperatures (Tables 2.10 & 2.11).

Absorption of fertiliser N during the first 31 days (primary) was the highest at 12 and 18 °C for ryegrass and clover respectively, highlighting the ability of ryegrass to absorb N under relative cool conditions.

Leaf N yield of all treatment combinations was higher than the amount of fertiliser N applied. This apparently 100% recovery of applied fertiliser N could be the result of N mineralisation, optimal water supply and zero N leaching losses. The total leaf N yield of ryegrass at the 0 kg N ha^{-1} treatments were 0.121 (6 °C), 0.152 (12 °C) and 0.160 g pot^{-1} (18 °C) indicating the growth medium's capacity to mineralise N in excess of 50 kg N ha^{-1} (0.121 g N pot = 56 kg N ha^{-1}).

Root dry mass

Perennial ryegrass and white clover root dry mass was influenced (P=0.05) only by soil temperature (Tables 2.12 & 2.13). The highest ryegrass root dry mass was recorded at 12 °C followed by 6 °C with the lowest at 18 °C. Root dry mass at 6 °C did not differ significantly (P>0.05) from root mass recorded at 12 and 18 °C, indicating, firstly, the ability of ryegrass to develop roots under relative cold (6 °C) soil conditions. Secondly the possible suppressing effect of higher (18 °C) soil temperatures on ryegrass root mass.

Table 2.12 Root dry mass (g pot^{-1}) of perennial ryegrass as influenced by soil temperature (°C) and fertiliser N application rate (kg ha^{-1}) under controlled conditions at Elsenburg

	Fertiliser N (kg ha^{-1})				
Soil temperature	**0**	**50**	**100**	**150**	**Mean (T)**
6 °C	5.958	7.898	6.448	7.028	**6.833 ab***
12 °C	8.12	7.498	8.103	7.888	**7.902 a**
18 °C	7.838	6.973	6.275	5.473	**6.639 b**
Mean (N)	**7.305**	**7.456**	**6.942**	**6.796**	
LSD(0.05) NxT=NS					
LSD(0.05) N mean=NS					
LSD(0.05) T mean=1.0756					
CV = 21.06					

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

Table 2.13 Root dry mass (g pot^{-1}) of white clover as influenced by soil temperature (°C) and fertiliser N application rate (kg ha^{-1}) under controlled conditions at Elsenburg

	Fertiliser N (kg ha^{-1})				
Soil temperature	**0**	**50**	**100**	**150**	**Mean (T)**
6 °C	1.548	1.670	1.298	1.803	**1.579 a***
12 °C	1.210	1.148	1.280	1.328	**1.241 b**
18 °C	1.205	0.920	1.073	0.783	**0.995 b**
Mean (N)	**1.321**	**1.246**	**1.217**	**1.304**	
LSD(0.05) NxT=NS					
LSD(0.05) N mean=NS					
LSD(0.05) T mean= 0.3346					
CV = 36.69					

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

The highest (P=0.05) white clover root dry mass was produced at 6 °C. Similar to ryegrass, the lowest clover root mass was also recorded at 18 °C although not significantly lower than at 12 °C.

Conclusion

This study showed that the application of fertiliser N resulted in increased ryegrass productivity at temperatures as low as 6 °C. However a significant reduction in dry matter production can be expected if soil temperature decreases from 18 to 12 as well as from 12 to 6 °C. The application of fertiliser N at 6 °C may still produce higher ryegrass dry matter yields (between 30 and 38% in the first regrowth cycle) compared to treatments that did not receive fertiliser N. This study furthermore showed that white clover could tolerate fertiliser N levels of 150 kg N ha^{-1} without reducing dry matter production, leaf N content or root dry mass. The lack of clover response (dry matter production) to fertiliser N as clearly seen at the 100 and 150 kg N ha^{-1} rates creates the opportunity to stimulate perennial ryegrass dry matter production in a ryegrass-white clover pasture without direct negative effects on the clover component. The reaction of clover in a mixed pasture under field conditions may however be altered as a result of the competitive effect of ryegrass stimulated by increased inorganic nitrogen supply.

The application of fertiliser N to ryegrass can stimulate ryegrass tiller development. In a ryegrass-white clover mixture this could however disturb the grass clover balance as the initiation of stolon growing points were not stimulated by fertiliser N application placing white clover at a possible competitive disadvantage relative to perennial ryegrass.

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Chapter 3

The effect of high soil water levels and fertiliser N rate on soil nitrogen dynamics and growth of perennial ryegrass *(Lolium perenne)* and white clover (*Trifolium repens)* grown under controlled conditions

Abstract

The effects of five fertiliser N rates (0, 30, 60, 120 and 180 kg N ha^{-1}) and four water potential levels (-10, -20, -25 and -35 kPa) on perennial ryegrass and white clover grown in separate pots in a glasshouse at 12/18 °C night/day temperatures were investigated. Primary- (PDM) and residual dry matter production (RDM) were recorded 30 and 60 days after fertiliser N application. The number of ryegrass tillers/clover stolons, root dry mass and leaf N content were recorded. Soil ammonium and nitrate content were monitored during a 45 and 31 day period following fertiliser N application for ryegrass and clover respectively.

The soil ammonium-N and nitrate-N levels with applications of 30 and 60 kg N ha^{-1} in both ryegrass and clover decreased within 11 to 14 days after fertiliser N application to levels slightly higher (P>0.05) than at the 0 kg N ha^{-1} treatments. Applying 120 kg N ha^{-1} to ryegrass however resulted in soil ammonium levels remaining relatively high (P=0.05) for a period of 30 days at water potential levels of -10 and -20 kPa and 14 days at -35 kPa. The same responses were recorded with soil nitrate-N although the higher (P=0.05) levels at 120 kg N ha^{-1} were extended to 37 (-10 and -20 kPa) and 45 (-25 kPa) days after fertiliser N application.

Increasing fertiliser N levels from 0 to 120 kg N ha^{-1} resulted in increased (P=0.05) perennial ryegrass primary dry matter production (PDM) at the end of the first regrowth cycle (30 days) followed by a non-significant increase as N rate was increased to 180 kg N ha^{-1}. Ryegrass PDM yield was influenced (P=0.05) by soil water potential with higher yields recorded at the -10 and -20 kPa treatments. White clover PDM production was not influenced by N rate or soil water potential. Carry-over fertiliser N caused increased (P=0.05) ryegrass RDM at 120 and 180 kg N ha^{-1} rates. Clover RDM production increased as fertiliser N rate was increased from 0 to 180 kg N ha^{-1}. Higher (-10 and -20 kPa) soil water levels caused increased ryegrass RDM production, a response not observed in clover. Total dry matter (TDM) production (the accumulative dry matter production during a 60 day period following fertiliser N application) of ryegrass was significantly increased as fertiliser N rate was increased from 0 to 180 kg N ha^{-1}. Higher ryegrass TDM yields at -10 kPa and -20 kPa were recorded at the higher (120 and 180 kg N ha^{-1}) N application rates but water content did not influenced TDM production at the lower (0, 30 and 60 kg N ha^{-1}) fertiliser N application rates. Clover TDM production was not influenced by the treatments applied. The number of ryegrass tillers per plant increased (P=0.05) as fertiliser N rate increased from 0 to 120 kg N ha^{-1}. The number of stolons produced was not influenced by the treatments. Increased fertiliser N rate resulted in increased ryegrass and, to a lesser extend, clover leaf N content at the end of the first regrowth cycle. Only 120 kg N ha^{-1} resulted in higher ryegrass leaf N content at the end of the second regrowth cycle. Best conditions for ryegrass (P=0.05) and clover (P>0.05) root development were found to be at -35 kPa soil water potential.

The study has shown that significant increases in dry matter production of perennial ryegrass can be achieved with fertiliser N applications under high (-10 kPa) soil water conditions. Clover dry matter production was not influenced by fertiliser N rate and soil water levels covered in this study. The highest ryegrass leaf N content after the first regrowth cycle, as measured at the -10 kPa soil water potential, suggest that the absorption of soil N was not restricted by the high soil water levels. The study also showed that the application of fertiliser N to stimulate ryegrass productivity under relative wet conditions without negatively affecting a companion clover crop in a mixed pasture could be successful.

Keywords: dry matter production, glasshouse, leaf N content, NH_4^+, NO_3^-, perennial ryegrass, root dry mass, soil water potential, stolons, tillers, white clover

Introduction

The window of opportunity to increase perennial ryegrass-white clover dry matter production during the cool season in the Western Cape Province is from April to September, seasons characterized by high rainfall that can result in soil water levels at field water capacity for several days. Average rainfall measured over a 30 year period for May, June, July and August were 83.7, 106, 99.7 and 84.7 mm respectively (Anon, 2004). Aggravating factors includes low evapotranspiration as low temperatures reduce evaporation and transpiration by plants, leaving the soil water at higher water levels for longer periods. Quantifying the effect of high soil water content on the fertiliser N applied and the resultant response of perennial ryegrass and white clover are therefore important to develop an efficient strategic fertiliser N programme for implementation during the cool season.

Apart from moving topdressed fertiliser N into the rooting zone soil water is also required to move the nitrogen to the root surface from where absorption into the root takes place (Miles & Manson, 2000). Increased soil water content may therefore increases fertiliser N supply to the plant root.

High water contents may influence, directly and indirectly, the ability of the plant to respond to the fertiliser N applied as well as possible N losses as a result of restricted soil aeration. Denitrification losses [(the biochemical reduction of NO_3- to N_2 and nitrous oxide under warm (above 5 °C), anaerobic conditions (poorly drained soils)] can contribute to fertiliser N losses when grown in soils with a water content between saturation point and field water capacity (Tinker, 1979; Miles & Manson, 2000). Limiting or excessive soil water may also seriously impair the N fixing processes in legumes (Ryden, 1984).

The dry matter production of potentially high yielding crops grown under conditions of high levels of soil available nutrients, including nitrogen, can be reduced by minor fluctuations in water supply. The N-use efficiency of kikuyu decreased by between 35 and 50% as rainfall decreased by 34% (Miles, 1997). Plants receiving high levels of fertiliser N on fertile soils can only produce at maximum levels if the stomata remain open, especially during midday when the rate of photosynthesis is at a maximum. Plants grown under these high potential conditions are easily affected by wilting (Russell, 1988). Stomatal resistance of grass-clover pastures at Rothamsted remained low at transpiration rates of 2 – 3 mm day^{-1} until soil suction in the top 250 mm soil had risen to between -300 and - 400 kPa after which resistance increased linearly with increase of suction up to -1200 kPa when it began to increase more rapidly causing severe reductions in transpiration rate (Szeicz & Long, 1969 cited by Russell, 1988).

Low levels of fertiliser N will result in plants developing less leaf surface area, thereby reducing the transpiration area, resulting in a more favorable root:shoot ratio enabling the plant to maintain optimal production levels. Relative short water stress periods can result in increases in root mass of certain grasses as well as carbohydrate content of roots and stubble suggesting decreased utilization of carbohydrates (Opperman & Human, 1976). Danckwerts (1988) found that leaf expansion ceased after 40% soil water depletion and significant leaf senescence at 70% soil water depletion in *Themeda* and *Sporobolus* species. Danckwerts (1988) came to the conclusion that even partial soil water stress can reduce maximum growth and assimilation rates in the species under consideration.

The objectives of this study were to 1) evaluate the response of perennial ryegrass and white clover to fertiliser N in a sandy loam soil at relative high soil water levels and 2) monitor soil N fractions after fertiliser N application.

Materials and methods

Locality

Perennial ryegrass (*Lolium perenne* cv. Ellet) and white clover (*Trifolium repens* cv. Haifa) were established in a glasshouse under natural photoperiod and light intensity conditions at the Institute for Plant Production, Elsenburg (altitude 177m, 18^{o}50'E, 33^{o}51'S). Day and night temperatures were regulated at 18 ° and 12 °C for 10 and 14 hours respectively.

Growth medium preparation

Topsoil (0-150 mm layer) from the orthic A horizon of an Oakleaf soil (Soil Classification Working Group, 1991) derived mainly from granite (Anon, 1996) was collected, as growth medium. To achieve a relative uniform bulk density for all pots the soil was gathered in heaps, mixed and ran through a 5 mm screen to separate the larger clods (aggregates) and crop residues from the soil used as growth medium. A composite soil sample was collected and analysed for both physical- and chemical properties (Table 3.1). Analysis methods for measuring the physical and chemical properties of the soil are referred to in Chapter 2. Soil fertility levels were corrected through application of single superphosphate and potassium chloride to levels recommended by Beyers (1983). Copper and manganese were sufficient with zinc and boron marginally low. Foliar nutrition was applied 4 times over a 14 week period to prevent any deficiencies. The C content of 1.46 was moderate and some N mineralisation could be expected. An equivalent of 40 kg N ha^{-1} was applied at seeding to maintain plant growth during the pre-treatment growing period.

Table 3.1 Chemical analysis and selected physical properties of the soil used as growth medium

Chemical properties			Physical properties		
pH (KCl)		6.1			
Resistance	**Ohms**	810	**Clay**	**%**	14
Phosphorus	**mg/kg**	45	**Silt**	**%**	13.1
Potassium	**cmol(+)/kg**	0.27	**Fine Sand**	**%**	49.8
Calcium	**cmol(+)/kg**	2.42	**Medium Sand**	**%**	15.9
Magnesium	**cmol(+)/kg**	1.09	**Course Sand**	**%**	7.2
Sodium	**cmol(+)/kg**	0.45			
Total cations	**cmol(+)/kg**	4.23	**Classification**		SaLm
Copper	**mg/kg**	1.15			
Zinc	**mg/kg**	0.79			
Manganese	**mg/kg**	54			
Boron	**mg/kg**	0.47			
Carbon	**%**	1.46			
Ammonium-N	**mg/kg**	2.729			
Nitrate-N	**mg/kg**	12.341			

Ammonium-N and nitrate-N contents of 2.73 and 12.34 mg kg^{-1} were measured before the N treatments were done. The soil was dried in stainless steel bins at 60 °C. To facilitate non-destructive soil sampling during the growth period, pots were lined with plastic bags and filled with 6.6 kg of the oven dried soil occupying a volume of 4324.57 cm^3 resulting in a bulk density of 1.53 g cm^{-3}. Pots were watered and left for ten days to enable weed seed to germinate. After removing the weeds a template with five holes, one in the centre and one in each quarter of the pot, was used to plant the seed in a predetermined configuration at 5-10 mm depth. A few seeds were planted per hole followed by water application to fill the soil to field water capacity (0.253 mm^3 mm^{-3}). Seedlings were thinned after emergence to five plants per pot. Pots were watered daily through weighing pots and adding water to the predetermined weight. Changes in plant weight as a result of growth, on a weekly basis, and soil removal due to soil sampling were considered when pot weight after watering was calculated. Pots were randomised after watering.

Plants were allowed to grow for an accumulative photoperiod of *ca* 691 hours from planting and clipped at 50 mm height. The clippings were dried at 60 °C for *ca*18 h upon which pots showing least variation in DM production were at random allocated to the treatment combinations.

Experimental design and treatments

The experiment was laid out as a completely randomised design with four replicates (Snedecor & Cochran, 1967). The treatment design was a factorial with four soil water levels namely 0.24, 0.19, 0.18 and 0.14 mm^3 water mm^{-3} soil, representing soil water potentials of -10, -20, -25 and -35 kPa, and five N levels equivalent to 0, 30, 60, 120 and 180 kg N ha^{-1} used as treatments. To simplify watering, the pots were grouped together according to water treatments with pots rotating within water treatments when watered. The groups of pots that remained together according to water treatments were rotated within the glasshouse after watering.

To determine soil water content, soil suction or water potential (Russell, 1988) was measured by placing three tensiometers in pots with perennial ryegrass at saturation point and tensiometer water potential recorded as pot mass decrease as a result of evapotranspiration. Pot mass (kg) versus tensiometer water potential (kPa) was plotted and described by the logarithmic function ($P<0.0001$):

$$\text{Pot mass (kg)} = 8.6417114 + (- 0.32044674 * \ln \text{Tensiometer water potential})$$

Soil water contents in mm^3 water mm^{-3} soil at predetermined soil water potentials (kPa) were calculated. These values were used to calculate the total weight of the watered pot at predetermined soil water potentials. The amount of water needed to reach a predetermined water potential was calculated using the formula: water needed (g) = volumetric water content * soil volume. The values derived from above mentioned equation were tested using a soil water-characteristic curve representing a soil with a clay + silt content of 26% (Bennie *et al.*, 1988). Pots were allowed to dry to the pre-determined pot weights representing the different water levels before fertiliser N treatments (as LAN dissolved in 200ml water) were applied. Watering took place at 24 hour cycles through weighing pots and maintaining a pot weights as close as possible to the predetermined weight.

Data collection

DM production was recorded by cutting the ryegrass at 50 mm height 31 (primary dry matter production) and 60 days (residual dry matter production) after nitrogen treatments had been applied. Fresh weight was recorded, cuttings ovendried at 60 °C for 72 hours and dry weight noted. Dry matter production was recorded as primary - (PDM) at 31 days, residual – (RDM) production from 31 to 60 days and total dry matter production (TDM, which is the cumulative DM production over 61 days). The number of tillers at 61 days was recorded. Dried cuttings were ground to pass through a 1 mm mesh screen and analyzed for nitrogen content (AOAC, 1970). Leaf N yield was calculated as the product of leaf N % and dry matter produced. After residual dry matter production was recorded roots were removed by wet sieving using a 2 and 1 mm combination sieve, dried at 60 °C for 72 h and dry mass recorded.

Soil samples were collected at 7, 14, 31, 37 and 45 days after the N treatments were applied in ryegrass and 11, 18, 21 and 31 days in clover. Soil samples were collected from the 0 to 120 kg N ha^{-1} treatments at the -10, -20 and -25 kPa soil water potentials. Soil samples were collected through pulling the plastic bag containing the soil and plants from the pot. Four sub samples per pot were taken, at the soil surface, 3 cm from top, center (9 cm) and 3 cm from the bottom. Sub samples were taken through cutting holes in the bags at the front, back, left and right hand side of the pot and bulked as one sample. After sealing the sample-holes with 50mm cello tape the bags were returned to the pots. To minimize any changes in ammonium-N and nitrate-N content samples were immediately dried using electric fans and stored in a freezer (Westfall, Henson & Evans 1978) until analyzed for NH_4^- and NO_3^--N content using the Auto Analyzer method (Bessinger, 1985).

Statistical procedures

Analysis of variance (ANOVA) was performed using SAS version 8.2 (SAS, 1999). The Shapiro-Wilk test was used to test for non-normality (Shapiro & Wilk, 1965). Student's t-Least Significant Difference (LSD) test was calculated at the 5% confidence level to compare treatment means (Ott, 1998).

Results and discussion

Soil ammonium-N and nitrate-N content

The influence of fertiliser N rate and soil water level on soil ammoniun-N and nitrate-N in ryegrass and clover are summarised in Tables 3.2 & 3.3; Figures 3.1 to 3.4. The highest soil ammonium-N and nitrate-N content for both perennial ryegrass and white clover were recorded at the 120 kg N ha^{-1} rates (Tables 3.2 and 3.3). To compare mean soil N levels at the end of the first regrowth cycle mean ammonium-N and nitrate-N were calculated over the first 31 days. In ryegrass the mean soil ammonium-N contents (over the first 31 days) were 8.13, 16.02 and 9.14 mg kg^{-1} and nitrate-N were 31.04, 41.10 and 25.21 mg kg^{-1} at -10, -20 and -25 kPa water potentials respectively. However for clover the means recorded were 10.16, 9.98 and 10.55 mg kg^{-1} (ammonium-N) and 27.70, 43.24 and 47.84 mg kg^{-1} (nitrate-N) at the respective soil water potentials. The slightly higher mean N levels normally recorded in clover is possibly the result of lower uptake of nitrate as symbiotic N fixation enable clover to be less dependent on soil-N.

Ammonium-N levels of ryegrass were relatively high at 7 days after fertiliser N application but dropped to levels generally lower than 10 mg kg^{-1} at the 30 and 60 kg N ha^{-1} rates at 14 days and thereafter (Table 3.2, Figure 3.1). Ammonium-N levels in clover at the 30 and 60 kg N ha^{-1} remained relative constant at levels between 2.75 and 9.15 mg kg^{-1} (except one treatment at 15.67 mg kg^{-1}) over the sampling period (Figure 3.2, Table 3.3). Application of 120 kg N ha^{-1} resulted in significantly higher ($P=0.05$) soil ammonium-N contents for a period of 30 (-10 and -20 kPa) and 14 days (-25 kPa) following fertiliser N application in ryegrass. Application of 120 kg N ha^{-1} resulted in significantly higher ($P=0.05$) soil ammonium-N contents for a period of 21 days (-10 and -20 kPa) and 31 days (-25 kPa) following fertiliser N application in clover.

Nitrate-N levels in ryegrass showed similar responses as observed for ammonium-N with nitrate-N levels decreasing between 7 and 14 days followed by relative constant levels from 14 to 45 days after fertiliser N application (Figure 3.3, Table 3.2). Nitrate-N contents in clover show a general decrease over the sampling period with 120 kg N ha^{-1} resulting in higher nitrate-N levels at all samplings (Figure 3.4, Table 3.3). The application of 120 kg N ha^{-1}

resulted in significantly higher (P=0.05) soil ammonium-N contents (compared to the 0 kg N ha^{-1}) during the first regrowth cycle (31 days) at all soil water levels tested.

The soil N content monitoring has shown that the application of 120 kg N ha^{-1} to ryegrass could result in significantly higher soil ammonium-N levels 7 days after N application. These higher levels can be maintained for up to 37 days at -10 and -20 kPa but only for 14 days at -25 kPa possibly due to increased nitrification in the better aerated soil at lower water levels (Foth, 1978). The application of 120 kg N ha^{-1} to ryegrass can result in significantly higher soil nitrate-N levels over a 37 day period following N application at -10 and -20 kPa and 45 days at -25 kPa. The application of 120 kg N ha^{-1} to clover resulted in higher (P=0.05) soil ammonium-N levels compared to the 0 kg N ha^{-1} rates for a period of 21 days at -10 and -20 kPa and 31 days at -25 kPa following fertiliser N application. Soil nitrate-N levels at 120 kg N ha^{-1} at all water levels covered by the study were higher (P=0.05) than the 0 kg N ha^{-1} rate over the 31 day sampling period.

Table 3.2 Soil ammonium and nitrate content (mg kg^{-1}) in perennial ryegrass as influenced by soil water potential (kPa) and fertiliser N rate (kg N ha^{-1}) under controlled conditions over a 45 day period following fertiliser N application at Elsenburg

Days after fertiliser N application	N rate (kg ha^{-1})	-10 kPa water potential Ammonium-N (mg kg^{-1})	-10 kPa water potential Nitrate-N (mg kg^{-1})	-20 kPa water potential Ammonium-N (mg kg^{-1})	-20 kPa water potential Nitrate-N (mg kg^{-1})	-25 kPa water potential Ammonium-N (mg kg^{-1})	-25 kPa water potential Nitrate-N (mg kg^{-1})	Mean Ammonium-N (mg kg^{-1})	Mean Nitrate-N (mg kg^{-1})	Mean Total-N (mg kg^{-1})
7	**0**	4.647 b*	19.89 b	3.85 b	74.10 a	4.08 b	7.31 b	**4.19**	**33.77**	**18.98**
	30	8.430 b	46.70 b	45.47 ab	126.83 a	15.83 b	40.54 ab	**23.24**	**71.36**	**47.30**
	60	14.403 ab	25.80 b	52.50 ab	113.53 a	22.91 b	107.34 ab	**29.94**	**82.22**	**56.08**
	120	22.723 a	109.90 a	68.69 a	142.82 a	118.80 a	152.07 a	**70.07**	**134.93**	**102.50**
	Mean	**12.55**	**50.57**	**42.63**	**114.32**	**40.41**	**76.82**			
14	**0**	3.763 b	3.127 b	3.400 b	1.03 b	3.463 b	1.750 b	**3.54**	**1.97**	**2.76**
	30	4.767 b	12.840 b	5.687 b	22.39 b	6.403 b	10.51 b	**5.62**	**15.25**	**10.43**
	60	5.697 b	19.297 b	9.737 b	23.80 b	8.073 b	39.01 b	**7.84**	**27.37**	**17.60**
	120	24.513 a	68.600 a	52.277 a	109.30 a	25.167 a	85.63 a	**33.99**	**87.84**	**60.91**
	Mean	**9.69**	**25.97**	**17.78**	**39.13**	**10.78**	**34.23**			
31	**0**	3.720 b	0.730 b	5.153 b	6.320 b	10.073 a	22.12 a	**6.32**	**9.72**	**8.02**
	30	5.017 b	10.084 b	3.817 b	3.157 b	3.953 a	1.29 a	**4.26**	**4.84**	**4.55**
	60	2.870 b	9.487 b	5.850 b	3.803 b	14.420 a	18.32 a	**7.71**	**10.54**	**9.13**
	120	19.883 a	103.033 a	16.487 a	108.433 a	9.413 a	27.38 a	**15.26**	**79.62**	**47.44**
	Mean	**7.87**	**30.83**	**7.83**	**30.43**	**9.46**	**17.28**			
37	**0**	3.560 a	0.500 b	3.9533 a	0.327 b	3.147 a	0.233 b	**3.55**	**0.35**	**1.95**
	30	3.217 a	3.560 b	3.280 a	0.503 b	3.620 a	1.105 ab	**3.37**	**1.72**	**2.55**
	60	3.323 a	0.820 b	3.660 a	0.733 b	3.303 a	0.613 ab	**3.43**	**0.72**	**2.08**
	120	6.310 a	62.23 a	4.320 a	20.800 a	3.265 a	13.990 a	**4.63**	**32.34**	**18.49**
	Mean	**4.10**	**16.78**	**3.80**	**5.59**	**3.33**	**3.99**			
45	**0**	6.437 a	3.58 a	3.283 a	0.52 a	2.997 a	0.190 b	**4.24**	**1.43**	**2.83**
	30	6.010 a	2.66 a	8.870 a	25.59 a	3.127 a	0.530 b	**6.00**	**9.59**	**7.80**
	60	3.250 a	34.54 a	3.180 a	0.35 a	3.363 a	0.587 b	**3.26**	**11.83**	**7.55**
	120	3.800 a	6.95 a	4.480 a	30.62 a	3.593 a	6.120 a	**3.96**	**14.56**	**9.26**
	Mean	**4.87**	**11.93**	**4.95**	**14.27**	**3.27**	**1.86**			
	Mean N fractions	**7.82**	**27.22**	**15.40**	**40.75**	**13.45**	**26.83**			
	Mean water potential	**17.52**		**28.07**		**20.14**				

***** *Means in the same column at a specified day following fertiliser N application followed by the same letter are not significantly different (P=0.05)***

Table 3.3 Soil ammonium and nitrate content (mg kg^{-1}) in white clover pots as influenced by soil water potential (kPa) and fertiliser N rate (kg N ha^{-1}) a under controlled conditions over a 31 day period following fertiliser N application at Elsenburg

Days after fertiliser N application	N rate (kg ha^{-1})	-10 kPa water potential Ammonium-N (mg kg^{-1})	Nitrate-N (mg kg^{-1})	-20 kPa water potential Ammonium-N (mg kg^{-1})	Nitrate-N (mg kg^{-1})	-25 kPa water potential Ammonium-N (mg kg^{-1})	Nitrate-N (mg kg^{-1})	Mean Ammonium-N (mg kg^{-1})	Mean Nitrate-N (mg kg^{-1})	Mean Total-N (mg kg^{-1})
11	**0**	3.447 b*	21.280 b	2.750 c	30.83 b	2.807 b	29.150 a	**3.00**	**27.09**	**15.04**
	30	5.967 b	47.880 ab	5.763 c	34.24 b	7.477 b	43.720 a	**6.40**	**41.95**	**24.17**
	60	8.800 b	21.15 b	15.670 b	70.900 ab	8.617 b	34.760 a	**11.03**	**42.27**	**26.65**
	120	47.783 a	65.100 a	37.480 a	92.930 a	31.240 a	86.22 a	**38.83**	**81.42**	**60.13**
	Mean	**16.50**	**38.85**	**15.42**	**57.23**	**12.54**	**48.46**			
18	**0**	7.790 b	14.640 b	7.357 b	28.550 b	6.210 b	15.890 b	**7.12**	**19.69**	**13.41**
	30	5.687 b	31.250 b	6.073 b	46.390 b	5.680 b	52.580 b	**5.81**	**43.41**	**24.61**
	60	5.273 b	20.910 b	9.040 b	69.600 ab	9.150 b	69.410 ab	**7.82**	**53.31**	**30.56**
	120	26.827 a	90.030 a	30.327 a	99.270 a	35.640 a	143.500 a	**30.93**	**110.93**	**70.93**
	Mean	**11.39**	**39.21**	**13.20**	**60.95**	**14.17**	**70.35**			
21	**0**	3.467 b	9.080 b	3.153 b	23.300 b	3.000 b	14.200 b	**3.21**	**15.53**	**9.37**
	30	3.803 b	28.470 ab	3.333 b	20.790 b	3.520 b	39.250 ab	**3.55**	**29.50**	**16.53**
	60	3.343 b	15.200 b	4.647 b	33.17 b	8.350 b	49.070 ab	**5.45**	**32.48**	**18.96**
	120	12.777 a	45.230 a	14.573 a	79.030 a	25.790 a	119.430 a	**17.71**	**81.23**	**49.47**
	Mean	**5.85**	**24.50**	**6.43**	**39.07**	**10.17**	**55.49**			
31	**0**	5.500 ab	1.613 b	4.057 a	5.070 b	4.807 b	3.000 b	**4.79**	**3.23**	**4.01**
	30	6.937 ab	5.467 b	4.8700 a	5.173 b	4.687b	7.810 b	**5.50**	**6.15**	**5.82**
	60	4.590 b	4.497 b	4.6967 a	17.837 b	4.770 b	5.910 b	**4.69**	**9.41**	**7.05**
	120	10.563 a	21.327 a	5.8367 a	34.770 a	7.003 a	51.560 a	**7.80**	**35.89**	**21.84**
	Mean	**6.90**	**8.23**	**4.87**	**15.71**	**5.32**	**17.07**			
	Mean N fractions	**10.16**	**27.70**	**9.98**	**43.24**	**10.55**	**47.84**			
	Mean Moisture	**18.93**		**26.61**		**29.19**				

Means in the same column at a specified day following fertiliser N application followed by the same letter are not significantly different (P=0.05)

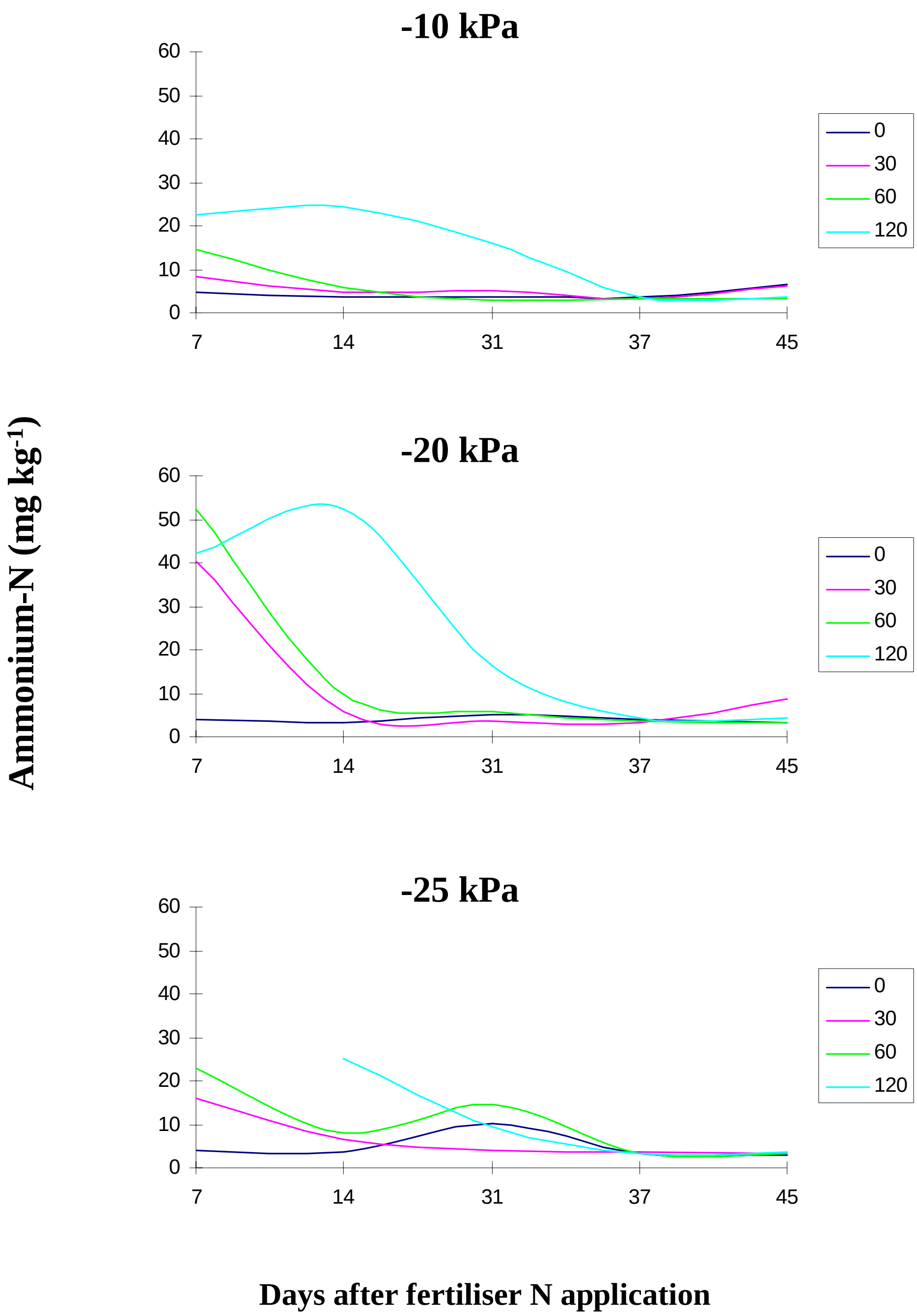

Figure 3.1 The effect of fertiliser N rate (kg N ha^{-1}) and soil water potential (kPa) on soil ammonium-N content (mg kg^{-1}) in perennial ryegrass over a 45 day period following fertiliser N application under controlled conditions at Elsenburg.

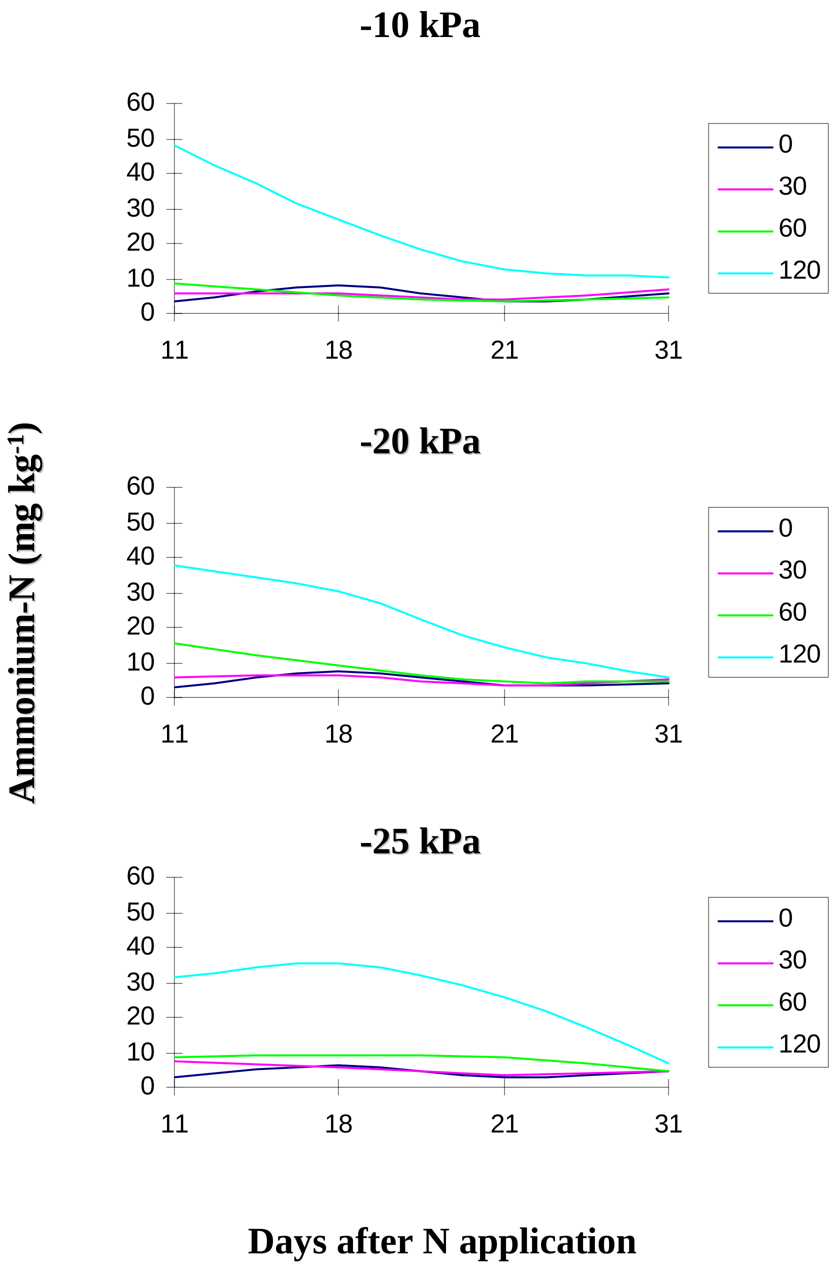

Figure 3.2 The effect of fertiliser N rate (kg N ha^{-1}) and soil water potential (kPa) on soil ammonium-N content (mg kg^{-1}) in white clover over a 31 day period following fertiliser N application under controlled conditions at Elsenburg.

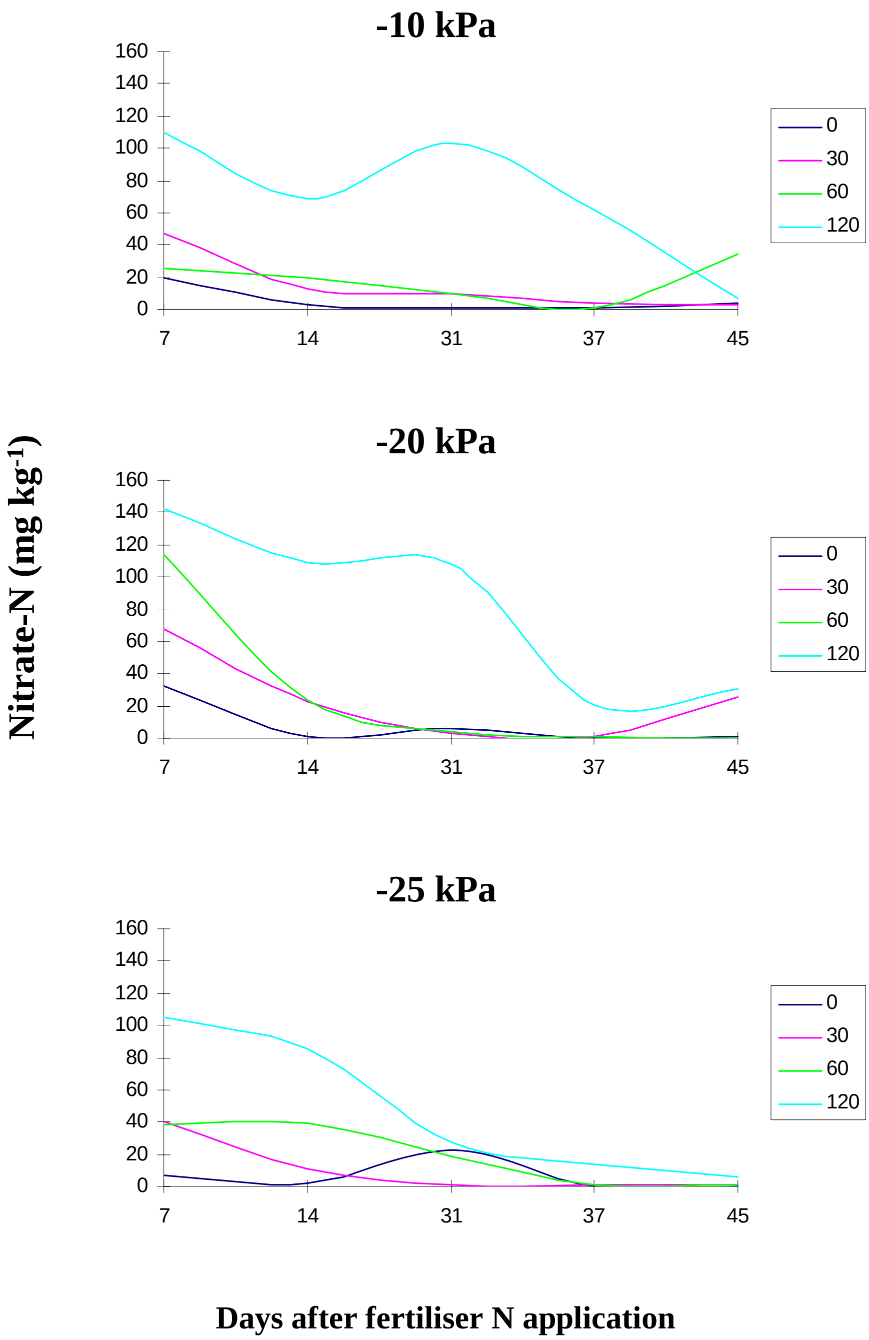

Figure 3.3 The effect of fertiliser N rate (kg N ha^{-1}) and soil water potential (kPa) on soil nitrate-N content (mg kg^{-1}) in perennial ryegrass over a 45 day period following fertiliser N application under controlled conditions at Elsenburg.

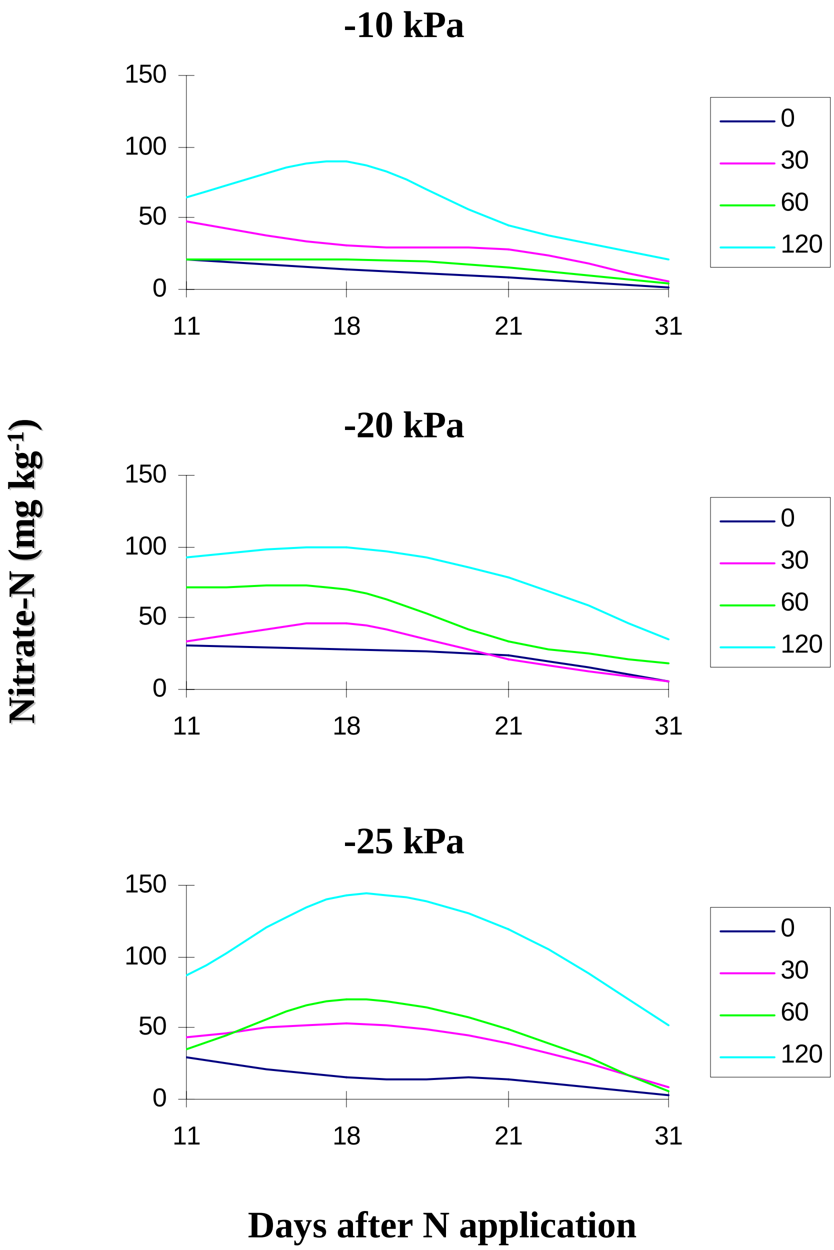

Figure 3.4 The effect of fertiliser N rate (kg N ha^{-1}) and soil water potential (kPa) on soil nitrate-N content (mg kg^{-1}) in white clover over a 31 day period following fertiliser N application under controlled conditions at Elsenburg.

Number of tillers/stolons

Increasing the fertiliser N rate from 0 to120 kg N ha^{-1} resulted in increased (P=0.05) number of ryegrass tillers per pot (Table 3.4). Soil water level however did not influence the number of ryegrass tillers produced. The number of white clover stolon growing points was not influenced by the treatments (Table 3.5).

Table 3.4 Number of perennial ryegrass tillers per pot 60 days after fertiliser N application as influenced by soil water potential (kPa) and rate of fertiliser N application (kg ha^{-1}) under controlled conditions at Elsenburg

	Fertiliser N rate (kg ha^{-1})					
Water potential	**0**	**30**	**60**	**120**	**180**	**Mean (M)**
-10 kPa	117.0	135.8	160.3	228.0	261.5	**180.5**
-20 kPa	88.3	145.0	147.5	203.8	242.0	**169.4**
-25 kPa	100.7	126.0	161.3	215.5	187.3	**161.2**
-35 kPa	94.3	132.0	156.0	208.5	227.3	**167.9**
Mean (N)	**101.38 d***	**134.69 c**	**156.27 b**	**213.94 a**	**229.5 a**	

LSD(0.05) Interaction=NS
LSD(0.05) N mean=21.155
LSD(0.05) M mean=NS
CV = 17.08

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

Table 3.5 Number of white clover stolon growing points per pot 60 days after fertiliser N application as influenced by soil water potential (kPa) and rate of fertiliser N application (kg ha^{-1}) under controlled conditions at Elsenburg

	Fertiliser N rate (kg ha^{-1})					
Water potential	**0**	**30**	**60**	**120**	**180**	**Mean (M)**
-10 kPa	24.5	26.3	25	23.8	22.5	**24.4**
-20 kPa	24.8	24	25.3	22.8	27.7	**24.7**
-25 kPa	22.5	21.8	23.8	23.7	23.8	**23.1**
-35 kPa	24	23	23.5	23.3	24.8	**23.7**
Mean (N)	**23.9**	**23.8**	**24.3**	**23.3**	**24.5**	

LSD(0.05) Interaction= NS
LSD(0.05) N mean=NS
LSD(0.05) M mean=NS
CV = 9.90

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

Eckard (1994) however found under field conditions that the number of stolons declined as fertiliser N rate increased. In the current study stolon growing points per pot were counted while Eckard (1994) counted the number of stolons crossing the sides of squares placed in the pasture.

Dry matter production

Primary-, residual- and total dry matter production of perennial ryegrass were significantly influenced by rate of fertiliser N application and soil water level. Total dry matter production was also significantly influenced by the interaction between fertiliser N rate and soil water level. White clover, however, was only influenced by fertiliser N rate and soil water level during the second regrowth cycle (RDM yield) following fertiliser N application.

Primary dry matter production (PDM)

Increasing fertiliser N application rate from 0 to 120 kg N ha^{-1} resulted in increased (P=0.05) ryegrass PDM production (Table 3.6) followed by a non-significant increase as N rate was increased to 180 kg N ha^{-1}. The non significant increases in ryegrass PDM yields as fertiliser N rates were increased from 120 kg N ha^{-1} to 180 kg N ha^{-1}, suggest that perennial ryegrass reached maximum productivity under the conditions that prevailed during the first regrowth cycle. In contrast to ryegrass, clover PDM production was not influenced by any of the treatments suggesting that fertiliser N rates of up to 180 kg N ha^{-1} may not influence clover productivity under similar conditions that prevailed during the first regrowth cycle (Table 3.7). This result shows that the nitrogen applied to a perennial ryegrass-white clover pasture will mainly be absorbed by the ryegrass fraction during the first regrowth cycle. The reduction of either clover % or clover DM production as reported by Stout, Weaver & Elwinger (2001) as well as van Heerden & Durand (1994) can therefore be ascribed to the stimulation of ryegrass by fertiliser N rather than suppressing clover growth directly. The mean clover PDM yield of 2.221 g pot^{-1} at 180 kg N ha^{-1} compared to 2.064 g pot^{-1} at 0 kg N ha^{-1} shows that the clover plants were not negatively influenced by the high levels of soil-N during the first regrowth cycle.

The highest ryegrass PDM production, although not significantly higher than at -10 kPa, was recorded at the -20 kPa soil water potential.

Table 3.6 Primary, residual and total dry matter production (g pot^{-1}) of perennial ryegrass as influenced by soil water potential (kPa) and fertiliser N application rate (kg ha^{-1}) under controlled conditions at Elsenburg

Primary DM Production (g pot^{-1})						
	Fertiliser N rate (kg ha^{-1})					
Water potential	**0**	**30**	**60**	**120**	**180**	**Mean (M)**
-10 kPa	2.383	2.930	3.240	4.848	5.145	**3.709 ab***
-20 kPa	1.993	3.398	3.725	4.713	5.343	**3.931 a**
-25 kPa	1.915	2.495	3.460	4.233	4.673	**3.355 c**
-35 kPa	1.970	2.980	3.733	4.578	3.993	**3.528 bc**
Mean (N)	**2.077 d**	**2.951 c**	**3.539 b**	**4.593 a**	**4.788 a**	
LSD(0.05) Interaction=NS						
LSD(0.05) N mean=0.3601						
LSD(0.05) M mean=0.3217						
CV = 13.83						
Residual DM Production (g pot^{-1})						
	Fertiliser N rate (kg ha^{-1})					
Water potential	**0**	**30**	**60**	**120**	**180**	**Mean (M)**
-10 kPa	0.790	1.130	1.100	4.550	6.495	**2.813 a**
-20 kPa	0.537	1.190	1.360	3.770	6.073	**2.694 a**
-25 kPa	0.483	0.743	1.165	2.918	5.470	**2.157 b**
-35 kPa	0.453	0.770	0.933	3.043	3.860	**1.883 b**
Mean (N)	**0.576 d**	**0.958 cd**	**1.139 c**	**3.570 b**	**5.474 a**	
LSD(0.05) Interaction=NS						
LSD(0.05) N mean=0.5381						
LSD(0.05) M mean=0.4807						
CV = 31.38						
Total DM Production (g pot^{-1})						
	Fertiliser N rate (kg ha^{-1})					
Water potential	**0**	**30**	**60**	**120**	**180**	**Mean (M)**
-10 kPa	3.173 hi	4.060 fgh	4.340 fgh	9.398 cd	11.640 a	**6.522 a**
-20 kPa	2.530 i	4.588 fgh	5.085 f	8.483 de	11.415 ab	**6.625 a**
-25 kPa	2.398 i	3.238 ghi	4.625 fgh	7.150 e	10.143 bc	**5.511 b**
-35 kPa	2.423 i	3.750 fghi	4.665 fg	7.620 e	7.853 e	**5.412 b**
Mean (N)	**2.653 e**	**3.909 d**	**4.679 c**	**8.163 b**	**10.263 a**	
LSD(0.05) Interaction=1.49						
LSD(0.05) N mean=0.7433						
LSD(0.05) M mean=0.6641						
CV = 17.21						

**** Means in the same column or row at a specified dry matter production followed by the same letter are not significantly different (P=0.05)***

Table 3.7 Primary, residual and total dry matter production (g pot^{-1}) of white clover as influenced by soil water potential (kPa) and fertiliser N application rate (kg ha^{-1}) under controlled conditions at Elsenburg

Primary DM Production (g pot^{-1})						
	Fertiliser N (kg ha^{-1})					
Water potential	**0**	**30**	**60**	**120**	**180**	**Mean (M)**
-10 kPa	2.145	2.700	2.868	2.648	1.950	**2.462**
-20 kPa	2.055	2.255	2.153	2.148	2.750	**2.252**
-25 kPa	2.108	2.238	2.130	2.110	2.110	**2.139**
-35 kPa	1.948	2.218	2.170	2.065	2.208	**2.122**
Mean (N)	**2.064**	**2.353**	**2.342**	**2.243**	**2.221**	
LSD(0.05) Interaction=NS						
LSD(0.05) N mean=NS						
LSD(0.05) M mean=NS						
CV = 28.07						

Residual DM Production (g pot^{-1})						
	Fertiliser N rate (kg ha^{-1})					
Water potential	**0**	**30**	**60**	**120**	**180**	**Mean (M)**
-10 kPa	4.383	5.078	4.583	5.338	5.653	**5.007 b**
-20 kPa	4.735	4.465	5.273	5.165	6.163	**5.098 b**
-25 kPa	5.478	5.408	5.205	6.088	5.805	**5.571 a**
-35 kPa	4.145	4.640	4.780	5.188	5.030	**4.757 b**
Mean (N)	**4.685 c**	**4.898 c**	**4.939 bc**	**5.401 ab**	**5.629 a**	
LSD(0.05) Interaction=NS						
LSD(0.05) N mean=0.5003						
LSD(0.05) M mean=0.4477						
CV = 13.58						

Total DM Production (g pot^{-1})						
	Fertiliser N rate (kg ha^{-1})					
Water potential	**0**	**30**	**60**	**120**	**180**	**Mean (M)**
-10 kPa	6.528	7.778	7.450	7.985	7.603	**7.469**
-20 kPa	6.790	6.720	7.427	7.313	8.913	**7.351**
-25 kPa	7.585	7.645	7.335	8.667	7.915	**7.785**
-35 kPa	6.093	6.858	6.950	7.253	7.238	**6.878**
Mean (N)	**6.749**	**7.250**	**7.281**	**7.747**	**7.851**	
LSD(0.05) Interaction=NS						
LSD(0.05) N mean=NS						
LSD(0.05) M mean=NS						
CV = 16.63						

***** *Means in the same column or row at a specified dry matter production followed by the same letter are not significantly different (P=0.05)*

Residual dry matter production (RDM)

Carry-over N to the second regrowth cycle (30 to 60 days) caused increased (P=0.05) ryegrass RDM production as N rate progressively increased from 60 to 120 and 180 kg N ha^{-1} (Table 3.6). No differences (P>0.05) in ryegrass RDM production between 0 kg N ha^{-1} and 30 kg N ha^{-1} were recorded suggesting no carry over of N fertiliser N to the second regrowth cycle at the 30 kg N ha^{-1} rate. Application of an equivalent of 120 and 180 kg N ha^{-1} resulted in higher (P=0.05) ryegrass RDM yields than achieved at the 0, 30 and 60 kg N ha^{-1} rates. These increases can largely be attributed to the higher (P=0.05) soil N levels measured at the 120 (and assumably the 180) kg N ha^{-1} rates. White clover showed similar reaction to fertiliser N rate with no difference in clover RDM production as N rate increased from 0 to 30 and 60 kg N ha^{-1} (Table 3.7). The application of 180 kg N ha^{-1} however resulted in significantly higher clover RDM yields compared to N rates of 0, 30 and 60 kg N ha^{-1}. The reaction of white clover to fertiliser N could be the result of increased productivity as mean dry matter production increased by 2.621 g pot^{-1} at the 0 kg N ha^{-1} rate between PDM and RDM production. The positive reaction to increased levels of fertiliser N confirmed that nitrogen levels of as high as 180 kg N ha^{-1} can be applied without reducing white clover productivity.

Increasing the soil water level to -10 kPa and -20 kPa, resulted in higher (P=0.05) ryegrass RDM yields than at -25 and -35 kPa. This reaction could be the result of increased mass flow of N to the root surface at the higher soil water contents as well as growth induced N demand as plant growth rate increased at the higher soil water levels.

Total dry matter production (TDM)

Increased fertiliser N application rate resulted in increased (P=0.05) ryegrass TDM yield over all N rates covered by the study (Table 3.6).

Higher (P=0.05) mean ryegrass TDM yields were recorded at the -10 kPa and -20 kPa soil water potentials, indicating that water potentials of -10 kPa will not reduce ryegrass TDM production. White clover TDM production was not influenced by the treatments used (Table 3.7).

Soil water levels did not influenced ryegrass TDM production at a specific N rate between 0 and 60 kg N ha^{-1}. However, the application of 120 kg N ha^{-1} and especially 180 kg N ha^{-1} resulted in higher (P>0.05) ryegrass PDM yields in the wetter soils (-10 and -20 kPa). The progressive reduction in ryegrass TDM yield at 180 kg N ha^{-1} as soil water level decreases from -10 to -35 kPa suggest that soil water potential as low as -25 kPa can significantly (P=0.05) reduces ryegrass productivity when plant production potential is high due to high soil N supply. A reduction of 3.787g (32.5%) in ryegrass TDM production was recorded when the soil water level was reduced from -10 to -35 kPa at the 180 kg N ha^{-1} application rate (Table 3.6). Reduced mass flow of water towards the ryegrass root surface as soil water tension increases could result in reduced contact between roots and N, restricting N absorption although high enough N concentrations to maintain maximum yields possibly occurred in the soil. The fact that no differences occurred between water levels at the 0 to 60 kg N ha^{-1} application rates confirms that high applications of N result in high productivity and therefore need high water availability, but as soon as N supply decreases, decreases in water supply will be less restricted. Penman (1962) reported that ryegrass on high nitrogen plots tolerated higher water deficits than low nitrogen plots but did not investigate the possible reason for this response.

Legumes like white clover is mostly self sufficient with regard to N nutrition due to symbiotic nitrogen fixation. It is therefore not dependent on the absorption of water to meet its N requirements as were proved by the absence of responses to the different N rates tested. Soil water level will however influence the supply of oxygen to nitrogen fixing bacteria and might influence clover production at higher soil water levels. The highest (-10 kPa) water level tested in the study however did not influence clover PDM and TDM production, which indicates that N-fixation was probably not affected.

Leaf N content (%)

Increased fertiliser N rate resulted in increased perennial ryegrass as well as white clover leaf N content at the end of the first regrowth cycle (Tables 3.8 & 3.9). The application of 120 kg N ha^{-1} resulted in the highest (P=0.05) ryegrass leaf N contents while the lowest (P=0.05) was recorded at 0 kg N ha^{-1} with no difference between 30 and 60 kg N ha^{-1}. Similar results were recorded in clover where the application of 60 and 120 kg N ha^{-1} resulted in higher (P=0.05) leaf N contents compared to 0 and 30 kg N ha^{-1}.

Table 3.8 Primary and residual leaf nitrogen content (%) of perennial ryegrass as influenced by soil water potential and rate of fertiliser N application (kg ha^{-1}) under controlled conditions at Elsenburg

	N in primary DM (%)				
Water potential	**Fertiliser N rate (kg ha^{-1})**				
	0	**30**	**60**	**120**	**Mean (M)**
-10 kPa	3.394	3.673	3.916	5.416	**4.100 a***
-20 kPa	2.907	3.490	3.730	5.261	**3.847 ab**
-25 kPa	2.450	3.189	3.519	5.340	**3.625 b**
Mean (N)	**2.917 c**	**3.451 b**	**3.722 b**	**5.339 a**	
LSD(0.05) interaction = NS					
LSD(0.05) N mean = 0.3584					
LSD(0.05) M mean = 0.3104					
CV = 9.55					
	N in residual DM (%)				
Water potential	**Fertiliser N rate (kg ha^{-1})**				
	0	**30**	**60**	**120**	**Mean (M)**
-10 kPa	2.956	2.763	2.772	3.133	**2.906**
-20 kPa		2.573	2.677	3.845	**3.032**
-25 kPa	2.522	2.336	2.270	3.697	**2.723**
Mean (N)	**2.783 b**	**2.557 b**	**2.573 b**	**3.558 a**	
LSD(0.05) interaction = NS					
LSD(0.05) N mean = 0.3778					
LSD(0.05) M mean = NS					
CV = 12.22					

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

Perennial ryegrass leaf N content at the end of the first regrowth cycle decreased as soil water level decreased with leaf N content at -10 kPa significantly higher than at -25 kPa. Ryegrass leaf N content measured at -20 kPa did not differ from values recorded at -10 and -25 kPa. Clover leaf N content was not influenced by soil water level for the same reasons as discussed under RDM production.

The only treatments resulted in ryegrass leaf N contents lower than the 2.0-3.2% suggested by Reuter & Robinson (1997) as the zone of marginal deficiency were 30 kg N ha^{-1} at -25 kPa and 0 kg N ha^{-1} at -20 and -25 kPa. Clover leaf N content was without exception higher than the 3.2 to 3.6 % regarded as marginal deficient (Reuter & Robinson, 1997).

Table 3.9 Primary and residual leaf nitrogen content (%) of white clover as influenced by soil water potential (kPa) and rate of fertiliser N application (kg ha^{-1}) under controlled conditions at Elsenburg

	N in primary DM (%)				
Water potential	**Fertiliser N rate (kg ha^{-1})**				
	0	**30**	**60**	**120**	**Mean (M)**
-10 kPa	4.604	4.669	4.988	5.035	**4.824**
-20 kPa	4.926	4.627	5.106	5.256	**4.979**
-25 kPa	4.535	4.905	5.203	5.223	**4.967**
Mean (N)	**4.688 b**	**4.734 b**	**5.099 a**	**5.171 a**	
LSD(0.05) Interaction = NS					
LSD(0.05) N mean = 0.266					
LSD(0.05) M mean = NS					
CV = 5.55					
	N in residual DM (%)				
Water potential	**Fertiliser N rate (kg ha^{-1})**				
	0	**30**	**60**	**120**	**Mean (M)**
-10 kPa	4.481	4.287	4.376	4.102	**4.312 a**
-20 kPa	4.143	4.098	4.012	4.207	**4.005 b**
-25 kPa	3.956	4.128	4.078	3.872	**4.009 b**
Mean (N)	**4.193**	**4.171**	**4.155**	**4.060**	
LSD(0.05) interaction = NS					
LSD(0.05) N mean = NS					
LSD(0.05) M mean = 0.1613					
CV = 4.62					

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

The only difference (P=0.05) recorded at the end of the second regrowth cycle were higher ryegrass leaf N contents at the 120 kg N ha^{-1} rate compared to the 0, 30 and 60 kg N ha^{-1} rates. Results confirmed by Ball (1979, cited by Eckard, 1994) reporting that white clover N content showed little response to fertiliser N. Eckard (1994) however reported that the average N content of both annual ryegrass and white clover increased linearly as fertiliser N increased.

Soil water level influenced (P=0.05) clover leaf N content only in the second regrowth cycle, resulting in a higher leaf N content at the -10 kPa level.

Leaf N yield

Perennial ryegrass leaf N yield (g pot^{-1}) at the end of the first regrowth cycle, increased as fertiliser N rates increased (Table 3.10).

Table 3.10 Primary, residual and total leaf nitrogen yield (g pot^{-1}) of perennial ryegrass as influenced by soil moisture potential (kPa) and rate of fertiliser N application (kg ha^{-1}) under controlled conditions at Elsenburg

N in primary DM (g pot^{-1})*					
Water potential	**Fertiliser N rate (kg ha^{-1})**				
	0	**30**	**60**	**120**	**Mean (M)**
-10 kPa	0.081	0.108	0.127	0.263	**0.144**
-20 kPa	0.058	0.119	0.139	0.248	**0.141**
-25 kPa	0.047	0.080	0.122	0.226	**0.119**
Mean (N)	**0.062**	**0.102**	**0.129**	**0.246**	
N in residual DM (g pot^{-1})					
Water potential	**Fertiliser N rate (kg ha^{-1})**				
	0	**30**	**60**	**120**	**Mean (M)**
-10 kPa	0.023	0.031	0.030	0.143	**0.057**
-20 kPa	**NA	0.031	0.036	0.145	**0.071**
-25 kPa	0.012	0.017	0.026	0.108	**0.041**
Mean (N)	**0.018**	**0.026**	**0.031**	**0.132**	
N in total DM (g pot^{-1})					
Water potential	**Fertiliser N rate (kg ha^{-1})**				
	0	**30**	**60**	**120**	**Mean (M)**
-10 kPa	0.104	0.139	0.157	0.405	**0.201**
-20 kPa	NA	0.149	0.175	0.393	**0.239**
-25 kPa	0.059	0.097	0.148	0.334	**0.160**
Mean (N)	**0.082**	**0.128**	**0.160**	**0.377**	

** No data available

Nitrogen yield was calculated as follows:

Nitrogen yield = DM production (g pot^{-1}) * Percentage N/100

No statistical analysis was done on this parameter as analysis were done on the components DM production and percentage N in the leaves harvested.

The increased ryegrass PDM production and higher plant N content are the reasons for the increased N removal at higher N rates. Clover showed a slight increase as fertiliser N was increased from 0 to 60 kg N ha^{-1} followed by a slight reduction at the 120 kg N ha^{-1} rate (Table 3.11). The lowest ryegrass primary leaf N yields were obtained at the -25 kPa water potential, possibly due to slower uptake of soil N as already discussed. The highest clover primary leaf N yield was measured at the -10 kPa water level, slightly higher than at -20 and -25 kPa.

Table 3.11 Primary, residual and total leaf nitrogen yield (g pot^{-1}) of white clover as influenced by soil moisture potential (kPa) and rate of fertiliser N application (kg ha^{-1}) under controlled conditions at Elsenburg

	N in primary DM (g pot^{-1})				
Water potential	**Fertiliser N rate (kg ha^{-1})**				
	0	**30**	**60**	**120**	**Mean (M)**
-10 kPa	0.099	0.126	0.143	0.133	**0.125**
-20 kPa	0.101	0.104	0.110	0.113	**0.107**
-25 kPa	0.096	0.110	0.111	0.110	**0.107**
Mean (N)	**0.099**	**0.113**	**0.121**	**0.119**	***0.113***
	N in residual DM (g pot^{-1})				
Water potential	**Fertiliser N rate (kg ha^{-1})**				
	0	**30**	**60**	**120**	**Mean (M)**
-10 kPa	0.196	0.218	0.201	0.219	**0.208**
-20 kPa	0.196	0.183	0.212	0.217	**0.202**
-25 kPa	0.217	0.223	0.212	0.236	**0.222**
Mean (N)	**0.203**	**0.208**	**0.208**	**0.224**	***0.211***
	N in total DM (g pot^{-1})				
Water potential	**Fertiliser N rate (kg ha^{-1})**				
	0	**30**	**60**	**120**	**Mean (M)**
-10 kPa	0.295	0.344	0.344	0.352	**0.334**
-20 kPa	0.297	0.287	0.322	0.330	**0.309**
-25 kPa	0.312	0.333	0.323	0.346	**0.329**
Mean (N)	**0.302**	**0.321**	**0.329**	**0.343**	***0.324***

Nitrogen yield was calculated as follows:

Nitrogen yield = DM production (g pot^{-1}) * Percentage N/100

No statistical analysis was done on this parameter as analysis were done on the components DM production and percentage N in the leaves harvested.

Ryegrass leaf N yields at the end of the second regrowth cycle followed the same trend as in the first although at much lower levels. In contrast to this, clover leaf N yields at the end of the second regrowth cycle were much higher than after the first regrowth cycle emphasising the ability of clover to fix N symbiotically and thereby restricting the negative effects of low soil-N content on clover productivity.

Increased fertiliser N rates resulted in increased ryegrass total N yields. Differences between total N yields of clover were negligible. Total leaf nitrogen yields of ryegrass after 60 days, with 0 kg N ha^{-1} at -10 and -25 kPa were 0.104 and 0.059 g pot^{-1} respectively (Table 3.10). These values represented N removal of 43.23 and 24.52 kg N ha^{-1} illustrating the negative effect of lower water levels on soil N availability and/or the capacity of ryegrass roots to absorb soil N at lower soil water contents. The same tendency was observed (total leaf N after a 60 day period) at the 120 Kg N ha^{-1} application rate where 168.33, 163.34 and 138.82 kg N ha^{-1} were removed at -10, -20 and -25 kPa respectively. A contributing factor to the differences measured, could be increased plant N uptake as dry matter production increase under more favourable environmental conditions.

Root dry mass (Root DM)

Both fertiliser N rate and soil water level influenced (P=0.05) the dry mass of ryegrass and clover roots (Tables 3.12 & 3.13). With the exception of 0 kg N ha^{-1}, the application of 180 kg N ha^{-1} resulted in lower (P=0.05) mean ryegrass root dry matter production compared to all other treatments. The increase in ryegrass root dry mass in treatments that received between 30 and 120 kg N ha^{-1} illustrated the importance of sufficient, but not an oversupply of N to the perennial ryegrass plant. In contrast to ryegrass, the highest clover root dry mass was recorded at the 180 kg N ha^{-1} rate, significantly higher than at 0, 30 and 60 kg N ha^{-1}. No differences in clover root dry mass were recorded between the 0, 30, 60 and 120 kg N ha^{-1} rates.

Table 3.12 Root dry mass (g pot^{-1}) of perennial ryegrass as influenced by soil water potential (kPa) and rate of fertiliser N application (kg ha^{-1}) under controlled conditions at Elsenburg

	Fertiliser N rate (kg ha^{-1})					
Water potential	**0**	**30**	**60**	**120**	**180**	**Mean (M)**
-10 kPa	3.3125	3.465	3.7925	3.8325	3.2875	**3.538 b***
-20 kPa	3.8033	4.6625	4.0725	3.6975	2.94	**3.8368 b**
-25 kPa	4.23	3.8825	4.395	3.6275	3.5425	**3.92 b**
-35 kPa	4.2133	5.4275	5.0433	5.17	3.675	**4.7144 a**
Mean (N)	**3.8454 ab**	**4.3594 a**	**4.278 a**	**4.0819 a**	**3.3613 b**	

LSD(0.05) Interaction=NS
LSD(0.05) N mean=0.6121
LSD(0.05) M mean=0.546
CV = 21.06

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

Table 3.13 Root dry mass (g pot^{-1}) of white clover as influenced by soil water potential (kPa) and rate of fertiliser N application (kg ha^{-1}) under controlled conditions at Elsenburg

	Fertiliser N rate (kg ha^{-1})					
Water potential	**0**	**30**	**60**	**120**	**180**	**Mean (M)**
-10 kPa	0.725	0.893	0.833	0.985	1.175	**0.922 c***
-20 kPa	1.023	1.313	1.000	1.108	1.820	**1.235 ab**
-25 kPa	0.838	1.030	0.923	1.300	1.488	**1.106 bc**
-35 kPa	1.443	1.313	1.550	1.520	1.565	**1.478 a**
Mean (N)	**1.007 b**	**1.137 b**	**1.081 b**	**1.223 ab**	**1.491 a**	

LSD(0.05) Interaction= NS
LSD(0.05) N mean=0.2964
LSD(0.05) M mean=0.2652
CV = 24.64

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

The highest (P=0.05) ryegrass root mass was found at the -35 kPa water potential. Although not significant lower than at -20 and -25 kPa, the lowest ryegrass root dry mass was recorded at the -10 kPa water potential indicating a possible reduction in root activity as a result of the high soil water content. However the higher ryegrass root mass could not increase dry matter production as TDM production at -35 kPa was significantly lower than at -10 and -20 kPa (Table 3.6). The relative low soil water level at -35 kPa resulted in significantly (P=0.05) higher ryegrass root dry mass compared to -10 kPa. In both perennial ryegrass and white clover pastures a soil water potential of -35 kPa will therefore result in the highest root dry mass production. Dry matter production (Tables 3.6 & 3.7) however showed that this benefit may not necessarily be reflected in higher foliage DM production. The benefit of increased root dry mass may however be of great benefit under less favourable soil conditions.

Conclusions

The study has shown that significant increases in PDM production of perennial ryegrass can be achieved with fertiliser N applications of up to 120 kg N ha^{-1} under high (-10 and -20 kPa) soil water levels. Perennial ryegrass could not maintain the significantly higher dry matter production at the 180 kg N ha^{-1} rates causing carry over N resulting in increased RDM yields. This carry-over N may be exposed to leaching under field conditions causing a possible reduction of N recovery rate and increasing the risk of contamination of natural resources. The N-uptake at the -25 and -35 kPa water levels were probably restricted by N supply as RDM yields were significantly reduced at these soil water levels. Lower mean ryegrass leaf N contents measured at 60 days support this conclusion. Clover PDM and TDM production were not influenced by fertiliser N rate and soil water levels covered in this study.

Although the highest root dry mass for both ryegrass and clover were recorded at soil water potentials of -35 kPa, it did not result in increased dry matter production. The application of 180 kg N ha^{-1} reduced, although not significant, ryegrass root dry mass illustrating the disadvantage of too high fertiliser N rates. The highest clover root dry mass was however recorded at the 180 kg N ha^{-1} application rate.

The highest ryegrass leaf N contents after the first regrowth cycle measured at the -10 kPa soil water potential suggested that the absorption of soil N were not restricted by the high soil water levels. However lower ryegrass leaf N content recorded with decreasing soil water levels furthermore illustrated the negative effect of reduced soil water content on N uptake.

The study has shown firstly, that the application of fertiliser N to stimulate ryegrass productivity under relative wet conditions without negatively affecting a companion clover crop in a mixed pasture could be successful. Secondly, that the positive effect of the high fertiliser N rate on ryegrass TDM was improved as soil water level increased, however TDM yields at low fertiliser N rates were not increased with increased soil water levels.

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Chapter 4

The influence of production season and fertiliser N rate on the growth and production of perennial ryegrass *(Lolium perenne)* and white clover *(Trifolium repens)* grown under controlled conditions

Abstract

The responses of perennial ryegrass and white clover grown in separate pots in a glasshouse at 12/18 °C night/day temperatures during production seasons in June/July (304.4 h photoperiod) and October/November (384.8 h photoperiod) to five fertiliser N rates (0, 30, 60, 120 and 180 kg N ha^{-1}) were investigated. Primary- (PDM) and residual dry matter production (RDM) were recorded 31 and 60 days after fertiliser N application. The number of ryegrass tillers or clover stolons and root dry mass were recorded 60 days after fertiliser N application.

Perennial ryegrass PDM, RDM and TDM yields were influenced by fertiliser N rate, production season as well as the fertiliser N rate and production season interaction. Increased fertiliser N rate resulted in increased (P=0.05) ryegrass dry matter production, the only exception a non-significant RDM production increase between 30 and 60 kg N ha^{-1}. PDM, RDM and TDM production of perennial ryegrass were higher (P=0.05) in October/November compared to June/July. White clover was influenced by production season only, resulting in increased (P=0.05) dry matter yields during October/November for PDM, RDM and TDM production compared to June/July. The number of ryegrass tillers increased (P=0.05) as fertiliser N rate was increased, with a non-significant increase measured between 30 and 60 kg N ha^{-1}. The October/November production season resulted in significantly more clover stolons than during June/July. Mean perennial ryegrass root dry mass at 180 kg N ha^{-1} was higher (P=0.05) than at 0 kg N ha^{-1}, the same reaction was observed during October/November. No differences in root dry mass as a result of fertiliser N rate were recorded during June/July. Both ryegrass and clover produced higher (P=0.05) root dry masses during October/November.

The study has shown that significant increases in dry matter production of perennial ryegrass and white clover can be expected if a 31 day regrowth period in June/July is compared to a 31

day regrowth period in October/November. The study also showed that the lack of sufficient accumulation of "sunlight hours" in June/July resulted in no differences in ryegrass dry matter production as fertiliser N was increased from 30 to 60 kg N ha^{-1} a response not observed during October/November.

Keywords: dry matter production, fertiliser N rate, perennial ryegrass, production season, root dry mass, stolons, tillers, white clover

Introduction

The opportunity to boost perennial ryegrass-white clover productivity in addressing the winter gap is restricted to autumn, winter and spring occurring between April and September. Daily photoperiod (daylength) during these seasons varies from 11.6 (April 1st) to a minimum of 9.8 (June 7th – July 4th) and 12.3 hours day^{-1} on September 30th (Anon, 2004).

Productivity, as a function of photosynthesis, of pastures grown under high potential conditions is normally limited by incoming solar energy, while transpiration is controlled by nett radiation (Russell, 1988). Most of the nett radiation is dissipated as transpiration under these conditions. Since netto radiation is closely correlated with incoming radiation, Russell (1988) suggested that rates of photosynthesis and transpiration would be closely related. It could therefore be assumed that the length of exposure to incoming solar energy would influence pasture productivity.

It can be expected that cloud cover during the rainy season will have similar effects on clover and ryegrass, because both are C_3 plants where photosynthesis reaches saturation point at about 66% of full sunlight (Ludlow, 1978). The frequency and duration of cloud cover will therefore be less critical to clover and ryegrass productivity.

In a review article Ludlow (1978) stated that short days stimulate tiller development and increase root:shoot ratio in some grasses while long days may increase leaf length and area in some temperate grasses effectively increasing the height and competitiveness of the grass. Short days may also promote a more prostrate and long days more erect growth pattern in some legumes. Nodulation and nitrogen fixation are also usually better under short than longer days. The advantage of N fixation under short day conditions can however not be taken advantage of as conditions in the Western Cape Province during the cool season (short days) normally coincides with low temperatures restricting *Rhizobia* activity.

The aim of this study was to evaluate the influence of production season on perennial ryegrass and white clover grown under controlled conditions. The information gathered will give us a better understanding of the response of ryegrass and clover to fertiliser N application during different seasons in the absence of temperature differences between seasons.

Materials and methods

Locality

Perennial ryegrass (*Lolium perenne* cv. Ellet) and white clover (*Trifolium repens* cv. Haifa) were established separately in pots in a glasshouse under natural photoperiod and light intensity conditions at the Institute for Plant Production, Elsenburg (altitude 177m, 18^{o}50'E, 33^{o}51'S). Day/night temperatures were regulated at 18° and 12 °C for 10 and 14 hours respectively. It was however difficult to maintain temperatures of 18 °C during November with day temperatures reaching 24 °C on numerous occasions.

Growth medium preparation

Topsoil (0-150 mm layer) from the orthic A horizon of an Oakleaf soil (Soil Classification Working Group, 1991) derived mainly from granite (Anon, 1996) was used as growth medium. To achieve a relative uniform bulk density over all pots the soil was gathered in heaps, mixed and ran through a 5 mm screen to separate the larger clods (aggregates) and crop residues from the soil used as growth medium. The soil was dried in stainless steel bins at 60 °C. Soil used in the October/November study was placed in bags and stored. A composite soil sample was collected and analysed for both physical- and chemical properties (Table 4.1). Analysis methods for measuring the physical and chemical properties of the soil are referred to in Chapter 2.

Table 4.1 Chemical analysis and selected physical properties of the soil used as growth medium at Elsenburg

Chemical properties			**Physical properties**		
pH (KCl)		6.4			
Resistance	**Ohms**	1360	**Clay**	**%**	14
P (citric acid)	**mg/kg**	34	**Silt**	**%**	13.1
K	**cmol(+)/kg**	0.37	**Fine Sand**	**%**	49.8
Ca	**cmol(+)/kg**	2.61	**Medium Sand**	**%**	15.9
Mg	**cmol(+)/kg**	1.16	**Course Sand**	**%**	7.2
Na	**cmol(+)/kg**	0.15			
Total cations	**cmol(+)/kg**	4.29	**Classification**		SaLm
Copper	**mg/kg**	0.77			
Zinc	**mg/kg**	0.79			
Manganese	**mg/kg**	107			
Boron	**mg/kg**	0.55			
Carbon	**%**	0.58			

Soil fertility levels were corrected through the application of single superphosphate to levels recommended by Beyers (1983). Copper, manganese and zinc were sufficient with boron marginally low. Foliar nutrition (N, P, K, Ca, Mg, S, B, Fe, Zn and Mo) were applied twice during the pre-treatment growth period and five days after each cut, a total of four times, to prevent any deficiencies. The organic C content of 0.58 % was low. An equivalent of 40 kg N ha^{-1} was applied at seeding to maintain plant growth during the pre-treatment growth period. To prevent N leaching, pots were lined with plastic bags and filled with 6.6 kg of the oven dried soil occupying a volume of 4324.57 cm^3 resulting in a bulk density of 1.53 g cm^{-3}. Pots were watered and left for ten days to enable weed seed to germinate. After removing the weeds a template with five holes, one in the centre and one in each quarter of the pot, was used to plant the seed in a predetermined configuration 5-10 mm deep. A few seeds were planted per hole followed by water application to fill the soil to field water capacity (0.253 mm^3 mm^{-3}). Seedlings were thinned after emergence to five plants per pot. Pots were watered daily through weighing and adding water to the predetermined weight at field water capacity. Changes in plant weight as a result of growth were considered on a weekly basis when pot weight after watering was calculated. Pots were randomised after watering.

Plants were allowed to grow for an accumulative photoperiod of *ca* 691 hours from planting and clipped at 50 mm height. The clippings were dried at 60 °C for *ca* 18 h upon which pots showing least variation in DM production were at random allocated to the treatment combinations.

Experimental design and treatments

A completely randomised experimental design was used. A factorial design was used to determine the effect of photoperiod (June/July and October/November) and fertiliser N rate (0, 30, 60,120 and 180 kg N ha^{-1} applied as LAN dissolved in 200 ml water) on ryegras and clover performance (Snedecor & Cochran, 1967). Fertiliser N rate was replicated four times.

Data collection

DM production was recorded by cutting the perennial ryegrass and white clover at 50 mm height 31 and 60 days after nitrogen treatments had been applied. Fresh weight was recorded, cuttings ovendried at 60 °C for 72 hours and dry weight noted. Dry matter production was recorded as primary - (PDM) at 31 days, residual – (RDM) production from 31 to 60 days and total dry matter production (TDM, which is the cumulative DM production over 60 days). The number of tillers and stolon growing points were recorded at 60 days after fertiliser N application. After residual dry matter production was recorded, roots were removed by wet sieving using a 2 and 1 mm combination sieve, dried at 60 °C for 72 h and dry mass recorded.

Statistical procedures

Analysis of variance (ANOVA) was performed using SAS version 8.2 (SAS, 1999). The Shapiro-Wilk test was used to test for non-normality (Shapiro & Wilk, 1965). Student's t-Least Significant Difference (LSD) test was calculated at the 5% confidence level to compare treatment means (Ott, 1998).

Results and discussion

Number of ryegrass tillers/clover stolons

The number of ryegrass tillers pot^{-1} was significantly influenced by fertiliser N rate and production season. Tiller numbers in June/July were 12.7 % (21.0 tillers pot^{-1}) higher than in October/November (Table 4.2).

Table 4.2 The influence of fertiliser N rate (kg ha^{-1}) and production season on the number of perennial ryegrass tillers 60 days after fertiliser N application grown under controlled conditions at Elsenburg

	Fertiliser N rate (kg N ha^{-1})					
Production season	**0**	**30**	**60**	**120**	**180**	**Mean (P)**
June/July	134.3	173.8	174.3	199.5	249.8	**186.3 a***
October/November	88.3	145.0	147.50	203.8	242.0	**165.3 b**
Mean (N)	**111.3 d***	**159.4 c**	**160.9 c**	**201.6 b**	**245.9 a**	
LSD (0.05) NxP = NS						
LSD (0.05) N = 22.665						
LSD (0.05) P = 14.319						
CV = 12.27						

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

Tiller number per plant increased with an increase in fertiliser N application rate between 0 and 180 kg N ha^{-1}, but no significant differences were found between 30 and 60 kg N ha^{-1}.

Only production season influenced clover stolon growing points with October/November resulting in the highest (P=0.05) number of stolons pot^{-1} (Table 4.3).

The assumption can be made that perennial ryegrass tiller development was stimulated by reduced exposure to photoperiod in winter whilest white clover stolon growingpoint initiation increases as photoperiod increased in summer.

Table 4.3 The influence of fertiliser N rate (kg ha^{-1}) and production season on the number of white clover stolon growing points 60 days after fertiliser N application grown under controlled conditions at Elsenburg

	Fertiliser N rate (kg N ha^{-1})					
Production season	**0**	**30**	**60**	**120**	**180**	**Mean (P)**
June/July	24.8	24.0	25.3	22.8	27.7	**24.7 b***
October/November	76.5	77.3	80.0	76.0	76.8	**77.3 a**
Mean (N)	**50.6**	**50.6**	**56.6**	**49.4**	**55.7**	
LSD (0.05) NxP = NS						
LSD (0.05) N = NS						
LSD (0.05) P = 5.6936						
CV = 16.33						

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

Dry matter production

Perennial ryegrass primary-, residual- and total dry matter production were significantly influenced by fertiliser N rate, production season as well as a fertiliser N rate x production season interaction (Table 4.4). White clover dry matter production was only influenced by production season (Table 4.5).

Primary Dry Matter Production

Mean ryegrass PDM production in October/November was 4.566 g pot^{-1} (116.15 %) higher than in June/July (Table 4.4). The longer exposure to sunlight within the 31 day regrowth cycle in October/November (384.8 hours versus 304.4 h in June/July), the possible reason for this higher mean PDM yields. Increased fertiliser N rate resulted in significant increases in mean ryegrass PDM production. Significant (P=0.05) increases in ryegrass PDM production as fertiliser N rate increased from 0 to 180 kg N ha^{-1} were measured in October/November. However no differences in ryegrass PDM production between 30 and 60 kg N ha^{-1} as well as 120 and 180 kg N ha^{-1} in June/July were noted. The significantly higher PDM production in October/November showed that increased exposure to light (photoperiod) could increase ryegrass productivity by a factor as high as 2.6 times of that achieved in June/July with the application of 180 kg N ha^{-1}. Even at the 0 kg N ha^{-1} rates the October/November treatments resulted in an increase of 1.966 g pot^{-1} (98.65%) in PDM yields compared to June/July. Increases of this magnitudes in ryegrass production under field conditions are unlikely as temperatures in the glasshouse were within the range of 12 to 18 °C (occasionally 24 °C) while temperatures under field conditions might be too high for perennial ryegrass to maintain this high productivity.

Table 4.4 The influence of fertiliser N rate (kg ha^{-1}) and production season on primary, residual and total dry matter production (g pot^{-1}) of perennial ryegrass grown under controlled conditions at Elsenburg

Primary dry matter production (g pot^{-1})						
	Fertiliser N rate (kg N ha^{-1})					
Production season	**0**	**30**	**60**	**120**	**180**	**Mean (P)**
June/July	1.993 h**	3.399 g	3.725 g	4.713 ef	5.343 de	**3.931 b***
October/November	3.959 fg	6.150 d	7.425 c	11.090 b	13.860 a	**8.497 a**
Mean (N)	**3.116 e***	**4.774 d**	**5.575 c**	**7.901 b**	**9.601 a**	
LSD (0.05) NxP = 0.8776						
LSD (0.05) N = 0.6191						
LSD (0.05) P = 0.3911						
CV = 9.52						
Residual dry matter production (g pot^{-1})						
	Fertiliser N rate (kg N ha^{-1})					
Production season	**0**	**30**	**60**	**120**	**180**	
June/July	0.537 f	1.190 ef	1.360 e	3.770 bc	6.073 a	**2.694 b**
October/November	1.798 e	2.865 d	3.210 cd	4.390 b	5.855 a	**3.624 a**
Mean (N)	**1.257 d**	**2.028 c**	**2.285 c**	**4.080 b**	**5.964 a**	
LSD (0.05) NxP = 0.7984						
LSD (0.05) N = 0.5632						
LSD (0.05) P = 0.3558						
CV = 17.13						
Total dry matter production (g pot^{-1})						
	Fertiliser N rate (kg N ha^{-1})					
Production season	**0**	**30**	**60**	**120**	**180**	
June/July	2.530 g	4.588 f	5.085 ef	8.483 d	11.415 c	**6.625 b**
October/November	5.755 e	9.015 d	10.635 c	15.480 b	19.715 a	**12.120 a**
Mean (N)	**4.373 e**	**6.801 d**	**7.860 c**	**11.981 b**	**15.565 a**	
LSD (0.05) NxP = 1.024						
LSD (0.05) N = 0.7224						
LSD (0.05) P = 0.4564						
CV = 7.38						

**** Means in the same column or row at a specified mean dry matter production followed by the same letter are not significantly different (P=0.05)***

***** Interaction means followed by the same letter at a specified DM production are not significantly different (P=0.05)***

White clover PDM production (Table 4.5) was only influenced by production season, resulting in significant higher (377.35%) PDM yields in October/November for the same reason as given for ryegrass namely increased exposure to sunlight.

Table 4.5 The influence of fertiliser N rate (kg ha^{-1}) and production season on primary, residual and total dry matter production (g pot^{-1}) of white clover grown under controlled conditions at Elsenburg

Primary dry matter production (g pot^{-1})						
	Fertiliser N rate (kg N ha^{-1})					
Production season	**0**	**30**	**60**	**120**	**180**	**Mean (P)**
June/July	2.055	2.255	2.153	2.148	2.750	**2.252 b***
October/November	9.833	10.273	10.788	11.453	11.403	**10.750 a**
Mean N	**5.944**	**6.264**	**7.087**	**6.800**	**7.694**	
LSD (0.05) NxP = NS						
LSD (0.05) N = NS						
LSD (0.05) P = 0.5003						
CV = 11.17						
Residual dry matter production (g pot^{-1})						
	Fertiliser N rate (kg N ha^{-1})					
Production season	**0**	**30**	**60**	**120**	**180**	
June/July	4.735	4.465	5.273	5.165	6.163	**5.098 b**
October/November	10.455	9.993	10.605	10.655	10.038	**10.349 a**
Mean N	**7.595**	**7.229**	**8.320**	**7.910**	**8.377**	
LSD (0.05) NxP = NS						
LSD (0.05) N = NS						
LSD (0.05) P = 0.7764						
CV = 14.84						
Total dry matter production (g pot^{-1})						
	Fertiliser N rate (kg N ha^{-1})					
Production season	**0**	**30**	**60**	**120**	**180**	
June/July	6.790	6.720	7.427	7.313	8.913	**7.351 b**
October/November	20.288	20.265	21.393	22.108	21.440	**21.099 a**
Mean N	**13.539**	**13.493**	**15.407**	**14.710**	**16.071**	
LSD (0.05) NxP = NS						
LSD (0.05) N = NS						
LSD (0.05) P = 1.1152						
CV = 11.49						

***** *Means in the same column or row at a specified mean dry matter production followed by the same letter are not significantly different (P=0.05)***

Residual Dry Matter Production

Mean ryegrass RDM production increased by 0.93 g pot^{-1} (34.52%) in October/November compared to June/July, a difference much less than measured during the PDM production cycle (Table 4.4). Increased fertiliser N rates resulted in significant (except between 30 and 60 kg N ha^{-1}) increases in ryegrass RDM production. Applications of 120 and 180 kg N ha^{-1} did not result in differences in RDM production if applied either in June/July or October/November however at the 0, 30 and 60 kg N ha^{-1} rates ryegrass RDM production during October/November were higher (P=0.05) than in June/July. The higher PDM production at the 120 (+6.377g) and 180 kg N ha^{-1} (+8.517 g) rates in October/November compared to June/July could result in more N absorbed from the soil, resulting in less N carried over to the second regrowth cycle. RDM yields at the 120 and 180 kg N ha^{-1} rates were therefore probably restricted by a shortage of soil N supply before the end of the second regrowth cycle. The advantage of the longer photoperiod in October/November during the second regrowth cycle could therefore probably not be utilized at the 120 and 180 kg N ha^{-1} rates possibly as a result of a N deficiency towards the end of the RDM production cycle.

White clover RDM production increased by 5.251 g pot^{-1} (102.99%) in October/November significantly higher than in June/July (Table 4.5). White clover RDM yield was not influence by fertiliser N rate.

Total Dry Matter Production

Ryegrass TDM production in June/July were on average 5.495 g pot^{-1} (82.95%) lower than in October/November (Table 4.4). Increased fertiliser N rates from 0 – 180 kg N ha^{-1} resulted in increased (P=0.05) TDM production in October/November. No differences in TDM production were recorded between 30 and 60 kg N ha^{-1} in June/July, however increasing fertiliser N rate to 120 and 180 kg N ha^{-1} resulted in significant TDM yield increases.

White clover TDM production in October/November was 187.0 % higher than in June/July. Fertiliser N rate did not influence white clover TDM production.

The increase in dry matter production as a result of spring applications of fertiliser N compared to winter applications should take total exposure (hours) to sunlight into consideration. Keeping regrowth cycles of properly managed pastures constant will benefit spring applications as increased photoperiod in spring may result in increased dry matter production.

Root dry mass

Ryegrass root dry mass (g pot^{-1}) was significantly influenced by fertiliser N rate, production season and the fertiliser N rate x production season interaction.

Mean ryegrass root dry mass in October/November was higher (P=0.05) than in June/July (Table 4.6).

Table 4.6 The influence of fertiliser N rate (kg ha^{-1}) and production season on root dry mass (g pot^{-1}) of perennial ryegrass grown under controlled conditions at Elsenburg

	Fertiliser N rate (kg N ha^{-1})					
Production season	**0**	**30**	**60**	**120**	**180**	**Mean (P)**
June/July	3.803 d**	4.663 d	4.073 d	3.698 d	2.940 d	**3.837 b***
October/November	18.698 c	25.620 b	25.605 b	27.105 ab	31.508 a	**25.707 a**
Mean (N)	**12.314 b***	**15.141 ab**	**14.839 ab**	**15.401 ab**	**17.224 a**	
LSD (0.05) NxP = 5.4922						
LSD (0.05) N = 3.8746						
LSD (0.05) P = 2.4478						
CV = 24.82						

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

***** Interaction means followed by the same letter are not significantly different (P=0.05)***

The application of 0 to 120 kg N ha^{-1} did not influence mean root dry mass. The only difference measured was a higher (P=0.05) ryegrass root dry mass at the 180 kg N ha^{-1} compared to 0 kg N ha^{-1}.

Ryegrass root dry mass was on average higher during the October/November production season with the highest root dry mass recorded at the 180 kg N ha^{-1} rate, significantly higher than at 0, 30 and 60 kg N ha^{-1}. The opposite trend was observed during the June/July production season with 180 kg N ha^{-1} resulting in the lowest (P>0.05) root dry mass although no significant differences between treatments were recorded in June/July.

Only production season significantly (P=0.05) influenced white clover root dry mass (Table 4.7).

Table 4.7 The influence of fertiliser N rate (kg ha^{-1}) and production season (hours) on root dry mass (g pot^{-1}) of white clover grown under controlled conditions at Elsenburg

	Fertiliser N rate (kg N ha^{-1})					
Production season	**0**	**30**	**60**	**120**	**180**	**Mean (P)**
June/July	1.023	1.313	1.000	1.108	1.820	**1.235 b***
October/November	7.130	6.350	7.747	8.025	6.275	**7.101 a**
Mean (N)	**4.076**	**3.471**	**4.373**	**3.413**	**3.602**	
LSD (0.05) NxP = NS						
LSD (0.05) N = NS						
LSD (0.05) P = 0.7597						
CV = 27.04						

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

Mean clover root dry mass was 5.866 g pot^{-1} lower during June/July compared to October/November. Nitrogen fertiliser application rate did not influenced clover root dry mass.

Conclusion

This study has shown that increased fertiliser N application rates increase perennial ryegrass dry matter production and tiller production. Increased photoperiod as the season progresses from autumn to spring will contribute to increased dry matter production of both ryegrass and clover especially if fixed cutting or grazing cycles are practised.

As soon as soil N content drops to levels where the nitrogen absorption rate is slowed down as a result of low nitrogen concentration in the soil solution, production season becomes more crucial. Reduced photoperiod (June/July production season) and low soil N content will therefore result in less time for N uptake to maintain a high growth rate and thereby reduce dry matter production.

The significant lower RDM yields in June/July at the lower N application rates clearly showed that, at low soil N levels, the effect of production season dominates. Production seasons during short photoperiods in combination with low soil N would therefore accelerate the reduction in RDM yields.

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SECTION 2

Field study

A field study was started with the establishment of a perennial ryegrass (*Lolium perenne* cv. Ellet)-white clover (*Trifolium repens* cv. Haifa) pasture during autumn 1999 at Elsenburg near Stellenbosch, South Africa. The study was designed to investigate the response of a perennial ryegrass-white clover pasture to a single strategic application of fertiliser N during the cool season. The aim was to develop an understanding of the response of a perennial ryegrass-white clover pasture to different fertiliser N rates during the cool season from autumn to late spring. Information gathered from such a study will enable scientists to develop an efficient strategic fertiliser N programme and thereby successfully reduce the impact of the feed gap (winter gap) on fodder flow management and thereby minimising potential N losses and contamination of natural resources.

The pasture was allowed to establish and grazing with sheep started in late spring 1999. The first fertiliser N treatments were applied during autumn 2000 and data recording commenced simultaneously.

The general materials and methods used are described in chapter 5 while detailed methods related to specific data sets as well as results obtained from the field study will be presented in chapters 6 to 9.

Chapter 5

Materials and methods

Location and site preparation

A perennial ryegrass (*Lolium perenne* cv. Ellet)-white clover (*Trifolium repens* cv. Haifa) pasture was established in May 1999 at the Institute for Plant Production (altitude 177m, 18°50'E, 33°51'S). The soil at the experimental site derived mainly from granite (Anon, 1996) and can be described as a sandy loam Oakleaf consisting of an Ortic A over a Neocutanic B horizon (Soil Classification Working Group, 1991). Selected chemical (0-200 mm) and physical (0-500 mm) properties of the soil are presented in Table 5.1. Analysis methods for measuring the physical and chemical properties of the soil are referred to in Chapter 2.

Table 5.1 Chemical and physical characterstics of the soil at the experimental site at Elsenburg

		Chemical properties				
Property	**Unit**	**Start**	**Feb-00**	**Jan-01**	**Jan-02**	**Jan-03**
pH (KCl)		6.1	6.2	6.3	6	6.4
Resistance	**Ohms**	853	850	2340	1250	630
Phosphorus	**mg/kg**	60	50	63	46	65
Potassium	**cmol(+)/kg**	0.23	0.30	0.21	0.17	0.36
Calcium	**cmol(+)/kg**	3.16	2.77	2.24	2.55	3.99
Magnesium	**cmol(+)/kg**	1.14	1.16	0.96	1	1.44
Sodium	**cmol(+)/kg**	0.24	0.42	0.21	0.28	0.41
Total cations	**cmol(+)/kg**	4.77	4.65	3.62	4.00	6.20
Copper	**mg/kg**	0.68	0.87	0.84	1.24	0.94
Zinc	**mg/kg**	0.74	1.3	0.83	1.6	2.14
Manganese	**mg/kg**	68.87	67.12	96.7	189.2	117.4
Sulphur	**mg/kg**	10.39	9.92	Not available	14.02	11.23
Boron	**mg/kg**	0.53	0.49	Not available	0.62	0.69
Carbon	**%**	0.86	0.81	Not available	Not available	1.07

	Soil particle size distribution				
	0-100 mm	**100-200 mm**	**200-300 mm**	**300-400 mm**	**400-500 mm**
Sand (%)					
Course	10.3	12.0	10.2	8.2	8.0
Medium	19.8	20.2	18.4	15.7	15.5
Fine	45.1	39.4	35.8	39.4	45.1
Silt (%)	14.8	11.2	13.0	13.6	13.2
Clay (%)	10.0	17.2	22.8	31.2	30.8

Climate

The climate at Elsenburg is typically Mediterranean, with a mean annual rainfall of 622.7 mm (30 year average) of which 84 % occurs between April and October (Anon, 2004). Annual climatic conditions, including the experimental period from May to November for 2000, 2001 and 2002 are compared with long-term averages in Figures 5.1 – 5.4. Maximum temperatures were higher in 2000 (22.04°C) than in 2001 (21.0°C) and 2002 (21.05°C) as well as the longterm average of 20.21°C (Figure 5.1).

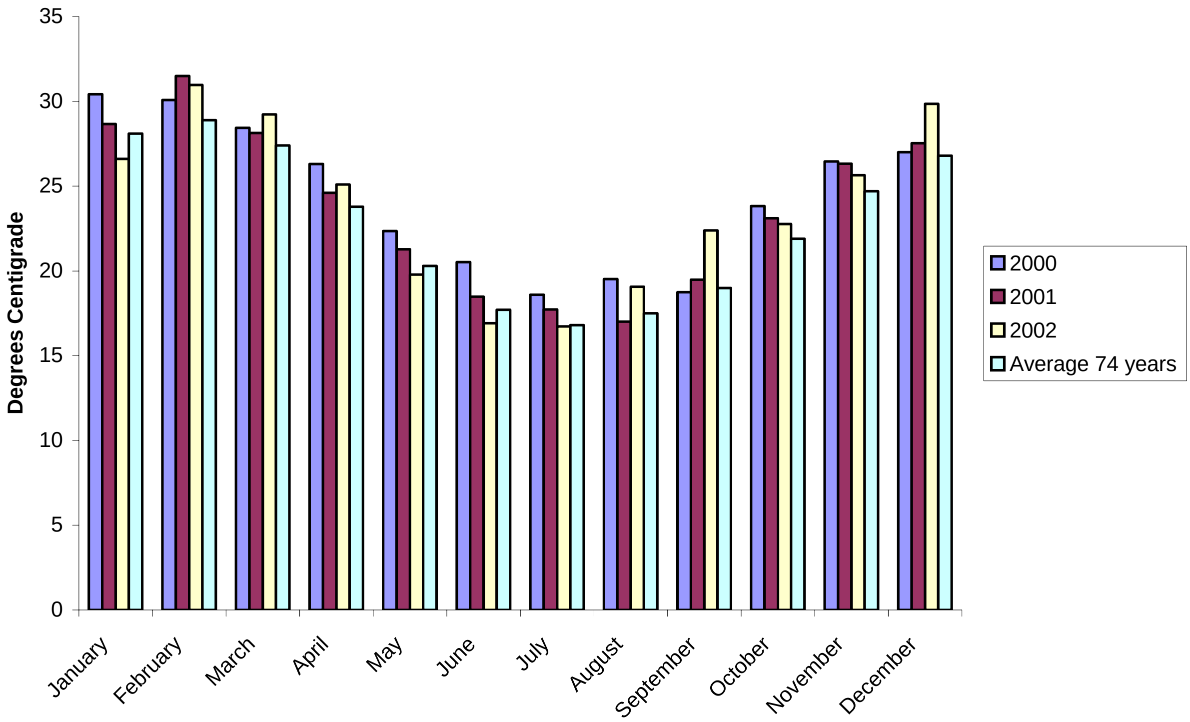

Figure 5.1. Monthly mean maximum temperatures (°C) during the experimental period (2000-2002) and the long term average at Elsenburg.

The average monthly minimum temperatures in 2002 (9.00°C) were lower than in 2001 (10.30°C) and 2000 (9.78°C) with a longterm average of 9.2°C (Figure 5.2). Monthly average temperatures for 2000 (15.57°C) and 2001 (15.33°C) were slightly higher than 2002 (14.59°C) and the long term average of 14.73°C (Figure 5.3).

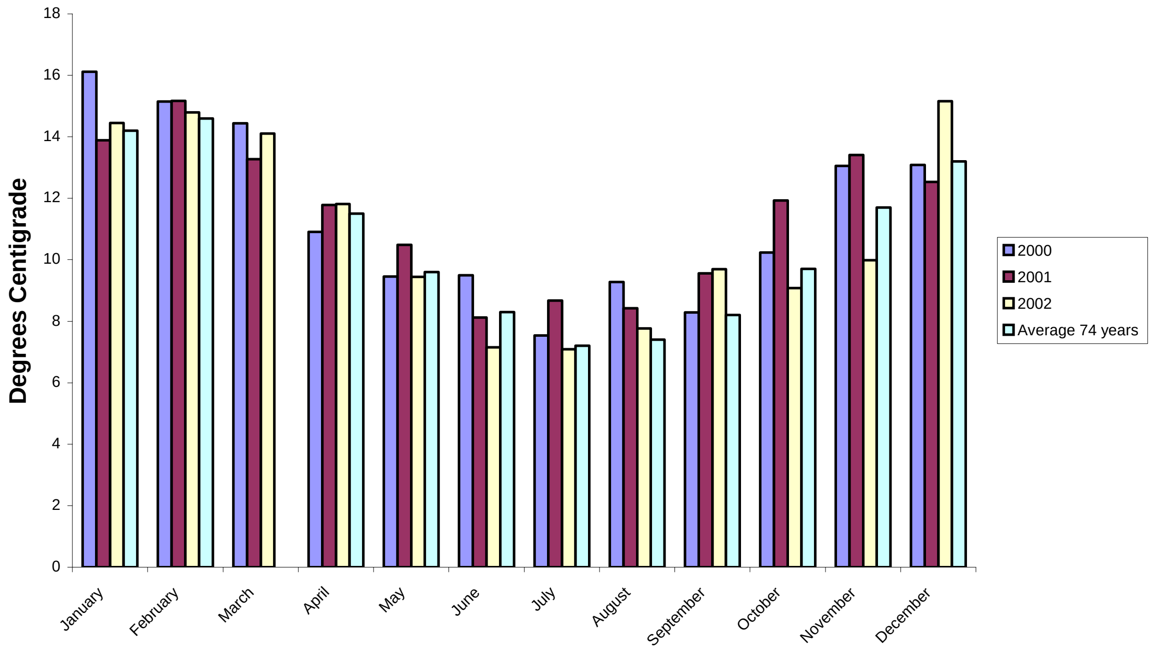

Figure 5.2. Monthly mean minimum temperatures (°C) during the experimental period (2000-2002) and the long term average at Elsenburg.

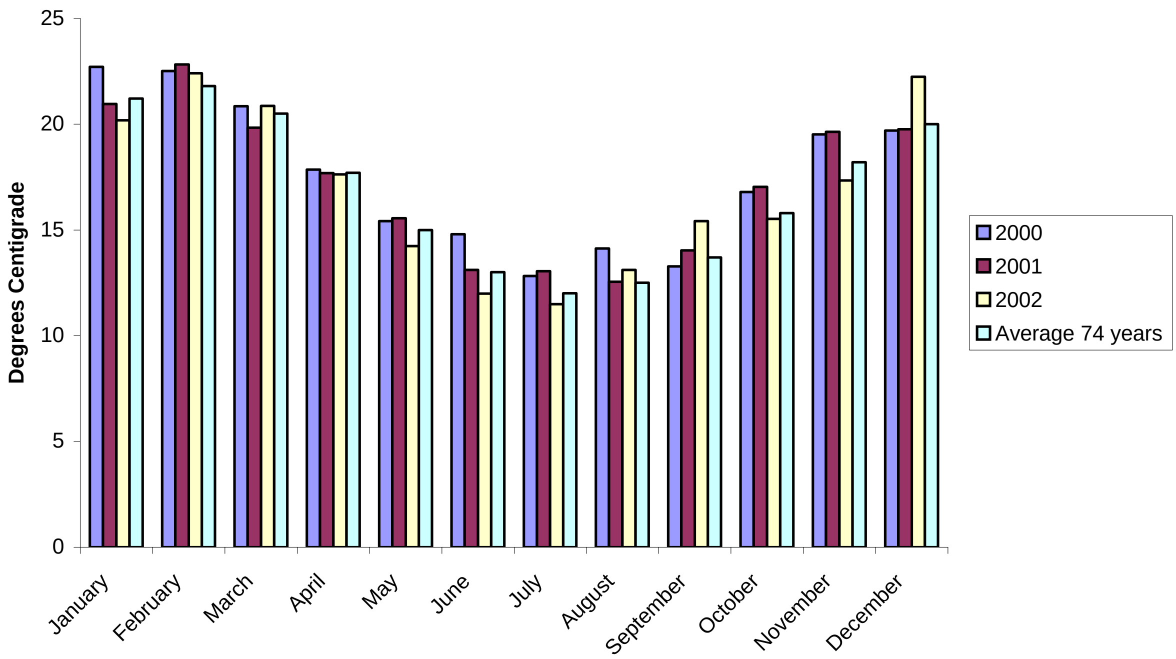

Figure 5.3. Average mean daily temperatures (°C) during the experimental period (2000-2002) and the long term average at Elsenburg.

Total rainfall of 933.8 mm in 2001 was extremely high if compared to 2000 (515.4 mm), 2002 (659.2 mm) and the long term average of 544.4 mm (Figure 5.4).

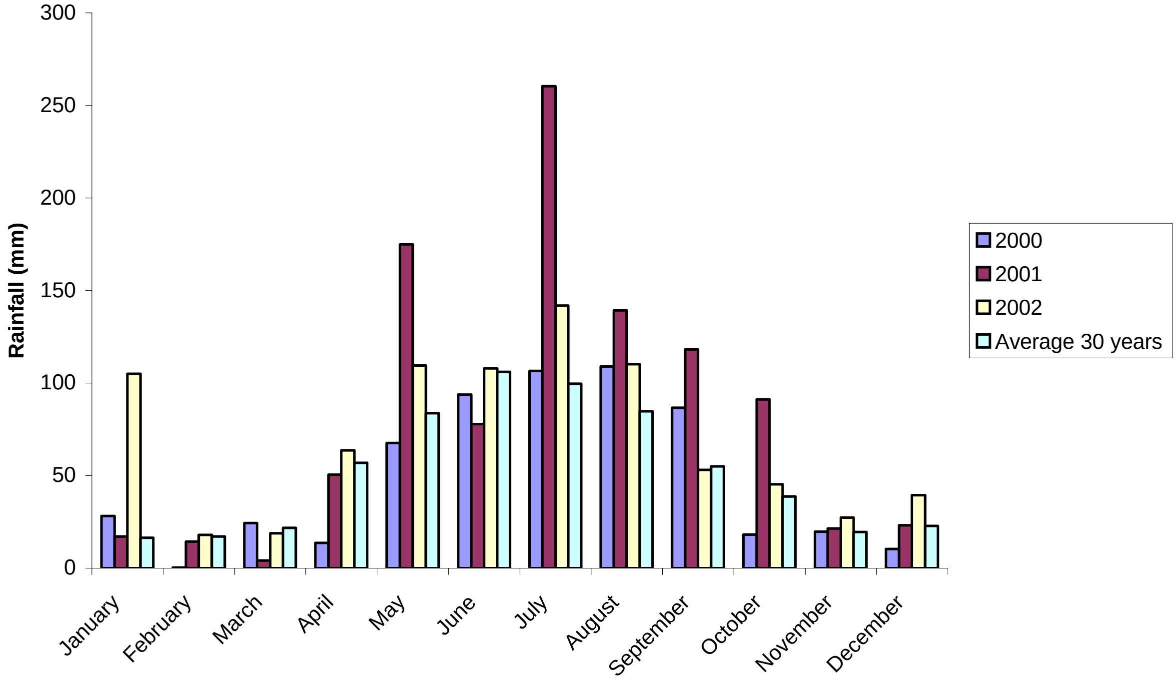

Figure 5.4. Monthly rainfall (mm) during the experimental period (2000-2002) and the long term average at Elsenburg.

The highest monthly rainfall recorded was 260.4 (July 2001), 175 (May 2001), 141.8 (July 2002) and 139.2 mm (August 2001). Supplementary irrigation was available to irrigate when necessary. Weekly water gains (rainfall and irrigation) are summarized in Table 5.2. The effect of the high rainfall in 2001 was not as prominent as expected (Fig 5.4). The exceptional high rainfall in May 2001 was evenly spread over a three week period between week 2 and 4 after fertilizer N application reducing the possible impact on N leaching. Furthermore the 176 mm received in week 4 early winter 2001 was also too late to cause extensive N leaching although some leaching losses could be expected. Data recorded in the glasshouse study (Chapters 2 and 3) also showed that soil N levels rapidly decreases within the first three weeks after feriliser N application, possibly as a result of plant uptake, and thereby reducing the risk of N leaching. The rapidly increasing clay content (10 to 30.8%) within the top 500 mm of the soil at the experimental site (Table 5.1) could also resulted in restricted N movement to the deeper layers of the soil.

Table 5.2. Weekly water gains (rainfall + irrigation in mm) to the pasture following fertiliser N applications during the seasons and years (2000-2002) covered by the study at Elsenburg

	Autumn	Early winter	Late winter	Early spring	Late spring
			Year 2000		
Week 1	NA	74	66	18	71
Week 2	NA	8	42	64	22
Week 3	19	30	0	27	37
Week 4	22	20	20	20	36
Week 5	20	32	49	51	24
Week 6	74	66	18	71	43
Week 7	8	42	64	22	50
Total	**143**	**272**	**259**	**273**	**283**
			Year 2001		
Week 1	8	33	87	70	38
Week 2	54	9	10	32	42
Week 3	50	25	51	20	32
Week 4	46	176	60	40	36
Week 5	8	18	53	28	38
Week 6	33	87	70	38	44
Week 7	9	10	32	42	15
Total	**208**	**358**	**363**	**270**	**245**
			Year 2002		
Week 1	36	33	38	28	10
Week 2	0	20	13	13	36
Week 3	0	8	72	6	32
Week 4	44	44	28	50	28
Week 5	54	11	18	35	40
Week 6	33	38	28	10	36
Week 7	20	13	13	36	40
Total	**187**	**167**	**210**	**178**	**222**

Evaporation in 2002 (690.7 mm) was lower than in 2001 (774.78 mm), 2000 (919.62 mm) as well as the long term average (918.9 mm) (Figure 5.5).

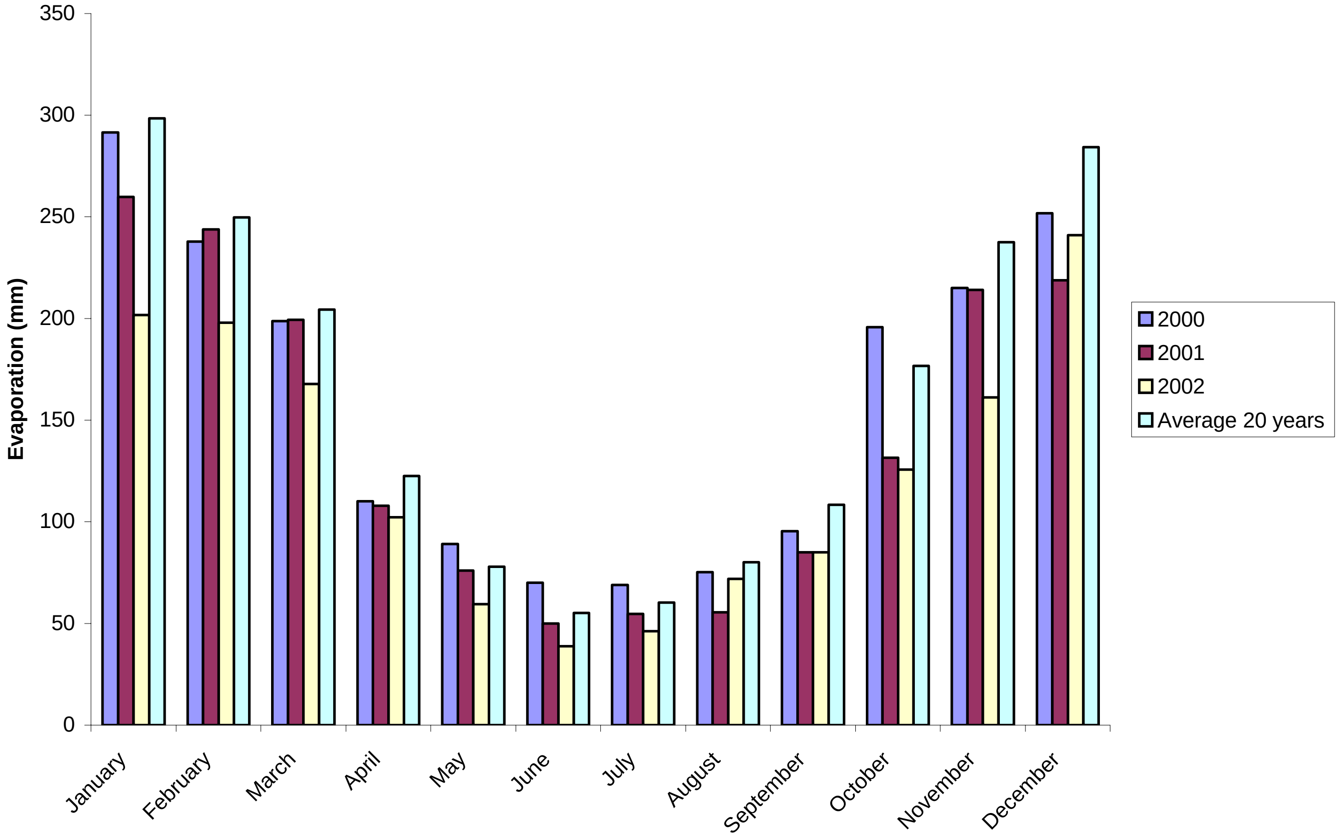

Figure 5.5. Monthly evaporation (mm) during the experimental period (2000-2002) and the long term average at Elsenburg.

Pasture Management

Soil fertility status was corrected to levels required by ryegrass-clover pastures as prescribed by Beyers (1983) before planting in 1999, while P, K and gypsum were added annually according to the soil analyses. Prior to seeding the clover seed was treated with an insecticide followed by inoculation with *Rhizobium* bacteria. A Brillion seeder was used to establish the pasture as a mixed sward at 12 kg (perennial ryegrass) and 6 kg (white clover) seed per hectare.

Irrigation was based on soil water potential as reflected by tensiometers (irrometers) with irrigation started as soon as tensiometer readings reached –25 kPa.

Each year was divided into a grazing cycle (pasture grazed by sheep – December to March) and a cutting cycle (April to November when the pastures were mowed to a 50 mm residue height and all cut material was removed from the pasture). Fertiliser N treatments were applied during the cutting cycle only. A fixed five week cutting interval was used during the cutting period and a four-weekly grazing interval during the summer cycle. To stimulate even regrowth after grazing the whole area was mowed to a height of 50 mm.

Experimental layout and treatments

The trial was laid out as a randomised complete block with a 4 x 5 factorial arranged in a split-plot design (four N levels 0, 50, 100, and 150 kg N ha^{-1} applied as LAN [28% N] and five seasons in which N was applied (late-April/early-May [autumn], early June [early winter], mid-July [late winter], late-August [early spring] and late-September/early October [late spring]) with four replicates (Snedecor & Cochran, 1967). Each plot received a single application of fertiliser nitrogen (as N treatment after cutting) per annum. The LAN was surface-applied followed by a light irrigation if necessary.

Subplot (N levels) dimensions were 3.6 x 7 m (25.2 m^2) of which a net area of 1.27 x 5.73m (7.28 m^2) was harvested during the cutting cycle using a sickle-bar mower. Soil sampling was restricted to the net plot area.

Data collection

Both plant and soil data were collected for the period 2000 – 2002. Soil samples taken in the 0-100, 200-300 and 400-500 mm soil layers at 7, 21 and 49 days after fertiliser N treatments were applied, were used to measure nitrate- and ammonium-N content of the soil. Plant samples were collected from each treatment combination during both the grazing and cutting cycles to determine dry matter production and pasture composition (clover content). Pasture quality was determined during the cutting cycle only. Two sub-samples (approximately 300 grams wet material) were collected from each treatment combination at harvesting. One was used to determine dry matter production and the second sub-sample was divided into clover and grass and dried for 72 h at 60 °C. The dried samples were used to determine the pasture composition on a dry matter basis. During the grazing cycle three 0.25 m^2 quadrants per treatment combination were cut and the same procedure as described for mowing was followed to determine pasture dry matter production and pasture composition. Root distribution was studied 18 months after pasture establishment.

Statistical procedures

Analysis of variance was performed using the SAS (Statistical Analysis System) version 8.2 (SAS, 1999) to analyse each year separately. The Shapiro-Wilk test was performed to test for non-normality of data analysed (Shapiro & Wilk, 1965). Least significant difference (LSD) was calculated at the 5% confidence level to compare treatment means using Student's t-test (Ott, 1998).

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Chapter 6

The effects of strategic fertiliser nitrogen application during the cool season on perennial ryegrass-white clover pastures in the Western Cape Province

1. Soil nitrogen dynamics

Abstract

The dynamics of soil inorganic nitrogen in an Oakleaf soil (clay content increasing from 10 to 30.8 % with depth in the profile) in reaction to a single, strategic application of fertiliser N was studied. Leaching of nitrogen after application to a perennial ryegrass-white clover pasture was monitored to a soil depth of 500 mm over a seven week period following fertiliser N application. Three levels of nitrogen (0, 50 and 150 kg N ha^{-1}) in the form of LAN (28) applied as a once off application during five different seasons (autumn, early winter, late winter, early spring or late spring) were used as treatments. Soil samples, using a 75 mm open sided Johnson bucket auger, were collected at 7, 21 and 49 days after fertiliser N application. Soil samples were collected at 0-100, 200-300 and 400-500 mm depths, placed in shallow trays and air- dried using electric fans. Both ammonium-N (NO_3^--N) and nitrate-N (NH_4^+-N) fractions were determined for all soil samples collected. It was however decided to present the data as soil inorganic nitrogen (ammonium-N + nitrate-N).

Mean inorganic nitrogen content increased from 2000 to 2002 possibly as a result of biological N fixation by the white clover in the pasture. The application of 50 kg N ha^{-1} did not result in significant differences in soil N content compared to the 0 kg N ha^{-1} treatments during almost the entire study and would therefore be regarded as a low risk treatment with regard to environmental pollution.

The effect of 150 kg N ha^{-1} lasted for minimum of 3 weeks in the 0-100 mm layer with the highest inorganic nitrogen levels normally measured in autumn and early winter. The effect of 150 kg N ha^{-1} lasted for 7 weeks in the 200-300 mm layers in years regarded as normal for the Stellenbosch area. Leaching of fertiliser N within one week after application to the 400-500 mm soil layers emphasised the possible negative effect of application rates exceeding the

absorption capacity of the pasture. This might result in reduced nitrogen response efficiencies (kg additional DM per kg N) and contamination of natural resources especially in winter when the uptake capacity of the pasture is low as a result of low pasture productivity.

Keywords: NH_4^+-N, NO_3^--N, perennial ryegrass, strategic nitrogen, white clover

Introduction

Applications of fertiliser N to boost grass production when the activity of the clover component in a perennial grass-clover pasture is restricted by low temperatures, is confined to applications in autumn, winter and spring (Laidlaw, 1984; Frame & Boyd, 1987; McKenzie, Ryan, Jacobs & Kearney, 1999; Stout, Weaver & Elwinger, 2001). Excessive rainfall at the time of N application may result in N losses due to runoff and leaching with a potential for N pollution to occur in the soil. Of major environmental concern is the flow of N from the pasture into water reserves and the atmosphere. The majority of ryegrass-clover pastures in the Western Cape Province are grown under irrigation near rivers increasing the risk of water pollution due to residual N following fertiliser N application. Excessive soil N levels will exacerbate the problem. The strategic application of fertiliser nitrogen, if managed wrongly, can therefore be regarded as an important environmental pollution hazard.

The recovery of fertiliser N by plants is generally low and rarely more than 50-75% (Allison, 1966; Ball & Ryden, 1984). This low recovery is mainly caused by the mineral N in the soil being exposed to potential leaching to depths below the active root zone, denitrification and volatilisation or rendered unavailable through immobilization (Olsen & Kurtz, 1982; Dinnes *et al*., 2002). Leaching losses are high in well-drained, temporary wet soils (Havlin *et al*., 1999). Denitrification losses, the biochemical reduction of NO_3- to N_2 and nitrous oxide under warm (above 5 °C), anaerobic conditions (poorly drained soils) in the presence of decomposing organic matter (Tinker, 1979; Miles & Manson, 2000), may result in N losses during early autumn, late spring and summer. Volatilisation due to the loss of gaseous ammonia (NH_3) from calcerious or alkaline moist soils (Havlin *et al*., 1999; Miles & Manson, 2000), may result in great losses especially when N is applied as urea or ammonium forms. Volatilisation losses increase as soil pH and fertiliser N application rate increases especially when applied to a wet soil which undergoes drying (Miles & Manson, 2000).

In order to reduce N losses from pastures the application of fertiliser N should aim to remain within the steepest portion of the response curve to ensure rapid absorption and efficient N use by plants. The rate at which applied N fertiliser is taken up by the plant is dependant on local environmental conditions, mainly temperature. Pasture productivity will increase as temperature and photoperiod increase with the onset of spring resulting in the ability of the pasture to respond and absorb more fertiliser N in a shorter period of time compared to winter applications. The implication of this is that the optimum amount of fertiliser N might differ when applied in different seasons. Under ideal conditions most uptake of fertiliser N occurs within four weeks of application (Whitehead, 1970). To achieve optimum efficiency the N fertiliser rate and season of application must be adapted to the pasture's N uptake capacity during selected time zones. Olsen & Kurtz (1982) state that N fertiliser applied to irrigated ryegrass during active pasture growth, will have little effect after the first harvest, with excessive free N in the soil possibly being leached out of the active root zone. In contrast Reid (1984) reported a positive residual effect of the applied N in the second regrowth cycle, resulting in production that was approximately 30-35% of the yield during the first regrowth cycle.

The use of LAN (as opposed to urea) as nitrogen source in this study reduced the potential of volatilisation losses to negligible levels (Whitehead, Pain & Ryden, 1986; Du Preez & Burger, 1987a & b). Some denitrification losses could however be expected. Ryden (1985) found that when ammonium nitrate was used as nitrogen source, denitrification losses seldom exceeded 8% even at soil nitrate contents in excess of 5 mg N kg^{-1} in combination with soil water contents of >20% and soil temperatures higher than 8 °C. Similar conditions occurred regularly during the duration of the study.

Hutchings & Kristensen (1995) reported nitrogen leaching losses of approximately 15 kg N ha^{-1} under unfertilised, grazed grass-clover pastures and predict losses of 90 kg N ha^{-1} on a well fertilized grass-clover pasture with a production potential of 11.5 t ha^{-1}. Ridley, Simpson & White (1999) recorded leaching losses of 6-16 kg N ha^{-1} for grass pastures receiving no N and 77-119 kg N ha^{-1} for grasses receiving 500 kg N ha^{-1}. Ryden, Ball & Garwood (1984) reported average leaching losses of 29 and 162 kg N ha^{-1} from cut and grazed pastures both receiving 420 kg N ha^{-1} in the form of ammonium nitrate. Leaching losses from grass swards is lower due to the high root density and high N uptake capacity during the growing season (Whitehead *et al.*, 1986).

Developing a strategic N fertilisation programme that will enhance nitrogen use efficiency requires a knowledge of the movement of nitrogen through a specific soil as well as the capacity of the pasture to absorb the applied fertiliser within the first regrowth cycle. Nitrogen supply in the soil can then be synchronized with pasture N requirements or the ability of the pasture to make maximum use of the additional N to enhance DM yield.

The objectives of this study were to monitor the mineral N content of the soil after fertiliser N application to develop strategic N fertilisation norms that will ensure 1) maximum N response efficiencies and 2) ensure minimum contamination of natural resources as a result of leaching.

Materials and methods

Experimental layout and treatments

The trial was laid out as a randomised complete block with a 4 x 5 factorial arranged in a split-plot design (four N levels 0, 50, 100, and 150 kg N ha^{-1} applied as LAN [28] and five seasons in which N was applied (late-April/early-May [autumn], early June [early winter], mid-July [late winter], late-August [early spring] or late-September/early October [late spring]) with four replicates (Snedecor & Cochran, 1967). Each plot received a single annual application of fertiliser nitrogen after cutting, the timing of application depending on season. The LAN was surface-applied followed by a light irrigation if necessary. Soil samples were only collected at the 0, 50, and 150 kg N ha^{-1} plots. Subplot dimensions were 3.6 x 7 m (25.2 m^2) of which a net area of 1.27 x 5.73m (7.28 m^2) was harvested using a sickle-bar mower. Soil sampling was restricted to the net plot area. Details on the experimental site and treatments applied are shown in Chapter 5.

Data collection

Soil data were collected over a three year period. Samples were taken in the 0-100, 200-300 and 400-500 mm layers 7, 21 and 49 days after fertiliser N application. Soil samples were taken only during the cutting cycle on plots where 0, 50 and 150 kg N ha^{-1} were applied. Three sample cores (45mm diameter) per plot were bulked, placed in shallow trays and air dried for 48 hours at room temperature using electric fans. The depth of soil sampling was chosen to cover the upper (0-100 mm) and lower (200-300 mm) part of the active root zone as well as a layer beneath (400-500 mm) to monitor leaching beyond the root zone. The first sampling was aimed at determining the soil N content before the onset of accelerated pasture growth in the first regrowth cycle after fertiliser N application (7 days). The second sampling was done at near the expected peak pasture productivity in the first regrowth cycle (21 days). The third sampling was done in the second regrowth cycle at the expected onset of increased N uptake at 49 days after N application, but 14 days into the second regrowth cycle. An autoanalyzer was used to determine the soil NH_4^+- and NO_3^--N content (Bessinger, 1985).

Root distribution was studied 18 months after pasture establishment. Three 0.0067 m^3 undisturbed soil cores were removed and sectioned into 0-100, 100-200 and 200-300 mm layers. The roots were separated from the soil by thoroughly washing the individual soil layers over a 2 and 1 mm combination sieve. Roots were dried at 60°C for 72 hours and dry mass recorded. Average daily soil temperatures were calculated from data recorded at 80 mm soil depth using a MCS 120-02EX datalogger.

Statistical procedures

Analysis of variance was performed using the SAS (Statistical Analysis System) version 8.2 (SAS, 1999). The Shapiro-Wilk test was performed to test for non-normality of data analysed (Shapiro & Wilk, 1965). Least significant difference (LSD) was calculated at the 5% confidence level to compare treatment means using Student's t-test (Ott, 1998).

Results and discussion

Soil temperatures

Average soil temperatures over the first seven weeks after fertiliser N application are shown in Table 6.1. Soil temperatures decreased from 15.0 °C in autumn to 11.9 °C in early winter followed by a gradual increase to 12.4, 14.1 and 16.4 °C in late winter, early spring and late spring respectively. The decreasing soil temperatures between autumn and early winter followed by the gradual increase could possibly play a major role in plant response to fertiliser N application and the total N content of the soil.

Table 6.1 Average weekly soil temperatures (°C) at 80mm depth over a nine week period following fertiliser N application in a perennial ryegrass-white clover pasture at Elsenburg

Season of application		Year			
Autumn		**2000**	**2001**	**2002**	**Mean**
	Week 1	17.2	17.7	23.1	19.3
	Week 2	14.1	15.8	20.0	16.6
	Week 3	14.7	13.0	18.4	15.4
	Week 4	16.2	12.4	17.1	15.2
	Week 5	14.4	12.5	14.8	13.9
	Week 6	13.4	12.8	11.6	12.6
	Week 7	12.8	12.0	11.7	12.2
	Week 8	11.7	11.1	NA*	11.4
	Week 9	13.0	10.1	10.9	11.3
Early Winter		**2000**	**2001**	**2002**	
	Week 1	14.1	12.8	11.8	12.9
	Week 2	13.1	12.0	11.9	12.3
	Week 3	12.7	11.1	NA	11.9
	Week 4	11.9	10.1	11.7	11.2
	Week 5	13.0	11.5	10.9	11.8
	Week 6	13.0	10.6	11.6	11.7
	Week 7	9.6	12.1	11.5	11.1
	Week 8	11.1	12.2	NA	11.7
	Week 9	12.1	12.6	NA	12.4
Late Winter		**2000**	**2001**	**2002**	
	Week 1	10.3	11.6	11.7	11.2
	Week 2	10.6	12.2	10.7	11.2
	Week 3	12.1	12.6	11.5	12.1
	Week 4	12.5	12.5	11.1	12.0
	Week 5	14.0	14.1	11.4	13.2
	Week 6	13.5	NA	11.6	12.6
	Week 7	15.1	NA	13.7	14.4
	Week 8	13.4	NA	12.6	13.0
	Week 9	13.2	NA	15.1	14.2
Early Spring		**2000**	**2001**	**2002**	
	Week 1	14.6	NA	11.4	13.0
	Week 2	13.7	NA	12.8	13.3
	Week 3	13.2	NA	12.9	13.1
	Week 4	13.1	14.1	14.1	13.8
	Week 5	15.4	15.1	12.8	14.4
	Week 6	17.6	13.4	14.5	15.2
	Week 7	17.2	12.8	18.7	16.2
	Week 8	18.3	14.0	16.3	16.2
	Week 9	18.4	15.6	15.8	16.6
Late Spring		**2000**	**2001**	**2002**	
	Week 1	16.4	14.7	13.1	14.7
	Week 2	15.9	13.8	15.1	14.9
	Week 3	17.9	13.2	18.5	16.5
	Week 4	17.0	13.7	15.9	15.5
	Week 5	16.7	15.6	16.2	16.2
	Week 6	19.1	16.9	18.7	18.2
	Week 7	21.3	18.7	16.6	18.9
	Week 8	23.1	18.0	15.7	18.9
	Week 9	23.4	19.2	17.9	20.2

* NA - no data available

Root study

The shallow root system of perennial ryegrass-white clover pastures (McKenzie, 1996) potentially reduces the duration that the applied N remains within the root zone, particularly in well drained soils. Increased leaching of N beyond the shallow root system may therefore result in reduced N use efficiencies. This indicates that the timing and amount of N applied must be carefully managed to minimize exposure of fertiliser N to potential losses.

The root study showed that the pasture was extremely shallow-rooted with 93.9% of the total root dry matter located in the top 100 mm of the soil profile (Figure 6.1).

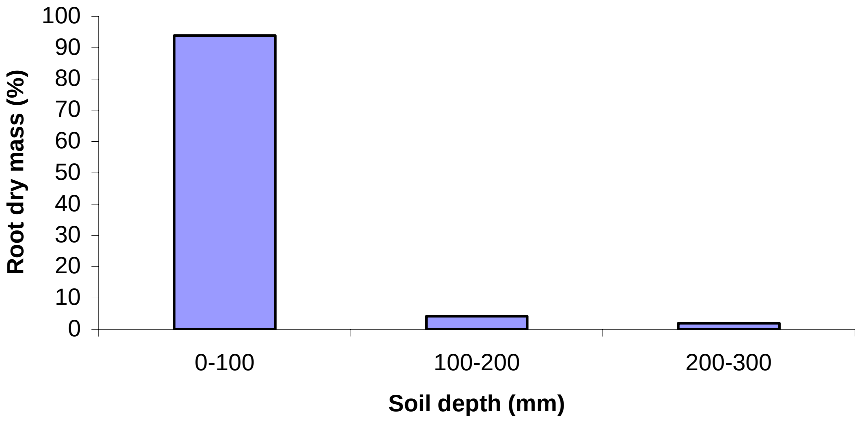

Figure 6.1 Relative root dry mass (%) at different soil depths (0-300mm) of a perennial ryegrass white clover pasture 18 months after establishment on an Oakleaf soil with clay content increasing from 10 to 22.8 % at 0-100, 100-200 and 200-300 mm soil depts respectively.

McKenzie (1996) reported perennial ryegrass where 75% of the root system occupied the top 50 mm of the soil. For reasons already mentioned, this root distribution must be kept in mind when mineral N content of the soil after N application, is discussed. It can be assumed that some of the fertiliser N that leached beyond the 100 mm top layer have been intercepted by the deeper roots as they are very fine and low in mass but considerably more efficient per unit weight of root (Wolfson & Tainton, 2000).

The gradual increase in clay content, from 10.0 % in the 0-100 mm layer to 30.8 % in the 400-500 mm layers, may also slow down the percolation of dissolved fertiliser N to layers beyond the root zone (Table 5.1).

Soil nitrogen content

General discussion

Levels of significance between treatments are summarised in Table 6.2.

Table 6.2 Partial ANOVA on concentration of soil nitrogen fractions in selected soil layers over time as influenced by season and rate of fertiliser N application at Elsenburg

	Year 2000			Year 2001			Year 2002		
	Season x N	**Season**	**N**	**Season x N**	**Season**	**N**	**Season x N**	**Season**	**N**
Sampling Depth 0-100mm-7 Days After N Application									
NH_4^+-N	*	***	***	*	NS	***	*	***	***
NO_3^--N	*	***	***	***	***	***	*	***	***
TOTAL N	*	***	***	*	***	***	*	***	***
Sampling Depth 0-100mm-21 Days After N Application									
NH_4^+-N	NS	NS	NS	NS	*	NS	*	***	*
NO_3^--N	***	***	***	*	*	NS	NS	*	***
TOTAL N	*	***	***	NS	*	NS	NS	*	***
Sampling Depth 0-100mm-49 Days After N Application									
NH_4^+-N	NS	***	*	NS	***	NS	NS	***	NS
NO_3^--N	*	*	NS	*	***	*	NS	NS	NS
TOTAL N	NS	NS	***	NS	***	*	NS	*	NS
Sampling Depth 200-300mm-7 Days After N Application									
NH_4^+-N	*	***	***	NS	***	NS	NS	***	NS
NO_3^--N	***	***	***	NS	*	*	NS	*	***
TOTAL N	***	***	***	NS	NS	*	NS	***	***
Sampling Depth 200-300mm-21 Days After N Application									
NH_4^+-N	NS	*	NS	NS	***	*	*	***	***
NO_3^--N	*	***	***	*	***	NS	*	*	***
TOTAL N	*	***	***	*	***	NS	*	***	***
Sampling Depth 200-300mm-49 Days After N Application									
NH_4^+-N	NS	*	*	*	***	NS	*	***	NS
NO_3^--N	NS	NS	NS	*	***	*	***	*	*
TOTAL N	NS	*	*	*	***	NS	*	***	*
Sampling Depth 400-500mm-7 Days After N Application									
NH_4^+-N	NS	*	NS	NS	***	NS	*	***	NS
NO_3^--N	*	*	*	NS	NS	*	NS	NS	NS
TOTAL N	NS	*	*	NS	***	*	NS	***	NS
Sampling Depth 400-500mm-21 Days After N Application									
NH_4^+-N	NS	*	NS	NS	*	NS	NS	***	NS
NO_3^--N	*	***	***	NS	*	NS	NS	*	***
TOTAL N	*	*	***	NS	*	NS	NS	***	***
Sampling Depth 400-500mm-49 Days After N Application									
NH_4^+-N	*	*	NS	*	***	*	NS	***	NS
NO_3^--N	*	*	NS	*	***	***	*	*	*
TOTAL N	NS	*	NS	***	***	***	*	***	NS

NS, not significant; *P< 0.05; **P<0.01;*P<0.0001**

From Table 6.2 it becomes clear that although both NH_4^+-N and NO_3^--N content were affected by both the fertiliser N application rate and season of application, the differences were inconsistent between years. Although both NH_4^+-N and NO_3^--N content were measured it was decided to present and discuss only inorganic nitrogen (NH_4^+-N + NO_3^--N) as indicator of inorganic-N content as NH_4^+-N may very rapidly be mineralised to NO_3^- -N (Wienhold & Halvorson, 1999). As measurements were done at quite long intervals (7, 21 and 49 days after N application) significant changes in NH_4^+-N and NO_3^--N ratios may for this reason occurred between samplings. The term inorganic nitrogen content in this discussion will thus refer to the inorganic-N content as explained.

Except for the 0-100 mm layer one week after fertiliser N application in year 2000 (18.37 mg kg^{-1}), mean soil inorganic-N content gradually increased with time with the highest means recorded in 2002 (Tables 6.4, 6.5 & 6.6). This trend supported results obtained by Eckard (1994) who showed that clover has potential in building soil N.

Soil inorganic nitrogen content

To simplify the discussion soil inorganic nitrogen content will be discussed as measured at the different sampling depths. Due to only two observations where 0 kg N ha^{-1} resulted in significantly lower soil inorganic nitrogen content than at 50 kg N ha^{-1} will the 0 and 50 kg N ha^{-1} treatment means not be discussed in the section that follows.

Layer 1: 0-100 mm

Increased fertiliser N rate caused increased mean soil inorganic nitrogen levels in the first week after N application in all three years covered by the study (Table 6.3). The application of 150 kg N ha^{-1} resulted in the highest (P=0.05) soil inorganic nitrogen levels one week after fertiliser N application. The same results were obtained in 2000 and 2002 three weeks after fertiliser N application indicating that, in the 0-100 mm soil layer, the effect of 150 kg N ha^{-1} will last for at least three weeks in normal seasons. No differences in total soil inorganic nitrogen levels were recorded seven weeks after fertiliser N applications in 2000 and 2002.

Soil inorganic nitrogen contents during the first week after fertiliser N application were the highest in autumn and early winter in all years covered by the study, possibly as a result of high residual N as measured at the 0 kg N ha^{-1} treatments in autumn and slow uptake in early winter due to low pasture N demand as a result of low pasture productivity. The lower soil inorganic nitrogen in late winter to late spring possibly the result of increased N uptake as pasture productivity increases as temperature and photoperiod increase. The increases in soil inorganic nitrogen content during late spring (week seven) could be the result of N mineralisation as soil temperatures increased. Except for year 2001, the same trend was observed three weeks after fertiliser N application.

Layer 2: 200-300 mm

An increase in soil inorganic nitrogen levels were recorded one week after fertiliser N application, in all years covered by the study, as fertiliser N rate was increased (Table 6.4). The application of 150 kg N ha^{-1} resulted in significantly higher soil inorganic nitrogen levels compared to the 0 kg N ha^{-1} rate during the first week in the 200-300 mm layers for all years covered by the study. This suggested that leaching of fertiliser N occured within one week after application. Except for 2001, the same reactions in terms of 150 kg N ha^{-1} were measured at three and seven weeks after fertiliser N application. The assumption can be made that fertiliser N leaching in 2000 and 2002 could be representative, because rainfall was on average, and resulted in significantly higher inorganic nitrogen levels at the 150 kg N ha^{-1} rates. The non-significant higher inorganic nitrogen levels in 2001 can be seen as a situation where fertiliser N had possibly been leached beyond the 200-300 mm layer because of the very high rainfall.

The effect of season of application was inconsistent although a weak tendency of higher soil N levels in autumn was observed especially one week after fertiliser N application.

Layer 3: 400-500 mm

Leaching of fertiliser N to the 400-500 mm layers was measured as the 150 kg N ha^{-1} rates resulted in significantly higher soil inorganic nitrogen levels one week after fertiliser N application in 2000 and 2001, three weeks in 2000 and 2002 as well as at seven weeks in 2000 and 2001 (Table 6.5). The high risk of applying 150 kg N ha^{-1} is emphasised by this rapid leaching (within one week) of fertiliser N to layers beyond the root zone of the shallow rooted pasture. The reaction to season of application was inconsistent.

Table 6.3 Inorganic-N (mg kg^{-1}) in the 0-100 mm soil layer at one, three and seven weeks in response to fertiliser N application (0, 50 and 150 kg N ha^{-1}) during different seasons (autumn to late spring) to a perennial ryegrass-white clover pasture at Elsenburg for the period 2000 to 2002

Sampling depth 1: 0-100mm — One week after fertiliser N application

YEAR 2000

Season	Fertiliser N rate (kg ha^{-1}) 0	50	150	Mean (S)
Autumn	9.89	27.04	49.39	**28.77 a***
Early winter	7.11	15.71	62.81	**28.54 a**
Late winter	3.68	9.29	22.44	**11.80 bc**
Early spring	1.34	7.40	48.13	**18.95 ab**
Late spring	1.40	2.55	7.45	**3.80 c**
Mean (N)	**4.68 b**	**12.40 b**	**38.04 a**	***18.37***

LSD interaction = 18.146
LSD N = 8.1153
LSD Season= 12.475

YEAR 2001

Season	Fertiliser N rate (kg ha^{-1}) 0	50	150	Mean (S)
Autumn	8.91	10.31	45.06	**21.42 a**
Early winter	7.52	14.03	55.91	**25.82 a**
Late winter	8.40	8.96	22.09	**13.15 b**
Early spring	4.83	6.45	16.46	**9.25 b**
Late spring	6.78	7.62	19.40	**11.27 b**
Mean (N)	**7.29 b**	**9.47 b**	**31.78 a**	***16.18***

LSD interaction= 11.207
LSD N = 5.012
LSD Season= 7.3707

YEAR 2002

Season	Fertiliser N rate (kg ha^{-1}) 0	50	150	Mean (S)
Autumn	16.60	24.53	85.95	**42.36 a**
Early winter	8.69	34.90	51.68	**31.76 ab**
Late winter	8.65	11.11	32.05	**17.27 bc**
Early spring	5.75	10.22	30.22	**15.39 c**
Late spring	12.02	20.19	39.90	**24.04 abc**
Mean (N)	**10.34 c**	**20.19 b**	**47.96 a**	***26.16***

LSD interaction = 12.243
LSD N = 5.3301
LSD Season= 15.479

Sampling depth 1: 0-100mm — Three weeks after fertiliser N application

YEAR 2000

Season	0	50	150	Mean (S)
Autumn	9.66	4.65	19.12	**11.14 a**
Early winter	4.67	6.71	21.64	**11.01 a**
Late winter	2.18	3.15	4.94	**3.42 b**
Early spring	3.52	4.67	7.32	**5.17 b**
Late spring	1.88	2.15	2.17	**2.07 b**
Mean (N)	**4.38 b**	**4.27 b**	**11.04 a**	***6.56***

LSD interaction = 5.2299
LSD N = 2.3389
LSD Season= 3.4897

YEAR 2001

Season	0	50	150	Mean (S)
Autumn	4.51	5.39	13.80	**7.90 b**
Early winter	4.92	6.79	10.46	**7.39 b**
Late winter	8.02	8.07	11.22	**9.10 b**
Early spring	16.41	18.44	9.74	**14.86 a**
Late spring	6.27	6.81	8.78	**7.29 b**
Mean (N)	**8.03**	**9.10**	**10.80**	***9.31***

LSD interaction= NS
LSD N = NS
LSD Season= 1.9215

YEAR 2002

Season	0	50	150	Mean (S)
Autumn	7.36	10.38	26.06	**14.60 a**
Early winter	8.29	12.95	21.90	**14.38 a**
Late winter	2.86	3.92	12.22	**6.33 c**
Early spring	8.39	9.05	14.33	**10.59 b**
Late spring	9.82	9.76	18.32	**12.63 ab**
Mean (N)	**7.34 b**	**9.21 b**	**18.57 a**	***11.71***

LSD interaction = NS
LSD N = 3.0323
LSD Season= 3.4774

Sampling depth 1: 0-100mm — Seven weeks after fertiliser N application

YEAR 2000

Season	0	50	150	Mean (S)
Autumn	7.11	5.06	6.31	**6.16 b**
Early winter	3.68	3.24	3.29	**3.40 c**
Late winter	5.10	15.95	8.03	**9.70 a**
Early spring	1.64	1.78	1.80	**1.74 c**
Late spring	2.86	3.19	2.80	**2.95 c**
Mean (N)	**4.076**	**5.845**	**4.445**	***4.79***

LSD interaction = NS
LSD N = NS
LSD Season= 2.6552

YEAR 2001

Season	0	50	150	Mean (S)
Autumn	5.91	5.26	6.32	**5.83 b**
Early winter	4.29	5.73	6.35	**5.46 bc**
Late winter	4.83	4.47	4.99	**4.76 c**
Early spring	5.43	5.45	5.76	**5.55 bc**
Late spring	10.52	12.36	12.63	**11.84 a**
Mean (N)	**6.20 b**	**6.65 ab**	**7.21 a**	***6.69***

LSD interaction= NS
LSD N = 0.6851
LSD Season= 1.051

YEAR 2002

Season	0	50	150	Mean (S)
Autumn	6.88	5.69	6.65	**6.40 b**
Early winter				
Late winter	5.94	6.96	5.97	**6.29 b**
Early spring	5.99	6.41	6.44	**6.28 b**
Late spring	23.21	12.06	10.80	**15.36 a**
Mean (N)	**8.40**	**6.22**	**5.97**	***8.58***

LSD interaction = NS
LSD N = NS
LSD Season= 4.3666

**** Means in the same year at a specific depth and time of sampling in the same column or row followed by the same letter are not significantly different (P=0.05)***

Table 6.4 Inorganic-N (mg kg^{-1}) in the 200-300 mm soil layer at one, three and seven weeks in response to fertiliser N application (0, 50 and 150 kg N ha^{-1}) during different seasons (autumn to late spring) to a perennial ryegrass-white clover pasture at Elsenburg for the period 2000 to 2002

Sampling depth 2:200-300 mm

YEAR 2000 — One week after fertiliser N application

	Fertiliser N rate (kg ha^{-1}) 0	50	150	Mean (S)
Autumn	3.06	4.18	9.95	**5.73 b**
Early winter	4.40	6.38	21.95	**10.91 a**
Late winter	2.55	3.17	8.97	**4.90 b**
Early spring	0.45	1.82	4.00	**2.09 c**
Late spring	1.25	1.79	2.81	**1.95 c**
Mean (N)	**2.34 b**	**3.47 b**	**9.54 a**	***5.12***

LSD interaction = 3.9243
LSD N = 1.755
LSD Season= 2.6167

YEAR 2001 — One week after fertiliser N application

	Fertiliser N rate (kg ha^{-1}) 0	50	150	Mean (S)
Autumn	4.31	4.48	10.18	**6.32**
Early winter	3.03	3.86	9.17	**5.35**
Late winter	4.92	5.49	6.63	**5.68**
Early spring	4.02	5.12	5.03	**4.72**
Late spring	5.52	5.45	5.61	**5.53**
Mean (N)	**4.36 b**	**4.88 b**	**7.32 a**	***5.52***

LSD interaction= NS
LSD N = 1.675
LSD Season= NS

YEAR 2002 — One week after fertiliser N application

	Fertiliser N rate (kg ha^{-1}) 0	50	150	Mean (S)
Autumn	16.41	14.60	18.63	**16.55 a**
Early winter	5.12	8.98	10.28	**8.13 b**
Late winter	5.65	6.42	10.90	**7.66 b**
Early spring	4.96	5.29	11.63	**7.29 b**
Late spring	8.30	7.31	10.80	**8.80 b**
Mean (N)	**8.09 b**	**8.52 b**	**12.45 a**	***9.69***

LSD interaction = NS
LSD N = 1.2793
LSD Season= 1.7448

Sampling depth 2:200-300 mm

YEAR 2000 — Three weeks after fertiliser N application

	0	50	150	Mean (S)
Autumn	3.97	4.17	11.76	**6.63 ab**
Early winter	2.03	5.89	14.94	**7.62 a**
Late winter	1.62	1.86	4.77	**2.75 c**
Early spring	1.82	3.07	10.77	**5.22 b**
Late spring	1.57	1.87	2.07	**1.84 c**
Mean (N)	**2.20 b**	**3.37 b**	**8.86 a**	***4.81***

LSD interaction = 3.3622
LSD N = 1.5036
LSD Season= 1.465

YEAR 2001 — Three weeks after fertiliser N application

	0	50	150	Mean (S)
Autumn	3.84	8.36	6.64	**6.28 b**
Early winter	2.36	4.40	6.61	**4.46 b**
Late winter	6.49	6.72	7.83	**7.01 b**
Early spring	33.06	32.49	5.78	**23.78 a**
Late spring	4.35	4.61	3.75	**4.24 b**
Mean (N)	**10.02 ab**	**11.31 a**	**6.12 b**	***9.15***

LSD interaction= 10.891
LSD N = NS
LSD Season= 5.2338

YEAR 2002 — Three weeks after fertiliser N application

	0	50	150	Mean (S)
Autumn	5.98	6.43	12.40	**8.27 ab**
Early winter	5.22	6.93	16.21	**9.45 a**
Late winter	0.86	1.35	7.20	**3.13 c**
Early spring	6.04	5.82	8.06	**6.64 b**
Late spring	7.76	7.61	11.69	**9.02 a**
Mean (N)	**5.17 b**	**5.63 b**	**11.11 a**	***7.30***

LSD interaction = 2.7456
LSD N = 1.2279
LSD Season= 1.7184

Sampling depth 2:200-300 mm

YEAR 2000 — Seven weeks after fertiliser N application

	0	50	150	Mean (S)
Autumn	4.40	2.44	9.66	**5.50 ab**
Early winter	2.55	1.84	7.68	**4.03 bc**
Late winter	5.92	6.70	10.57	**7.73 a**
Early spring	1.66	1.71	2.39	**1.92 c**
Late spring	3.71	2.81	3.13	**3.22 bc**
Mean (N)	**3.65 b**	**3.10 b**	**6.69 a**	***4.48***

LSD interaction = NS
LSD N = 2.8945
LSD Season = 3.5136

YEAR 2001 — Seven weeks after fertiliser N application

	0	50	150	Mean (S)
Autumn	2.63	4.18	2.98	**3.26 cd**
Early winter	2.70	2.84	2.66	**2.73 d**
Late winter	4.02	3.63	3.66	**3.77 bc**
Early spring	4.83	3.66	4.65	**4.38 b**
Late spring	7.86	10.10	12.40	**10.12 a**
Mean (N)	**4.41**	**4.88**	**5.27**	***4.85***

LSD interaction= 1.572
LSD N = NS
LSD Season= 0.8407

YEAR 2002 — Seven weeks after fertiliser N application

	0	50	150	Mean (S)
Autumn	4.58	3.67	6.34	**4.86 b**
Early winter				
Late winter	3.64	4.67	3.56	**3.96 c**
Early spring	3.71	3.99	4.22	**3.97 c**
Late spring	6.35	6.85	7.47	**6.89 a**
Mean (N)	**4.57 b**	**4.80 ab**	**5.40 a**	***4.92***

LSD interaction = 0.9631
LSD N = 0.4605
LSD Season= 0.6992

**** Means in the same year at a specific depth and time of sampling in the same column or row followed by the same letter are not significantly different (P=0.05)***

Table 6.5 Inorganic-N (mg kg^{-1}) in the 400-500 mm soil layer at one, three and seven weeks in response to fertiliser N application (0, 50 and 150 kg N ha^{-1}) during different seasons (autumn to late spring) to a perennial ryegrass-white clover pasture at Elsenburg for the period 2000 to 2002

Sampling depth 3:400-500 mm — One week after fertiliser N application

	YEAR 2000				YEAR 2001				YEAR 2002			
	Fertiliser N rate (kg ha^{-1})				Fertiliser N rate (kg ha^{-1})				Fertiliser N rate (kg ha^{-1})			
	0	**50**	**150**	**Mean (S)**	**0**	**50**	**150**	**Mean (S)**	**0**	**50**	**150**	**Mean (S)**
Autumn	1.94	3.60	5.30	**3.61 b**	5.51	4.46	6.53	**5.50 b**	18.10	14.65	16.98	**16.58 a**
Early winter	6.19	6.38	9.32	**7.30 a**	2.82	2.50	3.79	**3.04 c**	5.54	7.42	8.51	**7.15 c**
Late winter	2.37	3.44	7.95	**4.58 b**	5.35	7.50	9.51	**7.45 a**	8.58	7.04	5.92	**7.18 c**
Early spring	0.50	1.15	2.74	**1.46 c**	4.02	5.60	6.20	**5.27 b**	6.83	6.29	7.37	**6.83 c**
Late spring	1.27	1.90	3.33	**2.17 c**	5.61	6.54	6.17	**6.11 ab**	8.99	9.50	9.41	**9.30 b**
Mean (N)	**2.45 b**	**3.29 b**	**5.73 a**	***3.82***	**4.66 b**	**5.32 ab**	**6.44 a**	***5.47***	**9.61**	**8.98**	**9.64**	***9.41***

Year 2000: **LSD interaction = 2.04227; LSD N = 0.8435; LSD Season= 1.4326**

Year 2001: **LSD interaction= NS; LSD N = 1.2053; LSD Season= 1.362**

Year 2002: **LSD interaction = NS; LSD N = NS; LSD Season= 1.901**

Sampling depth 3:400-500 mm — Three weeks after fertiliser N application

	YEAR 2000				YEAR 2001				YEAR 2002			
	0	**50**	**150**	**Mean (S)**	**0**	**50**	**150**	**Mean (S)**	**0**	**50**	**150**	**Mean (S)**
Autumn	2.73	3.34	5.57	**3.88 ab**	5.52	5.92	13.71	**8.38 b**	5.25	5.80	9.17	**6.74 b**
Early winter	1.88	4.21	8.75	**4.94 a**	3.69	4.40	6.09	**4.72 b**	6.28	6.00	7.50	**6.59 b**
Late winter	1.50	2.46	4.33	**2.76 bc**	6.16	8.26	8.61	**7.68 b**	0.66	2.90	5.57	**3.04 c**
Early spring	1.53	3.28	9.67	**4.83 a**	20.82	18.16	14.24	**17.74 a**	4.92	5.97	7.25	**6.05 b**
Late spring	1.54	1.99	2.27	**1.93 c**	4.29	5.07	9.59	**6.32 b**	8.59	7.76	9.86	**8.73 a**
Mean (N)	**1.84 c**	**3.06 b**	**6.12 a**	***3.67***	**8.09**	**8.36**	**10.44**	***8.97***	**5.14 b**	**5.68 b**	**7.87 a**	***6.23***

Year 2000: **LSD interaction = 2.292; LSD N = 1.025; LSD Season= 1.1708**

Year 2001: **LSD interaction=NS; LSD N = NS; LSD Season= 5.0998**

Year 2002: **LSD interaction = NS; LSD N = 0.7982; LSD Season= 1.0063**

Sampling depth 3:400-500 mm — Seven weeks after fertiliser N application

	YEAR 2000				YEAR 2001				YEAR 2002			
	0	**50**	**150**	**Mean (S)**	**0**	**50**	**150**	**Mean (S)**	**0**	**50**	**150**	**Mean (S)**
Autumn	6.19	2.62	8.45	**5.76 a**	1.90	3.08	3.53	**2.84 c**	5.17	2.96	11.28	**6.47 ab**
Early winter	2.37	2.60	6.65	**3.87 ab**	2.10	3.03	4.07	**3.07 c**				
Late winter	3.61	6.80	4.47	**4.96 ab**	4.02	3.92	3.77	**3.90 c**	4.70	5.47	5.59	**5.25 b**
Early spring	1.41	1.80	2.08	**1.76 c**	5.94	4.65	5.64	**5.41 b**	4.64	4.19	4.17	**4.33 b**
Late spring	2.64	3.02	3.28	**2.98 bc**	6.70	9.13	14.21	**10.01 a**	7.83	8.54	7.38	**7.92 a**
Mean (N)	**3.24 b**	**3.37 b**	**4.99 a**	***3.87***	**4.13 b**	**4.76 b**	**6.24 a**	***5.04***	**5.58**	**5.29**	**7.10**	***5.99***

Year 2000: **LSD interaction = 3.7726; LSD N = 1.6833; LSD Season= 2.086**

Year 2001: **LSD interaction= 1.5413; LSD N = 0.6893; LSD Season= 1.0901**

Year 2002: **LSD interaction = 2.2889; LSD N = NS; LSD Season= 2.2315**

***** Means in the same year at a specific depth and time of sampling in the same column or row followed by the same letter are not significantly different (P=0.05)*

Conclusions

In this study the application of 50 kg N ha^{-1} did not increase inorganic-N to levels where leaching beyond the root zone could cause significant contamination of the water resources since the majority of the fertiliser N was absorbed within three weeks after application. This statement is supported by the fact that no differences in mean soil inorganic nitrogen levels between 0 and 50 kg ha^{-1} were reported for almost all data recorded. The application of 150 kg N ha^{-1} applied in autumn and early winter on the other hand may result in soil N levels exceeding the pasture's uptake capacity causing an accumulation of soil inorganic nitrogen and increasing the risk of contamination of soil water resources. Leaching of fertiliser N beyond the root zone, as possibly the case with applying 150 kg N ha^{-1} (especially in autumn and early winter), may result in lower N response efficiencies questioning the economic feasibility of these practices.

Leaching of fertiliser N to the 400 - 500 mm levels within one week after N application, as found in this study, clearly emphasise the risk of applying 150 kg N ha^{-1} as a single strategic application. These high levels were detected even seven weeks after fertiliser N application. The optimal N rate would clearly be the rate that results in sufficient N to last for only one regrowth cycle. This will require relative low rates of fertiliser N when pasture productivity is low and/or under high rainfall conditions and may be increased as pasture productivity increases in spring and water supply is managed by thorough irrigation scheduling as rainfall decreases with the onset of spring.

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Chapter 7

The effects of strategic nitrogen fertiliser application during the cool season on perennial ryegrass-white clover pastures in the Western Cape Province

2. Dry matter production

Abstract

The effect of four fertiliser nitrogen levels, (0, 50, 100 and 150 kg N ha^{-1}) applied in autumn (April/May), early winter (June), late winter (July), early spring (August) or late spring (September/October), on dry matter production of a perennial ryegrass-white clover pasture was investigated over a three year period. Primary dry matter (PDM) and residual dry matter production (RDM) were recorded five and ten weeks after fertiliser N application. Although reponses were affected by the age of the pasture, PDM production was dominated by fertiliser N application rate. On average the highest yields were obtained with spring applications and lowest when N was applied in early winter. Although RDM production generally also increased with increasing fertiliser N rates, responses were much smaller and were affected by the season of fertiliser N application. Best results were obtained when N was applied in autumn and winter while application in spring had no or even a negative affect. Due to this contrasting effect both total (PDM + RDM) and annual dry matter production were not much affected. Nitrogen application can therefore be used to improve dry matter production during a short predetermined period. Although the 150 kg N ha^{-1} applied in late spring generally gave the best results in terms of PDM produced the 50 kg N ha^{-1} treatments resulted in a more efficient conversion of N applied to additional DM produced.

Keywords: dry matter production, perennial ryegrass/white clover, strategic nitrogen

Introduction

Dry matter production of perennial ryegrass-white clover pastures grown in the Western Cape Province are characterized by low levels from April to September (Botha, 2002). Poor pasture performance and possible measures to minimize this negative effect on fodder flow management have been extensively investigated (Ball, Molloy & Ross, 1978; Laidlaw, 1984; Frame & Boyd, 1987; Eckard & Franks, 1998; McKenzie, Ryan, Jacobs & Kearney, 1999; Stout, Weaver & Elwinger, 2001). These authors concluded that the strategic use of fertiliser nitrogen, to boost grass productivity during this period of low dry matter production, could be an important management tool to manipulate pasture productivity.

In perennial ryegrass-white clover pastures soil temperatures below about 9°C will restrict soil N mineralisation and nitrification, as well as the growth of clover (MacDuff & Dhanoa, 1990; Hatch & MacDuff, 1991). Ryegrass is reported to grow and respond to fertiliser N application at soil temperatures as low as 5°C (Frame, 1994; Whitehead, 1995). This tendency creates an opportunity to increase pasture production by stimulating ryegrass growth when clover activity is restricted due to low soil temperatures. The negative effect of fertiliser N on clover activity may thus be minimal due to the inactivity of clover at relatively low temperatures.

Increasing soil nitrogen through the application of fertiliser N often results in a decrease in the clover content of grass-clover pastures (Frame & Boyd, 1987; van Heerden & Durand, 1994; Stout, *et al.*, 2001). High levels of soil nitrogen may restrict clover productivity to such low levels that the sward becomes N deficient later in the growing season (Thomas, 1992; Caradus *et al.*, 1993). High rates of fertiliser N applications may also result in an oversupply of soil N, leaving large amounts of residual N exposed to leaching and possible pollution of natural resources. High herbage N levels are also potentially toxic to ruminants (Eckard, 1990). The optimum fertiliser N application rate (kg N ha^{-1}) for each area representing a set of edaphic, environmental and management conditions can only be determined if a range of N application rates are evaluated.

The window of opportunity in which pasture, especially the grass component, productivity can be increased, includes autumn, winter and spring, complicating the decision of when to apply fertiliser N. The positive effect of fertiliser N applied, only lasts for about 4 weeks (Eckard, Bartholomew & Tainton, 1995), necessitating the testing of fertiliser N application over different seasons in order to identify the optimum combination(s) of fertiliser N rate and season of application.

The aim of this study was to determine the optimum response of the pasture in terms of dry matter production to fertiliser N rates and season of application treatment combination(s) as a possible management tool to increase pasture productivity when dry matter productivity is restricted by sub optimal environmental conditions.

Materials and methods

A field experiment was conducted at the Institute for Plant Production, Elsenburg during the period 2000 – 2002 to determine the effect of four nitrogen application rates (0, 50, 100 and 150 kg ha^{-1}), applied during autumn (April/May), early winter (early June), late winter (mid July), early spring (late August) or late spring (September/October) on the inorganic nitrogen content of the soil and subsequent dry mass (DM) production, pasture composition as well as the quality of a ryegrass-clover pasture. Details on the experimental site, climate, experimental design, lay-out, treatments applied and data analyses are discussed in Chapter 5.

Data collection

To determine dry matter (DM) production, each year was divided into a grazing cycle (pasture grazed by sheep – December to March) and a cutting cycle (April to November) when the pasture was mowed to a 50 mm residue height and all plant material removed from the pasture. A fixed five week cutting interval was used during the cutting cycle and a four-weekly grazing interval during the grazing cycle. Subplot dimensions were 3.6 x 7 m (25.2 m^2) of which a net area of 1.27 x 5.73 m (7.28 m^2) was harvested using a sickle-bar mower. N treatments were applied after cutting, the timing of application depending on season as a treatment. Two sub-samples (approximately 300 grams wet material) were collected from each treatment combination at harvesting. One sample used to determine dry matter production was dried for 72 h at 60 °C. During the grazing cycle three 0.25 m^2 quadrants per treatment combination were cut, bulked and the same procedure as described for mowing was followed to determine pasture dry matter production.

Dry matter production was divided into: Primary dry matter production (PDM) which refers to the dry matter produced during the first regrowth cycle (5 weeks) after fertiliser N treatments were applied; Residual dry matter production (RDM) which refers to the dry matter produced in the second regrowth cycle (5-10 weeks) after application of fertiliser N;

Total dry matter production (TDM) which refers to the cumulative dry matter produced during the first 10 weeks after fertiliser N application and annual dry matter production (ADM) which refers to all dry matter produced during the cutting and grazing cycle of a year and which were recorded during 2001 and 2002 only.

Results and discussions

Primary dry matter production (PDM)

The highest PDM yields were recorded during 2000 (1948.06 kg ha^{-1}), followed by 2001 (1879.85 kg ha^{-1}) and 1791.74 kg ha^{-1} in 2002 (Table 7.1). High rainfall could reduced N availability during 2001 (Chapter 6), but did not as expected, reduced PDM production, because PDM production decreased with season of production irrespective of rainfall.

A significant interaction between season of application and fertiliser nitrogen rate was observed in 2000 only, indicating that nitrogen application rate has a dominating effect on PDM production. Increased levels of fertiliser N therefore resulted in increased PDM production irrespective of season of application (Table 7.1), but the smallest response was generally obtained when N was applied in early winter. The mean PDM production for the autumn, late winter, early - and late spring treatments were respectively 792.5, 334.1, 1287.2 and 1513.7 kg ha^{-1} higher compared to early winter applications of fertiliser N. Highest yields of 3065.8 (2000), 3296.3 (2001) and 3144.7 kg DM ha^{-1} (2002) were obtained where 150 kg N ha^{-1} was applied during late spring, while the lowest yields of 945.4 (2000), 456.0 (2001) and 526.9 kg DM ha^{-1} (2002), were recorded in early winter where no nitrogen (0 kg N ha^{-1}) was applied. In general PDM production for the 0 kg N ha^{-1} treatments showed similar responses to season as fertilised plots, illustrating the normal seasonal effect on growth of a ryegrass-clover pasture.

The lack of response during autumn and early winter of 2000 and 2002 might have been the result of soil nitrate levels exceeding the pasture's (slow) nitrate absorption capacity at low temperatures (Chapter 2). Concentrations of plant available N above 28 mg kg^{-1} (2.0 mM) generally decrease N_2 fixation and high levels of soil N cause senescence of root nodules restricting N_2 fixation (Phillips & DeJong, 1984). The total soil-N levels of between 45 and 86 mg kg^{-1}, one week after 150 kg N ha^{-1} fertiliser N application in autumn and early winter

(Chapter 6), may therefore have resulted in a restriction of symbiotic N_2 fixation (Phillips & DeJong, 1984) in addition to the already low fixation rate as a result of low soil temperatures. This might attribute to poor responses in winter, even at high N application rates.

Table 7.1 Primary dry matter yield response (kg DM ha^{-1}) of perennial ryegrass-white clover to fertiliser N rates (kg N ha^{-1}) and time or season of application at Elsenburg

Year 2000					
	Nitrogen (kg ha^{-1})				
Season of application	**0**	**50**	**100**	**150**	**Mean (S)**
Autumn	2014.6 fgh*	2266.7 e	2450.5 de	2658.7 cd	**2347.6 a****
Early winter	945.4 m	985.4 lm	1215.9 jkl	1313.8 ijk	**1115.1 c**
Late winter	1126.0 klm	1501.5 i	1836.4 h	1998.7 gh	**1615.7 b**
Early spring	1425.0 ij	2254.7 ef	2686.9 bcd	2817.8 bc	**2296.1 a**
Late spring	1500.0 i	2230.8 efg	2906.5 ab	3065.8 a	**2425.8 a**
Mean (N)	**1402.2 d**	**1847.82 c**	**2219.24 b**	**2370.96 a**	***1960.1***
LSD (0.05) Year = 75.34					
LSD (0.05) NxS = 242.99					
LSD (0.05) S means = 168.73					
LSD (0.05) N means = 108.48					
CV = 8.68					
Year 2001					
Autumn	590.6 h	1537.6 ef	1886.4 d	2229.5 c	**1561.0 b**
Early winter	456.0 h	1259.3 g	1704.8 de	1748.5 de	**1292.1 c**
Late winter	586.5 h	1357.0 fg	1848.4 d	2228.2 c	**1505.0 bc**
Early spring	1427.8 fg	2291.6 c	2915.8 b	3068.6 ab	**2426.0 a**
Late spring	1749.9 de	2374.2 c	3039.9 b	3296.3 a	**2615.1 a**
Mean (N)	**962.2 d**	**1763.9 c**	**2279.1 b**	**2514.2 a**	***1879.8***
LSD (0.05) NxS = 232.3					
LSD (0.05) S means = 267.62					
LSD (0.05) N means = 103.89					
CV = 8.68					
Year 2002					
Autumn	1416.5 i	1676.8 gh	1847.9 fg	2035.1 ef	**1744.1 c**
Early winter	526.9 m	823.5 kl	982.0 jk	1138.7 j	**867.8 e**
Late winter	656.5 lm	1058.2 j	1390.2 i	1522.3 hi	**1156.8 d**
Early spring	1807.1 fg	2330.8 d	2687.8 c	2833.1bc	**2414.7 b**
Late spring	2197.8 de	2744.2 c	3014.7 ab	3144.7 a	**2775.4 a**
Mean (N)	**1321.0 d**	**1726.7 c**	**1984.5 b**	**2134.8 a**	***1791.7***
LSD (0.05) NxS = 232.22					
LSD (0.05) S means = 190.54					
LSD (0.05) N means = 103.85					
CV = 9.10					

***** Means in the same year followed by the same letter are not significantly different (P=0.05)***

*****Means (in bold) in the same year followed by the same letter in bold are not significantly different (P=0.05)***

PDM production data were subjected to multiple regression analysis (Draper & Smith, 1981). The linear function ($y = a + b_1x_1 + b_2x_2$) fitted the data best (Table 7.2). The data recorded shows great potential to develop regression models (Table 7.2) that can be instrumental in calculating the N rate required by a specific pasture (with a known initial clover content[1]) to produce at a predetermined level at the end of the first regrowth cycle. Due to variation in clover response at the 0 kg N ha^{-1} treatments it was decided to keep years separate with 2000 and 2002 representing pastures with decreasing and increasing clover contents, respectively. The N rate decided on will however also be dictated by predicted clover percentage, season of application and N response efficiencies.

Table 7.2. Relationship between predicted PDM production (Y), initial clover percentage (%) (X_1) and fertiliser N rate (kg N ha^{-1}) (X_2) in a perennial ryegrass-white clover pasture grown under irrigation in the Western Cape Province

Year*	Season	Predicted primary dry matter production (kg ha^{-1})	r^2
2000	**Autumn**	Y = 3426.189 - 20.297(X1) - 1.274(X2) + 0.087(X1*X2)	0.72
	Early winter	Y = 1188.642 - 4.127(X1) + 1.031(X2) + 0.024(X1*X2)	0.72
	Late winter	Y = 1409.093 - 4.335(X1) + 4.059(X2) + 0.034(X1*X2)	0.77
	Early spring	Y = 2602.931 - 19.320(X1) + 8.396(X2) + 0.014(X1*X2)	0.90
	Late spring	Y = 993.753 + 11.684(X1) - 3.184(X2) + 0.260(X1*X2)	0.55
2001	**Autumn**	Y = 456.762 + 16.099(X1) + 11.559(X2) - 0.068(X1*X2)	0.89
	Early winter	Y = 588.606 + 2.055(X1) + 9.782(X2) - 0.0424(X1*X2)	0.83
	Late winter	Y = 196.918 + 12.241(X1) + 18.404(X2) - 0.189(X1*X2)	0.93
	Early spring	Y = 2778.724 - 23.505(X1) + 2.401(X2) + 0.170(X1*X2)	0.85
	Late spring	Y = 2953.065 - 22.250(X1) + 18.036(X2) - 0.118(X1*X2)	0.76
2002	**Autumn**	Y = 1537.777 - 1.431(X1) + 15.621(X2) - 0.156(X1*X2)	0.75
	Early winter	Y = 421.955 + 2.635(X1) + 7.202(X2) - 0.060(X1*X2)	0.82
	Late winter	Y = 199.444 + 10.889(X1) + 12.302(X2) - 0.140(X1*X2)	0.87
	Early spring	Y = 2301.929 - 6.294(X1) + 0.258(X2) + 0.104(X1*X2)	0.80
	Late spring	Y = 1924.388 + 5.369(X1) + 37.295(X2) - 0.465(X1*X2)	0.84

**** Parameter values were not averaged over years as years 2000 and 2002 were characterised by a general decrease and increase in clover content between autumn and spring, respectively.***

[1] ***Refer to the clover content of the pasture when the fertiliser N treatments are applied***

Residual dry matter production (RDM)

Mean RDM productivity, over all treatment combinations, also differed between years with 2002 (1435.68 kg ha^{-1}) being the highest followed by 2000 (1333.50 kg ha^{-1}) and 2001 (1108.45 kg ha^{-1}) (Table 7.3). The low RDM yields in 2001 could be the result of leaching of fertiliser N caused by the abnormal high rainfall. Significant interactions in 2001 and 2002 between fertiliser N rate and season of application showed that the response to fertiliser N rate was affected by season of application in two of the three years tested, indicating that the effect of N rate on RDM production was less dominating than on PDM production. Although increased levels of fertiliser N still resulted in increased RDM production in general, mean increases in RDM production were much lower than at five weeks (PDM). For this reason differences between 0 and 50 kg N ha^{-1} were not significant and when applied in early or late spring N application generally had no or even a negative effect on RDM production.

Mean RDM production values for season of application, including the 0 kg N ha^{-1} treatment combinations, increased from a low in autumn to a high in early spring followed by a decrease in late spring. This response in early spring could be the result of increased mineralisation induced by higher soil temperatures (Olsen & Kurtz, 1982) and could explain the absence of or reduction in RDM production when N was applied in spring.

The general lack of pasture response to applied N in the second regrowth cycle (RDM) supported results obtained by Eckard *et al*. (1995) who found that the positive effect of fertiliser N applications normally last for four weeks. Because the objective of strategic N application is to stimulate pasture production for a short but specific period only (4-5 weeks), this lack of response can be seen as positive rather than negative.

Total dry matter production (TDM)

Mean TDM production in 2000 (3285.31 kg ha^{-1}) and 2002 (3227.42 kg ha^{-1}) were both higher than in 2001 (2988.31 kg ha^{-1}) for reasons already explained, showing a similar trend to that of RDM production (Table 7.4).

Table 7.3 Residual dry matter yield response (kg DM ha^{-1}) of perennial ryegrass-white clover to fertiliser N rates (kg N ha^{-1}) and time or season of application at Elsenburg

Year 2000					
	Nitrogen (kg ha^{-1})				
Season of application	**0**	**50**	**100**	**150**	**Mean (S)**
Autumn	945.4 j*	1041.7 ij	1072.1 hij	1167.0 ghi	**1056.57 c****
Early winter	1126.0 ghi	1163.1 ghi	1218.6 fgh	1431.9 cde	**1234.90 b**
Late winter	1425.0 de	1487.3 cde	1530.9 bcd	1727.9 a	**1542.79 a**
Early spring	1500.0 bcde	1477.0 cde	1578.9 abc	1645.0 ab	**1550.22 a**
Late spring	1368.9 ef	1139.9 ghi	1229.0 fg	1394.3 de	**1283.04 b**
Mean (N)	**1273.05 b**	**1261.81 b**	**1325.90 b**	**1473.25 a**	***1333.50***
LSD (0.05) Year = 41.63					
LSD (0.05) NxS= 151.17					
LSD (0.05) S means = 110.02					
LSD (0.05) N means = 67.603					
CV = 7.96					
Year 2001					
Autumn	456.0 j	524.6 j	723.5 hi	946.8 fg	**662.7 c**
Early winter	586.51 ij	779.8 gh	1043.7 f	1235.5 cde	**911.4 b**
Late winter	1427.9 b	1405.4 bc	1455.6 b	1682.6 a	**1492.9 a**
Early spring	1749.9 a	1456.4 b	1373.7 bc	1270.1 bcd	**1462.5 a**
Late spring	1081.6def	982.4 f	1079.5 ef	908.0 fgh	**1012.8 b**
Mean (N)	**1060.4 bc**	**1029.7 c**	**1135.2 ab**	**1208.6 a**	***1108.5***
LSD (0.05) NxS = 188.85					
LSD (0.05) S means = 154.52					
LSD (0.05) N means = 84.458					
CV = 11.96					
Year 2002					
Autumn	526.9 gh	506.1 h	549.3 fgh	745.7 f	**582.0 e**
Early winter	656.5 fgh	727.9 fg	995.4 e	1135.7 e	**878.9 d**
Late winter	1807.1 c	1821.4 c	1923.1 bc	2119.2 ab	**1917.7 b**
Early spring	2197.8 a	2199.3 a	2123.2 ab	2190.3 a	**2177.7 a**
Late spring	1819.4 c	1510.3 d	1403.6 d	1755.4 c	**1622.2 c**
Mean (N)	**1401.5 b**	**1353.0 b**	**1398.9 b**	**1589.3 a**	***1435.7***
LSD (0.05) NxS = 211.15					
LSD (0.05) S means = 137.5					
LSD (0.05) N means = 94.43					
CV = 10.33					

***** Means in the same year followed by the same letter are not significantly different (P=0.05)***

*****Means (in bold) in the same year followed by the same letter in bold are not significantly different (P=0.05)***

Table 7.4 Total dry matter yield (10 weeks) response (kg DM ha^{-1}) of perennial ryegrass-white clover to fertiliser N rates (kg N ha^{-1}) and time or season of application at Elsenburg

Season of application	Nitrogen (kg/ha) 0	50	100	150	Mean (S)
Year 2000					
Autumn	2960.0 e*	3308.3 d	3522.6 cd	3825.7 b	**3404.2 b**
Early winter	2071.4 i	2148.5 hi	2434.5 gh	2745.7 ef	**2350.0 d**
Late winter	2551.0 fg	2988.8 e	3367.2 d	3726.6 bc	**3158.4 c**
Early spring	2925.0 e	3731.6 bc	4265.7 a	4462.8 a	**3846.3 a**
Late spring	2868.8 e	3370.7 d	4199.5 a	4460.1 a	**3724.8 a**
Mean (N)	**2675.2 d**	**3109.6 c**	**3557.9 b**	**3844.2 a**	***3296.7***
LSD (0.05) Year = 96.94					
LSD (0.05) NxS = 299.54					
LSD (0.05) S means = 238.78					
LSD (0.05) N means = 133.73					
CV = 6.35					
Year 2001					
Autumn	1046.5 i	2062.2 h	2610.0 g	3176.3 de	**2223.8 c**
Early winter	1042.5 i	2039.0 h	2748.5 fg	2984.0 ef	**2203.5 c**
Late winter	2014.4 h	2762.4 fg	3303.9 d	3910.8 bc	**2997.9 b**
Early spring	3177.7 de	3748.0 c	4289.5 a	4338.7 a	**3888.5 a**
Late spring	2831.4 fg	3356.6 d	4119.4 ab	4204.3 ab	**3627.9 a**
Mean (N)	**2022.5 d**	**2793.6 c**	**3414.2 b**	**3722.8 a**	***2988.3***
LSD (0.05) NxS = 309.98					
LSD (0.05) S means = 305.74					
LSD (0.05) N means = 138.63					
CV = 7.28					
Year 2002					
Autumn	1943.3 j	2183.0 ij	2397.2 i	2780.8 gh	**2326.1 c**
Early winter	1183.4 l	1551.4 k	1977.4 j	2274.4 ij	**1746.7 d**
Late winter	2463.6 hi	2879.6 g	3313.2 f	3641.6 ef	**3074.5 b**
Early spring	4005.0 de	4530.0 bc	4811.0 ab	5023.4 a	**4592.3 a**
Late spring	4017.2 d	4254.6 cd	4418.3 c	4900.1 a	**4397.5 a**
Mean (N)	**2722.5 d**	**3079.7 c**	**3383.5 b**	**3724.0 a**	***3227.4***
LSD (0.05) NxS = 363.54					
LSD (0.05) S means = 241.42					
LSD (0.05) N means = 162.58					
CV = 7.91					

***** Means in the same year followed by the same letter are not significantly different (P=0.05)***

*****Means (in bold) in the same year followed by the same letter in bold are not significantly different (P=0.05)***

On average TDM production increased with increasing N application rates, but significant interactions between fertiliser N rate and season of application were recorded in 2000 and 2001. The highest TDM productions were recorded where 150 kg N ha^{-1} was applied in early spring (4462.8, 4338.7 and 5023.3 kg ha^{-1} for 2000, 2001 and 2002 respectively) and the lowest at the 0 kg N ha^{-1} treatment level in early winter (2071.4, 1042.5 and 1183.4 kg ha^{-1}). The application of 100 and 150 kg N ha^{-1} resulted in higher (P=0.05) TDM production compared to the 0 kg N ha^{-1} treatments during all seasons covered by the study, while applications of 50 kg N ha^{-1} resulted in higher (P=0.05) TDM production compared to 0 kg N ha^{-1}, with the exception of early winter in 2000 as well as autumn and late spring in 2002. As also found for the first five weeks after application (PDM), early spring and early winter proved to be most and least responsive seasons respectively.

The higher TDM production where N was applied in early- and late spring could be ascribed to increased soil N mineralisation and biological N_2 fixation, as average daily temperature of 12.5 °C during late winter increased to 16.5 °C in late spring (Table 5.3) and daylength (photoperiod) increased from 9.8 hours in early winter to 12.3 hours in late spring. This reaction in pasture productivity was also reflected in similar, but lower increases in PDM production on the zero N treatments. The lower PDM production during June/July suggest an ineffective uptake of applied N, leaving fertiliser N in the root-zone and are consistent with results obtained during the monitoring of soil nitrogen in this study (Chapter 6), confirming the need to monitor soil N distribution and pasture N recovery.

The increase in TDM production as a result of fertiliser N application indicates that a shortage of plant available N existed during the seasons covered by the study and therefore opens the opportunity to boost pasture productivity through applications of fertiliser N.

Stepwise regression analysis revealed that fertiliser N rate (kg N ha^{-1}) caused most of the variation in dry matter production (PDM and TDM) during a specified season with the contribution of initial clover percentage generally neglible (Table 7.5).

Annual dry matter production (ADM)

Although mean ADM production in 2002 (14 500.6 kg ha^{-1}) was higher than in 2001 (12 075.1 kg ha^{-1}), nitrogen applications did not influence ADM production in 2001, but in 2002 the 0 kg N ha^{-1} treatments produced significant (P=0.05) lower yields than the treatments that received fertiliser nitrogen (Table 7.6). The increase of 831.6 kg in ADM production when the 150 kg N ha^{-1} treatments were compared to 0 kg N ha^{-1} in 2002, supported earlier results by Ball *et al*. (1978), who showed annual yield increases of 1.1 and 3.5 t ha^{-1} where 112 and 448 kg N ha^{-1} were applied to grazed grass-clover pastures. The lower ADM production in 2001 most probably was, as already explained, the result of the high rainfall resulting in N leaching and low initial clover content restricting N fixation and recycling in the pasture.

Table 7.5 Contribution (%) of fertiliser N rate (kg ha^{-1}), applied in a specified season, and initial clover content (%), to variation measured in PDM and TDM production on perennial ryegrass-white clover pastures in the Western Cape Province

		PDM production			TDM production		
Year	**Season**	**N rate**	**Initial clover %**	**Interaction**	**N rate**	**Initial clover %**	**Interaction**
2000	**Autumn**	52.4	17.52	NS	53.12	19.99	NS
	Early winter	68.1	NS	NS	64.14	NS	NS
	Late winter	76.54	NS	NS	82.67	NS	NS
	Early spring	82.79	6.96	NS	83.39	4.79	NS
	Late spring	NS	NS	54.71	NS	NS	49.21
2001	**Autumn**	86.64	NS	NS	92.32	NS	NS
	Early winter	83.08	NS	NS	88.14	NS	NS
	Late winter	92.35	NS	NS	87.19	NS	NS
	Early spring	83.74	NS	NS	67.11	NS	NS
	Late spring	70.88	NS	NS	61.92	NS	NS
2002	**Autumn**	60.23	NS	14.65	55.62	NS	23.63
	Early winter	79.36	NS	NS	83.95	NS	NS
	Late winter	84.53	NS	NS	79.94	NS	NS
	Early spring	79.61	NS	NS	NS	NS	71.04
	Late spring	69.02	NS	15.03	53.48	NS	NS

Season of application also influenced ADM production in 2001, but not in 2002. The lowest ADM production in 2001 was recorded when fertiliser N was applied in early winter. Although differences (P=0.05) in ADM production were found due to different N-rates in combination with the season of application, no definite trend was observed. Frame & Boyd

(1987) also reported inconsistent results, while Schils, Vellinga & Kraak (1999) reported no difference in annual DM yields where 0 and 50 kg N ha^{-1} were applied in spring.

The fact that ADM production did not show any definite response to treatment combinations indicates that perennial ryegrass-white clover pastures can be manipulated to increased dry matter production during predetermined seasons without major negative effects on pasture annual dry matter production potential.

Table 7.6 Annual dry matter production of the pasture for 2001 and 2002 in response to strategic N fertilisation at at Elsenburg

			Year 2001		
	Nitrogen (kg ha^{-1})				
Season of application	**0**	**50**	**100**	**150**	**Mean (S)**
Autumn	12251.0 abcd *	13506.2 a	12509.5abcd	12350.2 abcd	**12654.2 ab****
Early winter	12251.0 abcd	11243.1 de	11290.3 de	10163.9 e	**11284.2 c**
Late winter	12251.0 abcd	12512.8 abcd	11641.2 bcd	12211.3 abcd	**12058.6 abc**
Early spring	12251.0 abcd	11398.8 cde	11420.4 cde	11319.1 de	**11597.3 bc**
Late spring	12251.0 abcd	12833.9 abc	12896.0 ab	12950.3 ab	**12732.8 a**
Mean (N)	**12251.0**	**12298.9**	**11951.5**	**11798.9**	*12075.1*
LSD (0.05) Year = 301.47					
LSD (0.05) NxS = 1461.8					
LSD (0.05) S means = 1111.9					
LSD (0.05) N means = NS					
CV = 8.65					
			Year 2002		
Autumn	13870.8 e	14978.1 abc	14737.8 abcd	14461.4 abcde	**14512.0**
Early winter	13870.8 e	14226.9 cde	14840.3 abcd	13673.9 e	**14153.0**
Late winter	13870.8 e	15024.7 abc	15286 a	15035.1 abc	**14804.1**
Early spring	13870.8 e	14719.7 abcd	14959.9 abc	15109.9 ab	**14665.1**
Late spring	13870.8 e	14321.1 bcde	14051.2 de	15231.5 a	**14368.7**
Mean (N)	**13870.8 b**	**14654.1 a**	**14775.0 a**	**14702.4 a**	*14500.6*
LSD (0.05) NxS = 847.1					
LSD (0.05) S means = NS					
LSD (0.05) N means = 378.83					
CV = 4.10					

***** *Means in the same year followed by the same letter are not significantly different (P=0.05)*

***Means (in bold) in the same year followed by the same letter in bold are not significantly different (P=0.05)*

The annual dry matter yield responses of the pasture are summarised in Figure 7.1. Results show that the positive effect of the applied fertiliser N on dry matter production lasted for a maximum of two regrowth cycles (10 weeks) mainly with application rates of 150 kg N ha^{-1} applied in early and late winter. This increase of dry matter production relative to the 0 kg N ha^{-1} treatments are generally followed by dry matter production levels slightly lower than the 0 kg N ha^{-1} levels generally over an extended period following fertiliser N application. The effect of season of application on addressing the winter gap is clearly shown in the results. Year 2002 showed that application of fertiliser N in autumn delayed the onset of the negative effect of the winter gap as pasture production was stimulated, especially at the 100 and 150 kg N ha^{-1} rates. The duration of the winter gap, period between sufficient forage availability in early autumn and sufficient forage availability in early summer, was therefore effectively reduced. The same effect was observed with late winter and early spring applications. Fertiliser N application in early winter increased dry matter production when shortages in available forage become crucial as pasture dry matter production reached a low resulting in severe forage shortages. Late spring applications were however too late to be of any significance in managing the winter gap as pasture productivity (without N application) was at the same – or higher – levels compared to dry matter production at the onset of the winter gap.

Nitrogen Response Efficiency (NRE)

The nitrogen response efficiency (kg additional DM produced per kg N applied) is the difference between DM produced at 0 kg N ha^{-1} and 50, 100 as well as 150 kg N ha^{-1} respectively divided by N application rate.

As also found by Eckard & Franks (1998), increasing fertiliser N rates resulted in progressively lower NRE values (Table 7.7). NRE values increased as the season progressed towards spring, reaching a maximum during early spring with trends obtained in this study similar to those reported in earlier studies (Frame & Boyd, 1987; McKenzie, Jacobs & Ryan, 1998; McKenzie *et al*., 1999).

Although no significant fertiliser N x season of application interaction was found, the application of 50 kg N ha^{-1} in early spring resulted in the highest NRE of 14.782 kg DM kg^{-1} N during the first regrowth cycle, while the lowest return (5.05 kg DM kg^{-1} N) was recorded where 150 kg N ha^{-1} was applied in early winter. These results supported the idea that nitrogen responses of grass-clover pastures are lower and less reliable compare to pure grasses (Whitehead, 1970 cited by Ball *et al*., 1978).

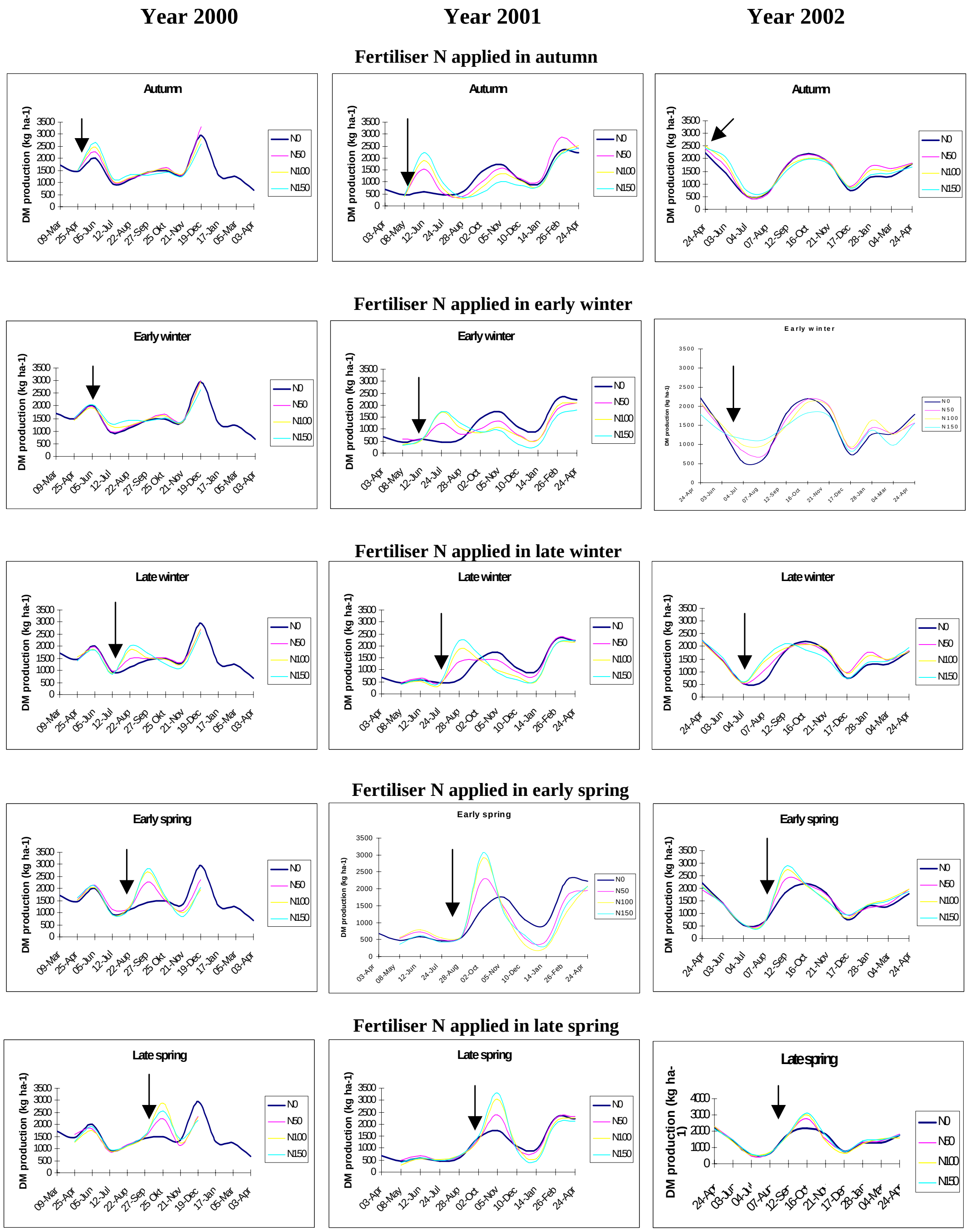

Figure 7.1 Long term effect of fertiliser N application rate (0, 50, 100 and 150 kg N ha^{-1}) and season of application on dry matter production (kg ha^{-1}) of a perennial ryegrass-white clover pasture from year 2000 to 2002 in the Western Cape Province (N application ⟶).

Table 7.7 Mean nitrogen response efficiency (kg additional DM per kg N applied) of perennial ryegrass-white clover as a result of different fertiliser N rates and season of application for the experimental period 2000-2002 at Elsenburg

Season of application	Nitrogen (kg ha^{-1}) 50	100	150	Mean (S)
PDM (First re-growth cycle)				
Autumn	9.730	7.213	6.448	**7.797 cd**
Early winter	7.599	6.581	5.050	**6.410 d**
Late winter	10.318	9.021	7.511	**8.950 bc**
Early spring	14.782	12.101	9.022	**11.968 a**
Late spring	12.677	9.929	9.020	**10.542 ab**
Mean (N)	**11.021 a**	**8.969 b**	**7.410 c**	
LSD (0.05) NxS = NS				
LSD (0.05) S means = 2.3999				
LSD (0.05) N means = 1.0773				
CV = 9.25				
RDM (Second re-growth cycle)				
Autumn	0.960	1.389	2.070	**1.473 b**
Early winter	2.012	2.968	3.187	**2.720 a**
Late winter	0.361	0.832	1.934	**1.042 b**
Early spring	-2.100	-1.240	-0.762	**-1.387 c**
Late spring	-4.249	-1.859	-0.471	**-2.193 c**
Mean (N)	**-0.603 b**	**0.417'a**	**1.1917a**	
LSD (0.05) NxS = NS				
LSD (0.05) S means = 1.0345				
LSD (0.05) N means = 0.8421				
CV = 10.18				
TDM (Total over two re-growth cycles)				
Autumn	10.691	8.601	8.518	**9.270**
Early winter	9.612	9.546	8.238	**9.132**
Late winter	10.677	9.852	9.444	**9.991**
Early spring	12.681	10.863	8.261	**10.602**
Late spring	8.428	8.070	8.549	**8.349**
Mean (N)	**10.418 a**	**9.386 ab**	**8.602 b**	
LSD (0.05) NxS = NS				
LSD (0.05) S means = NS				
LSD (0.05) N means = 1.3962				
CV =9.94				

** Means in the same year followed by the same letter are not significantly different (P=0.05)*

*** Means (in bold) in the same year followed by the same letter in bold are not significantly different (P=0.05)*

Carry over of fertiliser N to the second regrowth cycle where 150 kg N ha^{-1} was applied in autumn and winter caused, in contrast to the first re-growth cycle, an increase in the NRE resulting in less significant differences in NRE values between treatment combinations.

Conclusion

Results from this study show that dry matter production in a predetermined season can be increased through the application of fertiliser nitrogen without reducing annual dry matter production.

The application of 150 kg N ha^{-1} during early and late spring resulted in maximum pasture DM yield responses, but lower nitrogen response efficiencies and the possibility of a reduction in clover content and thus pasture quality may limit the economic feasibility of such treatments. According to this study, applications of 50 kg N ha^{-1} during any season will result in the most efficient conversion of applied fertiliser into additional fodder during the first regrowth cycle.

The overriding influence of climatic conditions and the resultant effect on the response capacity of a perennial ryegrass-white clover pasture impede the creation of a fixed strategic fertilisation programme. It is envisaged that clover content, quantity additional fodder required and season of application to be the major factors that will dictate the rate of fertiliser N recommended. Regression lines obtained shows great potential to develop regression models instrumental in predicting the resultant pasture dry matter production as influenced by selected environmental conditions and are presented in Table 7.2.

A clear limitation of this study is the absence of grazing animals for a period of eight months, due to the continuous sampling of soil to monitor soil-N as an accidental sampling of urine patches would render soil-N data not suitable for interpretation and N losses on grazed pastures due to volatilization and denitrification may be much higher in grazed pastures compared to cut swards (Whitehead, Pain & Ryden, 1986). Ball *et al.* (1978) however reported that mean daily yields of a ryegrass-clover pasture grazed by sheep were strikingly similar to results obtained from a mowed experiment in the same environment. In addition it can be said that the fixed five week cutting cycle might have benefited the August and

September applications mainly because of increased pasture growth due to higher average temperatures and increased day-length.

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Chapter 8

The effects of strategic nitrogen fertiliser application during the cool season on perennial ryegrass-white clover pastures in the Western Cape Province

3. Clover content

Abstract

The influence of a single application of fertiliser N (0, 50, 100 and 150 kg N ha^{-1}) applied in either autumn, early winter, late winter, early spring or late spring on the grass-clover balance in a perennial ryegrass-white clover pasture was studied over a three year period. Responses were measured over two regrowth cycles five and ten weeks after the application of N treatments.

Increased fertiliser N rates of up to 100 kg N ha^{-1} resulted in increasingly lower clover percentages. No differences in clover content were recorded between the 100 and 150 kg N ha^{-1} application rates five weeks after fertiliser N application. The effect of season of application was inconsistent due mainly to different initial clover contents and differences in environmental conditions amongst years. Partial recovery of clover content was noted at the second re-growth cycle but not to the same levels as at the 0 kg ha^{-1} treatment combinations.

Fertiliser N did not reduce ($P>0.05$) total clover DM production (kg clover ha^{-1}) over 10 weeks when applied in autumn and early winter. Clover DM yields were however reduced ($P=0.05$) when fertiliser N was applied during early and late spring, excluding 50 kg N ha^{-1} applied during early spring 2000.

The application of 50 kg N ha^{-1} during the relatively normal rainfall years in 2000 and 2002 did not cause the clover content to drop below 30%. The application of 150 kg N ha^{-1} resulted in numerous treatment combinations where the clover percentage were less than 30 %, the recommended minimum for the pasture to benefit from the clover. Data obtained shows great potential to develop regression models instrumental in predicting the resultant clover content

after application of selected fertiliser N rates to a perennial ryegrass-white clover pasture in the Western Cape Province.

Keywords: clover percentage, perennial ryegrass/white clover, strategic nitrogen

Introduction

Clovers are included in ryegrass-clover pastures for various reasons. Firstly, a high proportion of clover in the pasture improves pasture quality that results in increased milk, beef or wool production. Eerens & Ryan (2000) reported that mixed ryegrass-white clover pastures produced nearly 25% more dry matter, 40% (180 kg) more carcass weight and 25% (17kg) more wool than ryegrass pastures receiving 270 kg N ha^{-1}. Secondly, as a legume it is capable of fixing large quantities of atmospheric nitrogen and through recycling nitrogen becomes available for uptake by the ryegrass fraction (Martin 1960). Davidson & Robson (1990) reported that ryegrass in a grass/clover mixture consistently had higher N contents than pure grass grown in monoculture. Thirdly legumes improve seasonal distribution of the forage by being more productive later in the year than the companion grass crop (Sleugh *et al*., 2000). The only disadvantages of legumes in a pasture are the possibility of bloat in animals and the poor predictability of legume performance (Miles & Manson, 2000).

To ensure ryegrass-clover pastures of acceptable quality and quantity the percentage clover must remain between 30 and 50 percent on a dry matter basis (Martin, 1960). Pflimlin (1993, cited by Schils, Vellinga & Kraak, 1999) suggest an optimal clover content of between 25 and 50% for rotational grazing. Curll (1982) recommend an average clover content of at least 30% to have an impact on animal nutrition due to the superior feeding value of the clover plant. Harris (1994) recommended a clover content of 45% during optimum growth conditions (spring) to ensure optimum milk production per unit area and to ensure a 30% clover content in winter. McKenzie & Jacobs (1997, cited by McKenzie *et al.* 1999) and McKenzie, Jacobs & Ryan (1998) recommend a perennial ryegrass content of >50% when applying fertiliser N to stimulate pasture productivity.

Boosting the soil N pool through the application of fertiliser N often results in a decrease in the clover content of grass-clover pastures (Frame & Boyd, 1987; van Heerden & Durand, 1994; Stout, Weaver & Elwinger, 2001). Applying too high levels of nitrogen may reduce the clover fraction to such low levels that the sward becomes N deficient later in the growing season (Thomas, 1992; Caradus *et al*., 1993). Dennis & Woledge (1985) ascribed the decreased clover portion to the increased competition for light brought on by the stimulated growth of the grass, while Eckard (1994) cites the negative effect of fertiliser N on N_2 fixation as an additional cause of reduced clover content. Stout *et al*., (2001) found that the clover content decreased by 17 percent if the target sward harvest height was increased from 150 to 300 mm in a study where between 22.4 and 89.6 kg N ha^{-1} were applied in spring.

Applying moderate levels of fertiliser N during the cool season, when white clover activity is low, can increase DM production of grass-clover pastures without permanently suppressing clover growth (Laidlaw, 1980; Stout, *et al*., 2001). Laissus (1983, cited by Frame, 1987), agreed that fertiliser N use in spring can be profitable on swards with a reasonably high proportion of white clover but stressed the depressive effect of N late in spring as well as the intensified depressive effect on soils with high water-holding capacities and compact 'cold' soils. The value of the increased herbage production from fertiliser N must be weighed against the decline in white clover performance during the season and possibly thereafter.

The aims of the study were to develop a better understanding of the effect of a strategic N fertiliser application during the cool season on the grass-clover balance and to identify possible management guidelines (scenarios) that would maximise dry matter production without suppressing clover content to values lower than required to maintain the benefit of clover in the pasture.

Materials and methods

General procedures.

A field experiment was conducted during the period 2000-2002 at Elsenburg to determine the effect of a single application of four fertiliser N rates (0, 50, 100 and 150 kg N ha^{-1}) applied annually in either autumn, early winter, late winter, early spring or late spring on soil inorganic nitrogen content, pasture composition and quality as well as dry matter production of a perennial rye grass-white clover pasture. Details on the experimental site, experimental design, lay-out, treatments and data analyses are presented in Chapter 5.

Clover content

Fresh herbage samples of approximately 300 g wet material were collected from each treatment combination at harvesting following each of two consecutive re-growth cycles at five and ten weeks after the application of N treatments. Clover content was determined through dividing the sample into clover and grass fractions that were then dried for 72 h at 60 °C and dry weight recorded.

Primary- and Residual Clover Percentage were determined five and ten weeks after fertiliser N application respectively using the formula:

Clover % = (Clover dry mass / (Clover dry mass + Ryegrass dry mass))*100

The absolute clover content was calculated using the formula:

Absolute clover content (kg ha^{-1}) = DM production (kg ha^{-1}) * clover %

Results and Discussion

Primary Clover Percentage (PCP)

Highest mean PCP was recorded in 2002 (42.24%) followed by 2000 (38.75%) and 23.52% in 2001 (Table 8.1). The low clover content in 2001 could be due to a combination of two factors. Firstly, moisture stress during late summer 2001 (pastures were not irrigated due to pump failure) might reduced the clover content at the start of the cool season, because root nodules have been shown to senescence under dry soil conditions reducing clover growth potential (Chu & Robertson, 1974). Evans (1978) and Thomas (1984, cited by Frame & Boyd, 1987) also found that white clover production fell by a mean of 78%, while ryegrass production increased by 25%, under dry conditions suggesting the smaller root mass of clover in comparison to perennial ryegrass as the possible cause. Secondly, the above average rainfall during the cool season most probably, could have resulted in prolonged water logging of soils in the trial area. These conditions could also reduced clover growth due to the possible adverse effects on the N fixing bacteria.

Table 8.1 Primary clover percentage (PCP) (percentage clover on a DM basis) of a perennial ryegrass-white clover pasture after the first regrowth cycle (5 weeks) at Elsenburg

			Year 2000		
	Fertiliser N rate (kg ha^{-1})				
Season of application	**0**	**50**	**100**	**150**	**Mean (S)**
Autumn	66.47	49.79	47.06	39.65	**50.75 a**
Early winter	55.48	46.89	41.07	39.12	**45.64 ab**
Late winter	52.91	36.24	32.66	26.31	**37.03 bc**
Early spring	47.4	29.06	21.18	19.04	**29.17 c**
Late spring	50.42	32.24	22.02	20.00	**31.17 c**
Mean (N)	**54.53 a****	**38.84 b**	**32.80 c**	**28.82 c**	***38.75***
LSD (0.05) NxS = NS					
LSD (0.05) S = 10.457					
LSD (0.05) N = 5.3505					
CV = 21.68					
			Year 2001		
Autumn	26.79 cd*	7.89 hij	4.69 ij	3.46 j	**10.71 d**
Early winter	40.79 b	21.61 def	15.25 fg	14.90 fgh	**23.14 c**
Late winter	52.73 a	28.13 cd	17.38 efg	11.26 ghi	**27.38 ab**
Early spring	51.17 a	22.84 cde	18.24 efg	13.43 gh	**26.42 bc**
Late spring	47.24 ab	28.83 c	21.21 def	22.48 cde	**29.94 a**
Mean (N)	**43.75 a**	**21.86 b**	**15.35 c**	**13.11 c**	***23.52***
LSD (0.05) NxS = 7.1768					
LSD (0.05) S = 3.4255					
LSD (0.05) N = 3.2096					
CV = 21.43					
			Year 2002		
Autumn	56.62	49.86	37.11	36.7	**45.07 a**
Early winter	47.99	34.41	25.11	19.54	**31.76 b**
Late winter	64.26	48.37	42.17	34.82	**47.40 a**
Early spring	65.76	43.43	36.43	33.87	**44.87 a**
Late spring	62.81	36.45	33.64	35.54	**42.11 a**
Mean (N)	**59.49 a**	**42.50 b**	**34.89 c**	**32.09 c**	***42.24***
LSD (0.05) NxS = NS					
LSD (0.05) S = 5.8449					
LSD (0.05) N = 4.7928					
CV = 17.81					

Means in the same year followed by the same letter are not signifficantly different (P=0.05)

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

Increased fertiliser N rates resulted in increasingly lower clover percentages irrespective of season of application (Table 8.1) during all years covered by the study. Mean PCP of treatments at the 0 kg N ha^{-1} rates were significantly higher than treatments receiving fertiliser N while the lowest (P=0.05) PCP values were found where 100 and 150 kg N ha^{-1} were applied.

PCP tends to decrease from late autumn to early spring in 2000 and late winter to late spring in 2002. In contrast, mean PCP tended to increase as season progressed in 2001 possibly as a result of clover recovering from the adverse conditions that prevailed during late summer. The decrease in PCP in 2000 and 2002 may be the result of overshadowing of clover by grass stimulated by fertiliser N, high soil-N levels (Chapter 6), as well as low N fixing rates of *Rhizobia* during the cooler months (Dennis & Wolledge, 1985; Wolledge, 1988).

Although a significant interaction ($P<0.05$) between season of application and fertiliser N rate was recorded in 2001, Table 8.1 clearly shows that increased fertiliser N rates resulted in a reduction in clover content over all seasons of N application. No differences ($P>0.05$) in clover content between 100 and 150 kg N ha^{-1} applied during the same season in 2001 were recorded, however the application of 50 kg N ha^{-1} resulted in a lower (P=0.05) clover content compared to the treatments that received no nitrogen during the same season.

During years 2000 and 2002 when the initial clover percentage (in autumn) was high (66.47 and 56.62% respectively) the clover percentage at the 0 kg N ha^{-1} treatments over season of application remained high with only early spring in 2000 and early winter in 2002 having clover percentages of less than 50 percent. This resulted in the majority of treatment combinations maintaining a clover content of 30% and more. The treatment combinations resulting in less than 30% clover were those where 50 kg N ha^{-1} was applied in early spring 2000; where 100 kg N ha^{-1} was applied in early and late spring 2000 or early winter 2002; and where 150 kg N ha^{-1} was applied in late winter, early and late spring 2000 as well as early winter 2002.

The initial clover percentage in autumn 2001 was low (26.79 %) at the 0 kg N ha^{-1} treatments due to water stress as already mentioned, but increased to levels comparable to those achieved in 2000 as season progressed to late spring. The suppressive effect of N, whether direct or indirect, on clover percentage was however much more pronounced causing clover percentages of less than 30 % in all treatments that received fertiliser N.

In an attempt to predict the possible effect of fertiliser N rate on the resultant clover percentage at the end of the first regrowth cycle regression functions were fitted to establish the best option (Draper & Smith, 1981). The linear function ($y = a + b_1x_1 + b_2x_2$) was decided on (Table 8.2). The funtion shows great potential to be instrumental in predicting the clover content at the end of the first regrowth cycle as a result of the N rate applied. The predicted clover content should be within the prescribed 30 – 50% range as suggested by Martin (1960). This method will exclude certain N rates, however final N rate will also be a function of the season of application, N response efficiencies and quantity additional fodder required.

Table 8.2. Relationship between predicted RCP (%) (Y), initial clover percentage (%) (X_1) and fertiliser N rate (kg N ha^{-1}) (X_2) in a perennial ryegrass-white clover pasture grown under irrigation in the Western Cape Province

Year*	Season	Predicted primary clover percentage (%)	r^2
2000	**Autumn**	Y = -33.828 + 1.410(X1) + 0.257 (X2) - 0.007(X1*X2)	0.56
	Early winter	Y = 18.626 + 0.523(X1) - 0.066(X2) - 0.0003(X1*X2)	0.65
	Late winter	Y = 25.230 + 0.448(X1) - 0.296(X2) + 0.003(X1*X2)	0.74
	Early spring	Y = - 5.478 + 0.939(X1) - 0.045(X2) - 0.003(X1*X2)	0.86
	Late spring	Y = 57.057 - 0.224(X1) - 0.387(X2) + 0.004(X1*X2)	0.80
2001	**Autumn**	Y = 25.286 - 0.181(X1) - 0.214(X2) + 0.003(X1*X2)	0.65
	Early winter	Y = 22.238 + 0.504(X1) + 0.003(X2) - 0.006(X1*X2)	0.68
	Late winter	Y = 24.310 + 0.582(X1) - 0.255(X2) - 0.0003(X1*X2)	0.90
	Early spring	Y = - 15.216 + 1.180(X1) + 0.085(X2) - 0.006(X1*X2)	0.84
	Late spring	Y = 97.089 - 1.034(X1) - 0.995(X2) + 0.015(X1*X2)	0.79
2002	**Autumn**	Y = - 26.920 + 1.220(X1) + 0.190(X2) - 0.005(X1*X2)	0.75
	Early winter	Y = 44.991 + 0.020(X1) - 0.340(X2) + 0.003(X1*X2)	0.72
	Late winter	Y = 51.351 + 0.185(X1) - 0.337(X2) + 0.004 (X1*X2)	0.66
	Early spring	Y = 39.974 + 0.350(X1) - 0.721(X2) +0.008(X1*X2)	0.81
	Late spring	Y = -28.109 + 1.270(X1) - 0.064(X2) - 0.002(X1*X2)	0.62

** Parameter values were not averaged over years as years 2000 and 2002 were characterised by a general decrease and increase in clover content between autumn and spring, respectively.*

Stepwise regression results revealed that most of the variation in clover percentage within a specified season was caused by fertiliser N rate (Table 8.3). The contribution of initial clover percentage to RCP was restricted to year 2000, with neglible effects in 2001 and 2002.

Table 8.3 Contribution (%) of fertiliser N rate (kg ha^{-1}), applied in a specified season, and initial clover content (%), to variation measured in pasture clover percentage (%) at the end of the first regrowth cycle on perennial ryegrass-white clover pastures in the Western Cape Province

Year	Season	Fertiliser N	Initial clover %
Year 2000	**Autumn**	26.56	25.16
	Early winter	18.05	46.95
	Late winter	63.52	10.27
	Early spring	65.1	19.87
	Late spring	79.71	NS
Year 2001	**Autumn**	62.45	NS
	Early winter	62.82	NS
	Late winter	85.52	4.52
	Early spring	75.3	6.75
	Late spring	68.28	NS
Year 2002	**Autumn**	40.61	30.53
	Early winter	66.44	NS
	Late winter	56.52	NS
	Early spring	68.73	NS
	Late spring	50.72	NS

Residual Clover Percentage (RCP)

Mean RCP values for all treatment combinations, except for 0 kg N ha^{-1} in 2000, were higher than the PCP values (Table 8.4).

Table 8.4 Residual clover percentage (RCP) (percentage clover on a DM basis) of a perennial ryegrass-white clover pasture after the second re-growth cycle (10 weeks) at Elsenburg

Season of application	**Fertiliser N rate (kg ha^{-1}) 0**	**50**	**100**	**150**	**Mean (S)**
Year 2000					
Autumn	55.48	47.21	46.95	43.82	**48.37 a****
Early winter	52.91	49.06	43.39	36.11	**45.37 ab**
Late winter	47.4	38.93	35.58	22.41	**36.08 bc**
Early spring	50.42	35.79	23.44	19.99	**32.41 c**
Late spring	57.42	41.44	30.11	27.05	**39.00 abc**
Mean (N)	**52.73 a**	**42.48 b**	**35.89 c**	**29.88 d**	*40.25*
LSD (0.05) NxS = NS					
LSD (0.05) S = 9.6844					
LSD (0.05) N = 4.2662					
CV = 16.64					
Year 2001					
Autumn	40.79 cd*	19.57 hij	10.19 kl	8.71 l	**19.81 c**
Early winter	52.73 ab	30.92 efg	15.12 jkl	11.75 jkl	**27.69 b**
Late winter	51.17 ab	41.17 cd	25.37 ghi	13.96 jkl	**32.92 b**
Early spring	47.24 bc	27.22 fgh	20.31 hij	17.50 ijk	**28.07 b**
Late spring	58.36 a	46.33 bc	35.79 def	39.30 cde	**44.94 a**
Mean (N)	**50.06 a**	**33.04 b**	**21.35 c**	**18.24 c**	*30.67*
LSD (0.05) NxS = 8.6535					
LSD (0.05) S = 7.5803					
LSD (0.05) N = 3.8699					
CV = 19.81					
Year 2002					
Autumn	47.99 efgh	47.11 efghi	36.69 ijkl	30.96 klm	**40.69 b**
Early winter	64.26 ab	45.65 fghi	30.76 klm	25.59 m	**41.57 b**
Late winter	65.76 ab	53.02 cdefg	44.18 ghij	27.02 lm	**47.49 b**
Early spring	62.81 abc	55.60 bcdef	39.92 hijk	34.48 jklm	**48.20 b**
Late spring	71.05 a	58.90 bcd	50.53 defg	57.06 bcde	**59.39 a**
Mean (N)	**62.37 a**	**52.06 b**	**40.41 c**	**35.02 d**	*47.47*
LSD (0.05) NxS = 10.432					
LSD (0.05) S = 7.8005					
LSD (0.05) N = 4.6652					
CV = 15.43					

Means in the same year followed by the same letter are not signifficantly different (P=0.05)

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

These results confirmed earlier findings (Frame, 1987) showing that decreases in clover content due to fertiliser N applications were followed by a partial recovery of clover content later in the season.

RCP values showed the same trend as PCP namely the highest mean clover percentage in 2002 (47.47%) and the lowest (30.67%) in 2001 (Table 8.4).

The highest mean RCP values were recorded where no fertiliser N was applied. Residual clover percentage tends to decrease as fertiliser N rate increases. The magnitude of this decrease however differed between years and seasons of application.

The highest RCP was recorded during late spring in 2001 and 2002, a reaction anticipated because clover requires higher temperatures than ryegrass to reach maximum growth rates (Williams, 1970 cited by Frame & Newbould, 1986). RCP measured in the late spring of 2000 did not differ ($P>0.05$) from RCP in other seasons of the same year. Due to inconsistent results, the seasons of application that resulted in the lowest clover content could not be established. Differences in initial clover content, climatic conditions (high rainfall in 2001) and carryover fertiliser N might have attributed to this inconsistency.

Although a significant interaction between fertiliser N rate and season of application was recorded in 2001 and 2002, an increase in fertiliser N rate resulted in decreases in clover content in all seasons. The only exception was a non-significant increase in clover content between 100 and 150 kg N ha^{-1} applied in late spring in both years. The application of 50 kg N ha^{-1} in 2001 resulted in a decrease ($P=0.05$) in clover content relative to treatments receiving no fertiliser N. Similar results were obtained in winter (early and late) and late spring in 2002. The only significant ($P=0.05$) difference in clover content found between the 100 and 150 kg N ha^{-1} application rates were during late winter in both years. The highest clover contents in 2001 and 2002 tend to be where 0 or 50 kg N ha^{-1} were applied in late winter or spring.

The percentage clover of treatments that received fertiliser N was always less than that of the 0 kg N ha^{-1} treatments emphasising the suppressive effect of fertiliser N on clover relative to grass production. Whether this suppressive effect is direct, by causing a reduction in clover growth or indirect, through increased competitiveness of the ryegrass fraction is still to be determined. Results from the glasshouse studies however revealed that fertiliser N applications of up to 180 kg N ha^{-1} did not suppress clover production (Chapter 3). The measured reduction in clover percentage (percentage points) after fertiliser N application during the different seasons at the Institute for Plant Production Elsenburg is summarised in Table 8.5. The application of fertiliser N will result in the least reduction of clover percentage if applied in autumn and early winter. The highest reductions in clover content were observed with fertiliser N applications in spring (2000 and 2002) and late winter, early spring 2001. Increased fertiliser N rate resulted in increased suppression of the clover content. This clearly shows the negative effects of injudicious application of fertiliser N on the clover content of pastures.

The recovery of white clover following fertiliser N application depends both on the fertiliser N rate and season of application (Figure 8.1).

Increasing the fertiliser N rate to 150 kg N ha^{-1} resulted in increasingly lower clover contents however in the study even the 150 kg N ha^{-1} recovered to levels comparable to the 0 kg N ha^{-1} levels although recovery time between fertiliser rates differed. After recovery the clover percentage of the treatments that received fertiliser N tend to be higher than the 0 kg N ha^{-1} treatments.

The recovery period for the 50 and 100 kg N ha^{-1} treatments to the same clover percentages as at the 0 kg N ha^{-1} treatments, especially in autumn, early and late winter tend to be shorter than at the 150 kg N ha^{-1} treatments. It is however important to realise that the recovery of clover to the same levels as at the 0 kg N ha^{-1} treatments may extent over months after N application. The consequences of injudicious application of fertiliser N can be eminent over several months after fertiliser N application.

Decreases, relative to 0 kg N ha^{-1}, in PCP as a result of fertiliser N application in the current study tends to be highest in spring and lowest in autumn and early winter (Table 8.5).

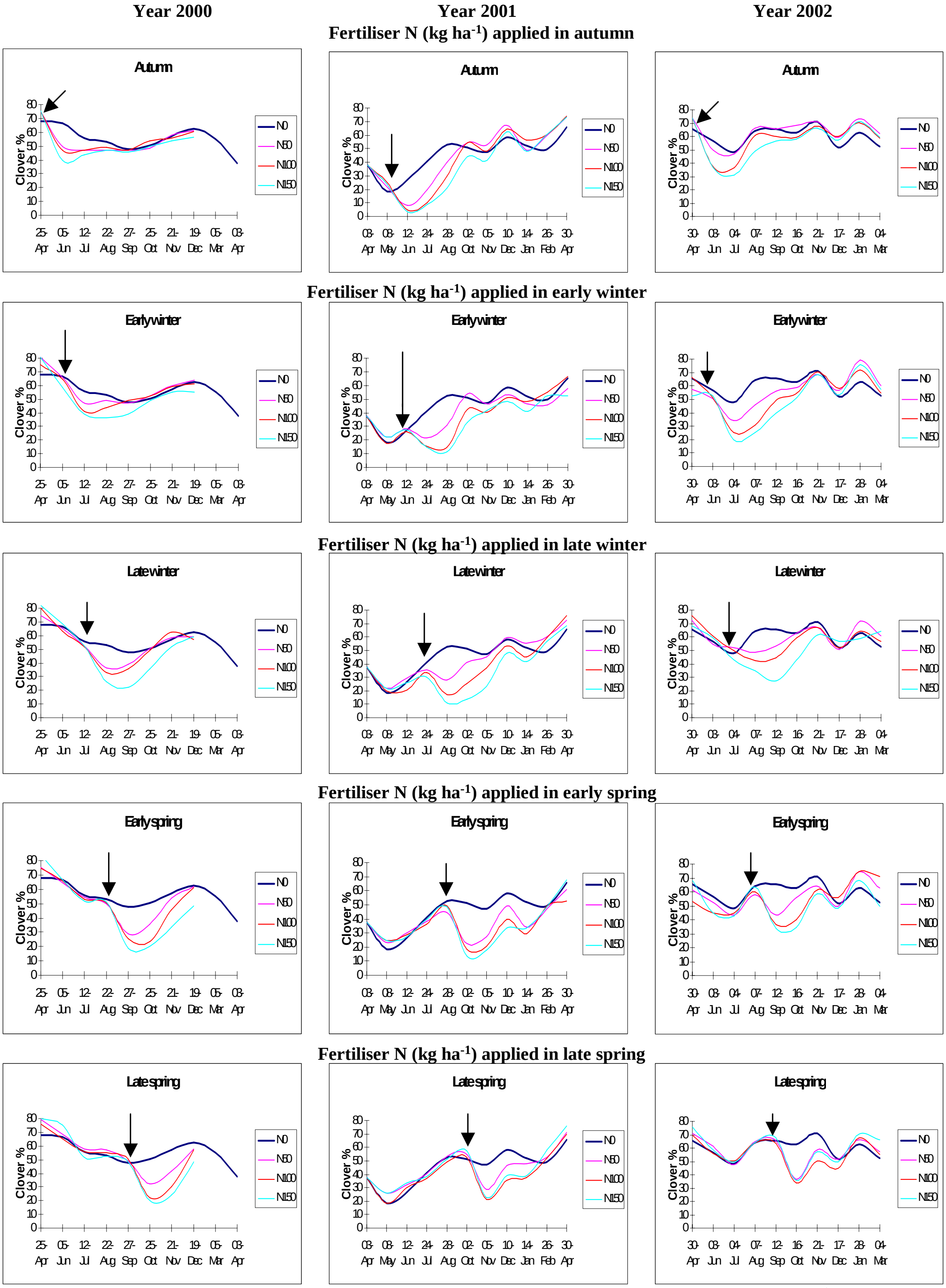

Figure 8.1 Long term effect of fertiliser N application rate (0, 50, 100 and 150 kg N ha^{-1}) and season of application on the percentage clover in a perennial ryegrass-white clover pasture at Elsenburg (Fertiliser N application : ⟶)

Table 8.5 Decrease in clover content (percentage points) in a perennial ryegrass-white clover pasture as a result of fertiliser N application during different seasons at Elsenburg during the period 2000 – 2002

Season of application	**Fertiliser N rate (kg N ha^{-1})** 50	100	150	Mean (S)
Year 2000				
Autumn	16.68	19.39	26.81	**20.96**
Early winter	8.60	14.41	16.37	**13.13**
Late winter	16.67	20.25	26.60	**21.17**
Early spring	18.34	26.22	28.36	**24.31**
Late spring	18.18	28.39	30.43	**25.67**
Mean (N)	**15.69**	**21.73**	**25.71**	***21.05***
Year 2001				
Autumn	18.91	22.10	23.33	**21.45**
Early winter	19.18	25.54	25.88	**23.53**
Late winter	24.60	35.35	41.47	**33.81**
Early spring	28.34	32.93	37.74	**33.00**
Late spring	18.41	26.03	24.76	**23.07**
Mean (N)	**21.89**	**28.39**	**30.63**	***26.97***
Year 2002				
Autumn	6.77	19.51	19.92	**15.40**
Early winter	13.57	22.87	28.44	**21.63**
Late winter	15.89	22.10	29.44	**22.48**
Early spring	22.33	29.33	31.90	**27.85**
Late spring	26.35	29.17	27.27	**27.60**
Mean (N)	**16.98**	**24.60**	**27.39**	***22.99***

No statistical analysis was done on this data as analysis was done on clover percentage.

Absolute clover content (ACC)

Many studies compared annual or seasonal yields of clover in a mixed sward given fertiliser N with those given none to demonstrate the adverse effects of N on the legume (Chestnutt & Lowe, 1970; Frame & Newbould, 1986). Not much attention was given to the absolute clover content (ACC) and whether it was increasing, decreasing or remaining unchanged. ACC therefore relates to the ability of the clover component to maintain productivity levels, irrespective of the influence of the companion ryegrass component, under the conditions that prevailed during the years covered by the study. The highest mean ACC of 1432.57 kg ha^{-1} was recorded in 2002, followed by 1210.3 kg ha^{-1} in 2000 and the lowest of 728.08 kg ha^{-1} in 2001 (Table 8.6).

Table 8.6 Accumulative (absolute) white clover DM production (kg DM ha^{-1}) over a ten week period following fertiliser N application in a perennial ryegrass-white clover pasture at Elsenburg

	Year 2000				
	Fertiliser N rate (kg N ha^{-1})				
Time of application	**0**	**50**	**100**	**150**	**Mean (S)**
Autumn	1837.0	1604.3	1622.8	1557.8	**1655.5 a**
Early winter	1108.9	1018.6	1015.1	1027.2	**1042.4 b**
Late winter	1264.6	1122.7	1129.1	905.1	**1105.4 b**
Early spring	1430.1	1176.1	939.0	843.6	**1097.2 b**
Late spring	1548.1	1191.3	885.5	979.0	**1151.0 b**
Mean (N)	**1437.73 a**	**1222.60 b**	**1118.31 bc**	**1062.52 c**	***1210.3***
LSD (0.05) NxS = NS					
LSD (0.05) S = 250.32					
LSD (0.05) N = 136.65					
CV = 17.73					
	Year 2001				
Autumn	358.3 ghi*	224.3 hi	163.0 i	160.4 i	**226.49 e**
Early winter	505.1 efg	517.7 efg	422.7 fgh	405.4 fgh	**462.73 d**
Late winter	1039.3 bc	960.6 bc	680.2 de	480.3 efg	**790.07 c**
Early spring	1555.8 a	921.6 bc	809.9 cd	625.9 def	**978.28 b**
Late spring	1464.0 a	1135.3 b	1024.8 bc	1107.1 b	**1182.82 a**
Mean (N)	**984.51 a**	**751.89 b**	**620.12 c**	**555.81 c**	***728.08***
LSD (0.05) NxS = 238.98					
LSD (0.05) S = 147.56					
LSD (0.05) N = 106.87					
CV = 23.05					
	Year 2002				
Autumn	1064.9 f	1057.5 f	886.1 fgh	949.5 fg	**989.49 c**
Early winter	676.9 ghi	615.6 hi	529.7 i	522.2 i	**586.09 d**
Late winter	1609.6 de	1491.0 e	1429.1 e	1092.4 f	**1405.53 b**
Early spring	2566.0 a	2231.7 b	1822.0 cd	-	**2085.55 a**
Late spring	2675.9 a	1886.7 cd	1715.4 de	2106.7 bc	**2096.18 a**
Mean (N)	**1718.66 a**	**1456.48 b**	**1276.46 c**	**1278.67 c**	***1432.57***
LSD (0.05) NxS = 297.52					
LSD (0.05) S = 156.35					
LSD (0.05) N = 133.05					
CV = 14.58					

****Means in the same year followed by the same letter are not signifficantly different (P=0.05)***

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

The extremely low ACC values in 2001 may be the result of the adverse environmental conditions as discussed earlier. The relative low soil K content (Table 5.1) could also contributed to the lower clover productivity as clover roots have lower cation exchange capacities than grasses resulting in clover experiencing K deficiencies at levels still sufficient to maintain high grass production (Havlin *et al*., 1999).

Increased fertiliser N rates resulted in increasingly lower mean ACC values during all three years covered by the study. Mean ACC values decreased as fertiliser N rate increased with no differences ($P>0.05$) in mean ACC values recorded between the 100 and 150 kg N ha^{-1} application rates. Treatments that did not receive fertiliser N resulted in the highest ($P=0.05$) ACC values.

The ACC values in 2001 and 2002 showed a slight increase from late autumn to spring with no trend visible in 2000.

A significant interaction between fertiliser N application and season of application was noted in 2001 and 2002. Application of fertiliser N in autumn and early winter in 2001 and 2002 did not significantly reduced clover DM content (Table 8.6). Soil temperatures during the cooler months remained between 9 and 13 °C for a considerable time (Table 6.1). These low temperatures could result in a reduction in clover activity (MacDuff & Dhanoa, 1990; Hatch & MacDuff, 1991) and therefore reduce the magnitude of the negative effect of fertiliser N, if any, on clover productivity. The reversed situation occurred during early and late spring where all fertiliser N rates tend to cause significant reductions in ACC, confirming that clover DM production was negatively affected as a result of the stimulation of ryegrass productivity following fertiliser N application in spring. These results suggest that the application of increased fertiliser rates applied in autumn and early winter will probably not reduce clover DM content, but rather stimulate grass productivity (higher N resulted in lower clover percentage) and therefore resulted in less pronounced changes to absolute clover content. Early and late spring fertiliser N applications may however tend to reduce ACC. These results are similar to those reported by Ball, Molloy & Ross (1978). Late winter applications of 150 kg N ha^{-1} reduced ($P=0.05$) ACC relative to treatments receiving no fertiliser N in both 2001 and 2002.

In general, clover content increased as temperatures increased (Tables 8.1 & 8.2) during late winter and early spring in treatments that received no fertiliser N. The increased clover fraction might be either the result of higher temperatures that stimulate clover growth (Davidson & Robson, 1990) and symbiotic N fixation (Russell, 1988) and/or a reduction in ryegrass production because of low nitrogen availability to the ryegrass fraction, due to a lack in return of excreta by grazing animals. Increased N mineralisation with rising temperatures will increase supply of N to ryegrass but it may be too low to maximise ryegrass growth.

Mean decreases over seasons of application, in ACC relative to 0 kg N ha^{-1} were 4.73, 3.75 and 2.39 kg DM kg^{-1} N applied for 50, 100 and 150 kg N ha^{-1} respectively. Mean decreases in clover content over all fertiliser N rates were 1.92, 0.92, 2.57, 6.83 and 6.68 kg DM kg^{-1} N applied in autumn, early-, late winter, early- and late spring. These results supported those of Frame & Boyd (1987) who reported decreases in absolute white clover content of 16.0, 7.6 and 6.3 kg DM if 25, 50 and 75 kg N were applied per hectare compared to 0 kg N ha^{-1}. In the same study the white clover production declined by 0.6 and 9.1 kg DM for every kg N ha^{-1} applied in autumn and in spring respectively.

The increase in mean clover content in 2002 as compared to 2001 in this study is in accordance with results reported by several authors. Davidson & Robson (1990) reported a 5% increase in clover content where the equivalent of 80 kg N ha^{-1} was applied and 29% where no N was applied to ryegrass-clover mixtures grown under controlled conditions. Frame & Boyd (1987) however stress the point that white clover under grazing is more subjected to competition from grasses and grazed pastures are likely to have lower clover content compared to mowed pastures. In addition, under permanent grazing conditions clover content tends to decrease (Schils, Vellinga & Kraak, 1999) due to the increased supply of N through decomposition of trampled forage and recycling of N by the grazing animal. Frame & Peterson (1987) stated that if the soil N becomes depleted due to continuous crop removal (silage) the clover performance would be sustained or even improved. The N made available through recycling seems to benefit the ryegrass to the disadvantage of the clover component. In this study the lack of re-circulation could cause sub optimum ryegrass leaf N contents, especially during spring. This might give ryegrass a competitive disadvantage to clover. Schils, Vellinga & Kraak (1999) reported a 12% higher mean clover percentage when the pasture was mowed and forage removed compared to rotational grazing plus mowing. The same study showed a 6% reduction in clover percentage where 50 kg N ha^{-1} was applied as compared to no fertiliser N application.

Conclusion

Increasing fertiliser N application rate resulted in decreases in pasture clover percentage. The effect of season of application was inconsistent and was influenced by climatic and environmental conditions such as rainfall and pasture productivity.

This study showed that in years when moisture stress due to drought or waterlogging do not occur, 50 kg N ha^{-1} did not reduce the clover content to less than 30% during any season if an initial clover content of at least 47% was present before fertiliser N application. The application of 100 and 150 kg N ha^{-1} resulted in lower clover contents than the 50 kg N ha^{-1} treatments, but at various treatment combinations remained well above the minimum of 30% clover. The application of 150 kg N ha^{-1} in the current study resulted in numerous treatment combinations to have clover percentages of less than 30%, the minimum required to maintain the benefit of the clover in the pasture. Winter applications of 150 kg N ha^{-1} resulted in clover percentages to decrease to less than 30 percent possibly as clover percentages were naturally low in winter. Initial clover content of the pasture, the season when N application is planned and pasture productivity will dictate the fertiliser N rate to be applied.

The application of fertiliser N to a pasture exposed to adverse environmental conditions that resulted in tiller and stolon deaths is not recommended because studies done under controlled conditions (Chapters 2 & 3) showed that only ryegrass will benefit through the production of daughter tillers.

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Chapter 9

The effects of strategic fertiliser nitrogen application during the cool season on perennial ryegrass-white clover pastures in the Western Cape Province

4. Selected nutritive characteristics and mineral content

Abstract

The influence of a single application of fertiliser N (0, 50, 100 and 150 kg N ha^{-1}) applied in either autumn, early winter, late winter, early spring or late spring on selected nutritive and mineral parameters of a perennial ryegrass-white clover pasture was investigated over a three year period. Responses were measured over one regrowth cycle five weeks after the application of N treatments. A sample of the plant material harvested was separated into grass and clover fractions. Dry matter (DM), crude protein (CP), in vitro organic matter digestibility (IVOMD), phosphorus- (P) and calcium (Ca) content were determined for each sample.

Winter applications of fertiliser N, in combination with the higher N rates, resulted in higher ryegrass CP contents. Clover CP content was decreased (P=0.05) with spring (early and late) applications, but fertiliser N rate did not appear to affect clover CP content. The highest IVOMD values for both ryegrass and clover were recorded in winter. Increased fertiliser N rate resulted in lower DM conents with the lowest DM produced during winter, while the highest DM contents were produced where no fertiliser N was applied in spring.

P content was significantly influenced by season of application and fertiliser N rate in ryegrass and only by season of application in clover. The highest mineral concentrations were found in winter. N recovery (kg N ha^{-1} in forage) was the lowest during early winter and the highest during autumn, late winter and early spring.

The rate and season of fertiliser N application as used in this study did not negatively affect pasture mineral content and quality, but care must be taken to protect the grazing animal from the potential negative effects of high N on animal health.

Keywords: calcium, crude protein, dry matter content, in vitro organic matter digestibility, nitrogen, phosphorus,

Introduction

The use of fertiliser N to increase pasture productivity when environmental conditions restrict nitrogen absorption and availability to perennial ryegrass-white clover pastures has been widely studied in Australia and New Zealand. This research showed that fertiliser N applications not only resulted in increased dry matter production (Ball, Molloy & Ross, 1978; McKenzie, Ryan, Jacobs & Kearney, 1999; Stout, Weaver & Elwinger, 2001), but also influenced the sensitive grass-clover balance (Ball *et al.*, 1978; Laidlaw, 1984) and fodder quality as well as mineral content (Frame, 1987; McKenzie, Jacobs & Kearney, 2003).

The nutritive characteristics of a pasture are influenced by environmental conditions including soil N supply and the season of pasture utilization. McKenzie *et al*. (1999) reported an improvement in the nutritive value of a perennial ryegrass-white clover pasture, receiving fertiliser N in autumn to mid winter by increasing metabolisable energy (ME) and crude protein (CP) and decreasing neutral detergent fiber (NDF).

Increasing rates of fertiliser N normally result in increased herbage crude protein content (Ball *et al*., 1978; McKenzie *et al*., 2003). High rates of fertiliser N application can also lead to high levels of soil N resulting in luxury uptake of N by plants. These high herbage N levels are potentially toxic to ruminants (Eckard, 1990). Nitrogen levels higher than 4% of total dry matter produced, exceed plant as well as animal nutrition requirements, can cause ammonia-induced bloat as well as ammonia- and nitrate toxicity especially where hungry or unadapted ruminants are allowed to graze on moisture stressed pastures (Eckard & Dugmore, 1993). Delaying grazing to a minimum of 21 days after fertiliser N application will reduce the possibility of nitrate toxicity as most nitrate would have been incorporated as part of the protein component of the plant cell (Meissner, Zacharias & O'Reagain, 2000).

The in vitro organic matter digestibility (IVOMD) of clover is generally higher than that of grasses (Botha, 2003) suggesting that increased clover content will result in higher IVOMD values. The expected decrease in cell wall thickness as fertiliser N rate increases can also result in increased IVOMD (Wilman & Martins, 1977; Frame & Boyd 1987). In vitro organic matter digestibility decreases as harvesting date is postponed and the pasture left to mature (Frame, 1987).

The application of fertiliser N can decrease dry matter content and as a result dry matter intake by the grazing animal (Meissner *et al*., 2000), causing a restriction in the intake of plant nutrients that eventually results in lower animal production potential per unit area.

Data supplied by the NRC (1989) recommend a pasture with a Ca and P content of 5.4 and 3.4 g kg^{-1} DM respectively, to satisfy the needs of a 600 kg diary cow producing 23 kg milk day^{-1}. Prevention of Ca:P disorders can be achieved by keeping the Ca:P ratio larger than 1:1 (Miles, De Villiers & Dugmore, 1995) and as close as possible to 1.59:1 as recommended by the NRC (1989).

The objective of this study was to determine the influence of different rates of fertiliser N, applied as a single strategic application during the cool season from autumn to late spring, on selected herbage quality and mineral parameters after 5 weeks of regrowth following fertiliser N application.

Materials and methods

General procedures.

A field experiment was conducted during the period 2000-2002 at the Institute for Plant Production, Elsenburg to determine the effect of a single application of four fertiliser N rates (0, 50, 100 and 150 kg N ha^{-1}) applied annually in either autumn, early winter, late winter, early spring or late spring on soil inorganic nitrogen content, pasture composition and quality as well as dry matter production of a perennial rye grass-white clover pasture. Details on the experimental site, experimental design, lay-out, treatments and data analyses are presented in Chapter 5.

Quality and mineral content

Fresh herbage samples of approximately 300 g wet material were collected from each treatment combination at harvesting five weeks after the application of N treatments. The samples were separated into clover and grass fractions, dried for 72 h at 60°C and dry weight recorded. Dry matter production (DMP) (kg ha^{-1}) was calculated for each treatment plot harvested. Dried clover and grass were ground to pass through a 1 mm mesh screen and analysed according to AOAC (1970) standards as described by Anon (1998) for crude protein (CP), in vitro organic matter digestibility (IVOMD), phosphorus (P) and calcium (Ca) content. Crude protein was calculated as Dumas-N x 6.25. Dry matter content (DM) of the herbage on offer was calculated as the difference between the fresh and dry weight of the sample and expressed as a percentage.

Herbage CP content was calculated using the following method:

$$\text{Herbage CP (\%)} = ((DMP_{ryegrass} * CP\%_{ryegrass}) + (DMP_{clover} * CP\%_{clover})) / (DMP_{pasture}) * 100$$

Herbage N yields were calculated as:

$$\text{Herbage N yield (kg N ha}^{-1}) = (\text{Herbage CP\%} / 6.25) * DMP$$

Results and Discussion

Partial ANOVA indicating levels of significance between treatment combinations are summarised in Table 9.1.

Table 9.1 Partial ANOVA illustrating the levels of significance between treatment combinations, rate and season of fertiliser N applications, on selected quality and mineral parameters of a perennial ryegrass-white clover pasture at the Institute for Plant Production, Elsenburg

	Perennial Ryegrass					White clover				Pasture			
	N yield	**C P**	**OMD**	**P**	**Ca**	**N yield**	**C P**	**OMD**	**P**	**N Yield**	**Ca**	**DM**	**Apparent N recovery**
Year 2000													
Season of application	*	***	**	***	***	*	***	***	***	*	***	***	*
N	*	***	NS	***	NS	*	*	NS	NS	*	NS	***	*
Season x N	**NS**	**	NS	NS	NS	NS	NS	NS	NS	NS	NS	**	*
Year 2001													
Season of application	*	***	***	**	NS	*	***	***	**	*	NS	***	*
N	*	***	NS	***	**	NS	**	NS	NS	*	NS	***	*
Season x N	*	***	NS	NS	NS	NS	NS	NS	NS	*	NS	**	NS
Year 2002													
Season of application	*	***	**	**	***	*	***	***	***	*	**	***	*
N	*	***	NS	***	*	*	**	NS	NS	*	NS	***	*
Season x N	**NS**	**	NS	***	NS	*	NS	NS	NS	NS	NS	**	*

NS. Not significant; *P<0.05; **P<0.01; ***P<0.0001

Pasture Dry Matter Content (DM)

Mean DM contents of the pasture were similar among years with the highest DM content recorded in 2001 (14.455%) followed by 13.214% in 2002 and 13.176% in 2000 (Table 9.2). The slightly higher DM content in 2001 may be the result of a reduction in clover productivity during wet conditions as abnormal high rainfall was experienced during 2001 (Figure 5.4). Increased fertiliser N rates tend to result in decreased pasture DM content. Spring applications of fertiliser N tend to increase DM content in 2000 and 2001 however 2002 was characterised by the highest (P=0.05) DM content occurring during late winter.

A significant interaction between fertiliser N rate and season of application on DM content was noted for all years covered by the study due to differences in response to nitrogen when applied in autumn, winter or spring (Table 9.2). The highest DM contents tend to occur in late spring where no fertiliser N was applied and the lowest in autumn and winter where 100 or 150 kg N ha^{-1} were applied. The higher DM in spring is possibly the result of faster growth rates where plants reached maturity sooner than under conditions characterised by slow growth rates. The low (11 to 12%) DM content may reduce animal productivity as a result of low DM intake.

Crude protein (CP)

Pasture

Crude protein content of the pasture exceeded 15% in all treatment combinations except for late spring applications of 0 and 50 kg N ha^{-1} in 2001 (Table 9.3). General recommendations are that forage with a CP content of >15% will maintain high livestock production on grazed pastures (NRC, 2001). Animals may however, still respond to additional protein if a large percentage of the crude protein is in the form of non-protein constituents, a fraction not determined in this study (Meissner *et al*., 2000).

Table 9.2 Dry matter (DM) content (%) of a perennial ryegrass-white clover pasture as influenced by fertiliser N rate (kg N ha^{-1}) and fertiliser N application at Elsenburg

	Year 2000				
	Fertiliser N rate (kg ha^{-1})				
Season of application	**0**	**50**	**100**	**150**	**Mean (S)**
Autumn	13.248 cde*	11.788 h	11.883 h	11.868 h	**11.9888 b****
Early winter	12.968 def	12.663 efg	12.005 gh	12.310 fgh	**12.4863 b**
Late winter	13.153 cde	12.390 fgh	12.273 fgh	12.053 gh	**12.4669 b**
Early spring	14.988 b	14.553 b	13.533 cd	13.735 c	**14.2019 a**
Late spring	15.730 a	15.158 ab	14.518 b	13.328 cde	**14.7356 a**
Mean (N)	**13.893 a**	**13.31 b**	**12.842 c**	**12.6585 c**	***13.176***
LSD (0.05) NxS = 0.7354					
LSD (0.05) S means = 0.6796					
LSD (0.05) N means = 0.3278					
CV =3.90					
	Year 2001				
Autumn	17.202 a	15.200 cd	12.933 hij	12.858 ijk	**14.5138 b**
Early winter	15.938 bc	13.935 fgh	12.520 jk	11.843 k	**13.5588 c**
Late winter	15.313 cd	13.795 fghi	12.860 ijk	12.548 jk	**13.6288 c**
Early spring	14.993 cde	15.000 cde	14.153 efg	13.608 ghi	**14.4381 b**
Late spring	17.640 a	16.755 ab	15.378 cd	14.743 def	**16.1356 a**
Mean (N)	**16.195 a**	**14.937 b**	**13.5685 c**	**13.1195 c**	***14.455***
LSD (0.05) NxS = 1.0361					
LSD (0.05) S means = 0.7556					
LSD (0.05) N means = 0.4618					
CV = 5.01					
	Year 2002				
Autumn	13.060 efg	11.650 j	11.533 j	12.155 ij	**12.025 c**
Early winter	14.620 bc	13.535 def	12.848 fghi	12.923 fgh	**13.4813 b**
Late winter	16.288 a	15.098 b	14.695 bc	14.023 cd	**15.0256 a**
Early spring	12.688 ghi	12.840 fghi	12.208 hij	11.840 j	**12.3938 c**
Late spring	13.527 def	13.783 de	12.815 fghi	12.275 hij	**13.145 b**
Mean (N)	**14.013 a**	**13.381 b**	**12.8195 c**	**12.643 c**	***13.214***
LSD (0.05) NxS = 0.7634					
LSD (0.05) S means = 0.6239					
LSD (0.05) N means = 0.3403					
CV = 4.04					

Means in the same year followed by the same letter are not signifficantly different (P=0.05)

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

Table 9.3 Crude protein (CP) content (%) of a perennial ryegrass-white clover pasture as influenced by fertiliser N rate (kg N ha^{-1}) and season of fertiliser N application at Elsenburg

	Year 2000				
	Nitrogen (kg ha^{-1})				
Season of application	**0**	**50**	**100**	**150**	**Mean (S)**
Autumn	29.125 bc*	25.263 ef	26.945 de	28.203 cd	**27.384 b****
Early winter	28.890 bc	29.975 ab	30.320 ab	31.308 a	**30.123 a**
Late winter	25.545 ef	24.125 f	24.575 f	24.690 f	**24.734 c**
Early spring	21.993 g	19.695 hi	20.115 hi	20.770 gh	**20.643 d**
Late spring	21.088 gh	18.543 i	18.810 i	20.720 gh	**19.873 d**
Mean (N)	**25.328 a**	**23.782 b**	**24.153 b**	**25.138 a**	***24.61***
LSD (0.05) NxS = 1.7574					
LSD (0.05) S means = 1.4524					
LSD (0.05) N means = 0.7846					
CV = 4.97					
	Year 2001				
Autumn	21.115 e	19.108 f	21.390 e	23.625 bc	**21.309 b**
Early winter	23.503 c	23.595 bc	24.700 b	26.200 a	**24.364 a**
Late winter	22.810 cd	21.008 e	21.870 de	22.025 de	**21.914 b**
Early spring	19.360 f	16.355 gh	17.033 g	17.275 g	**17.506 c**
Late spring	15.250 hi	14.150 i	14.555 i	16.848 g	**15.201 d**
Mean (N)	**20.408 a**	**18.843 c**	**19.657 b**	**20.802 a**	***19.90***
LSD (0.05) NxS = 1.1145					
LSD (0.05) S means = 0.645					
LSD (0.05) N means = 0.4924					
CV = 3.77					
	Year 2002				
Autumn	24.443 de	23.408 ef	24.550 de	25.310 bcd	**24.428 c**
Early winter	25.758 bcd	26.158 bc	28.303 a	29.230 a	**27.362 a**
Late winter	25.325 bcd	25.015 cd	26.475 b	28.043 a	**26.214 b**
Early spring	22.700 fg	20.270 ij	20.480 hij	21.867 gh	**21.329 d**
Late spring	19.343 jk	16.865 l	18.605 k	21.186 hi	**19.000 e**
Mean (N)	**23.556 b**	**22.452 c**	**23.851 b**	**25.299 a**	***23.790***
LSD (0.05) NxS = 1.395					
LSD (0.05) S means = 0.7897					
LSD (0.05) N means = 0.6199					
CV = 3.98					

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

Perennial ryegrass

Mean ryegrass CP content varied between 22.24% in 2000, 18.69% in 2001 and 21.72% in 2002 (Table 9.4).

Table 9.4 Perennial ryegrass crude protein (CP) content (%) as influenced by fertiliser N rate (kg N ha^{-1}) and season of fertiliser N application at Elsenburg

	Year 2000				
	Fertiliser N rate (kg ha^{-1})				
Season of application	**0**	**50**	**100**	**150**	**Mean (S)**
Autumn	25.588 ef*	23.730 gh	25.887 de	26.893 cd	**25.626 b****
Early winter	24.483 fg	27.195 bc	28.213 b	30.025 a	**27.479 a**
Late winter	21.593 ij	21.905 i	22.690 hi	23.688 gh	**22.469 c**
Early spring	17.083 n	16.993 n	18.633 ml	20.578 jk	**18.321 d**
Late spring	16.258 no	15.688 o	17.505 mn	19.728 kl	**17.294 e**
Mean (N)	**20.999 c**	**20.963 c**	**22.412 b**	**24.181 a**	***22.24***
LSD (0.05) NxS = 1.2631					
LSD (0.05) S means = 0.7326					
LSD (0.05) N means = 0.5628					
CV = 3.93					
	Year 2001				
Autumn	18.750 ef	18.590 f	21.253 d	23.568 b	**20.540 b**
Early winter	19.833 e	22.560 bc	23.598 b	25.710 a	**22.925 a**
Late winter	19.483 ef	19.585 ef	21.385 cd	22.280 cd	**20.683 b**
Early spring	15.978 g	14.473 h	15.973 g	16.525 g	**15.737 c**
Late spring	12.330 i	12.315 i	13.488 hi	16.095 g	**13.557 d**
Mean (N)	**17.275 c**	**17.505 c**	**19.139 b**	**20.836 a**	***18.69***
LSD (0.05) NxS = 1.1894					
LSD (0.05) S means = 0.5958					
LSD (0.05) N means = 0.5319					
CV = 4.47					
	Year 2002				
Autumn	20.863 f	21.000 f	23.225 cde	24.340 bcd	**22.357 c**
Early winter	22.205 ef	24.870 bc	27.578 a	28.998 a	**25.913 a**
Late winter	20.603 f	22.918 de	25.350 b	27.528 a	**24.100 b**
Early spring	18.370 g	17.587 g	18.837 g	21.513 ef	**19.077 d**
Late spring	15.545 h	14.868 h	17.630 g	20.658 f	**17.175 e**
Mean (N)	**19.577 d**	**20.388 c**	**22.718 b**	**24.770 a**	***21.72***
LSD (0.05) NxS = 1.7122					
LSD (0.05) S means = 0.6392					
LSD (0.05) N means = 0.7607					
CV = 5.31					

Means in the same year followed by the same letter are not signifficantly different (P=0.05)

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

The lower CP content in 2001 is indicative of low fertiliser N availability as a result of leaching and/or the inability of ryegrass to absorb N in soil with moisture contents near saturation point (Russell, 1988) as very high rainfall was experienced in this year.

Increasing fertiliser N rates from 50 to 150 kg N ha^{-1} resulted in significant increases in mean CP content in all years covered by the study. Similar observations were reported by Eckard (1994) and Wilkins, Allen & Mytton (2000). No differences (P>0.05) in CP % between the 0 and 50 kg N ha^{-1} application rates were found in 2000 and 2001. However in 2002 the CP content of forage from the 50 kg N ha^{-1} application rate was higher (P=0.05) than from the 0 kg N ha^{-1} treatment.

The highest mean CP content was recorded where fertiliser N was applied in early winter and gradually decreased to a low in early and late spring.

A significant interaction between season - and rate of fertiliser N application was noted for all three years covered by the study due to the much larger response to nitrogen when applied in early winter compared to other seasons. On average the highest CP contents were recorded where 150 kg N ha^{-1} was applied in early winter and the lowest where 50 kg N ha^{-1} was applied in late spring. The crude protein levels at early and late spring applications of 50 and 100 kg N ha^{-1} tend to be low, but not deficient (Smith, 1986; Reuter & Robinson, 1997).

An important consideration with interpretation of this data set is that the pasture was only grazed in summer and all herbage was removed during the cutting cycle (April-November). The lack in return of N through excreta and mineralisation of trampled organic matter due to the said management may have resulted in a gradual decrease in nitrogen supply to the ryegrass as illustrated by the 0 kg N ha^{-1} treatments (Table 9.4).

White clover

Mean clover CP levels showed the same trend as ryegrass namely a lower CP content in 2001 (23.90 %) compared to 2000 (28.52%) and 2002 (25.88 %) (Table 9.5).

Table 9.5 White clover crude protein (CP) content (%) as influenced by fertiliser N rate (kg N ha^{-1}) and season of fertiliser N application at Elsenburg

	Fertiliser N rate (kg ha^{-1})				
Season of application	**0**	**50**	**100**	**150**	**Mean (S)**
Year 2000					
Autumn	30.955	30.373	30.235	30.188	**30.438 b****
Early winter	32.265	33.155	33.290	33.468	**33.044 a**
Late winter	29.093	27.978	28.423	27.418	**28.228 c**
Early spring	27.555	26.098	25.613	25.667	**26.271 d**
Late spring	25.848	24.397	23.435	24.728	**24.615 e**
Mean (N)	**29.143 a**	**28.611 ab**	**28.199 b**	**28.432 b**	***28.52***
LSD (0.05) NxS = NS					
LSD (0.05) S means = 1.2331					
LSD (0.05) N means = 0.6735					
CV = 3.65					
Year 2001					
Autumn	27.388	25.138	24.550	25.218	**25.573 b**
Early winter	28.883	27.345	28.107	27.487	**27.978 a**
Late winter	25.768	24.540	24.130	24.655	**24.790 c**
Early spring	22.640	22.810	21.940	22.173	**22.391 d**
Late spring	18.520	18.659	18.568	19.408	**18.788 e**
Mean (N)	**24.640 a**	**23.698 b**	**23.214 b**	**23.468 b**	***23.90***
LSD (0.05) NxS = NS					
LSD (0.05) S means = 0.7386					
LSD (0.05) N means = 0.6043					
CV = 3.87					
Year 2002					
Autumn	27.300	25.878	26.808	27.063	**26.762 c**
Early winter	29.643	28.790	29.973	30.230	**29.659 a**
Late winter	27.965	27.265	28.085	28.740	**28.014 b**
Early spring	24.803	24.063	23.570	22.823	**23.815 d**
Late spring	21.598	20.445	20.318	22.305	**21.166 e**
Mean (N)	**26.338 a**	**25.353 b**	**25.865 ab**	**26.412 a**	***25.88***
LSD (0.05) NxS = NS					
LSD (0.05) S means = 0.8053					
LSD (0.05) N means = 0.584					
CV = 3.43					

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

The effect of fertiliser N was inconsistent although treatments that did not receive fertiliser N tend to have higher CP contents. The highest CP contents were recorded where fertiliser N was applied in early winter, declining to the lowest CP contents during late spring. Eckard (1994) reported that white clover N content increased marginally in late winter and declined with the onset of summer. The summer decline in N content can be ascribed to a possible dilution effect as clover growth rate increases. In contrast to ryegrass, mean clover CP content at 0 kg N ha^{-1} tended to be higher than treatments receiving fertiliser N, possibly as a result of symbiotic N fixation.

No interaction (P>0.05) was recorded between season of application and fertiliser N rate. CP content of clover receiving fertiliser N during early and late spring tended to be low (< 27.5 %). Smith (1986) recommended a CP content of 27.5 to 29.4% to maintain optimal clover productivity while Reuter & Robinson (1997) regard tissue N contents of more than 3.2 to 3.6% (20 to 22.5% CP) as adequate. Clover CP content in late spring 2001 was marginally lower than the minimum levels suggested by Reuter & Robinson (1997). The CP content of the white clover in the current study was higher than that of the companion perennial ryegrass highlighting the positive effect of clover on pasture quality.

In vitro organic matter digestibility (IVOMD)

IVOMD in both ryegrass and clover was influenced (P<0.05) only by season of fertiliser N application (Tables 9.6 & 9.7). These results supported the findings of Schils, Vellinga & Kraak, (1999) who reported no difference in IVOMD and CP where 0 and 50 kg N ha^{-1} were applied in spring. Mean IVOMD of clover tended to be higher than perennial ryegrass only in 2000.

Perennial ryegrass

Mean IVOMD varied between 74.625% in 2000, 75.601% in 2001 and 81.923% in 2002 (Table 9.6). Frame (1987) also reported an increase in IVOMD over time. The high rainfall in 2001 did not influence ryegrass IVOMD values.

Fertiliser N rate did not influence IVOMD in this study, but Valente, Borreani, Peiretti & Tabacco (2000) reported that nitrogen fertiliser may cause a slight decrease in digestibility in Italian ryegrass.

The only constant trend visible was lower IVOMD values of perennial ryegrass in late spring compared to the other seasons of fertiliser N application (Table 9.6).

Table 9.6 In vitro organic matter digestibility (IVOMD) (%) of perennial ryegrasas as influenced by fertiliser N rate (kg N ha^{-1}) and season of fertiliser N application at Elsenburg

			Year 2000		
	Fertiliser N rate (kg ha^{-1})				
Season of application	**0**	**50**	**100**	**150**	**Mean (S)**
Autumn	74.520	73.123	77.000	74.133	**74.641 b****
Early winter	76.403	77.013	77.875	78.638	**77.482 a**
Late winter	75.393	75.093	77.288	76.793	**76.141 ab**
Early spring	75.385	74.568	75.023	77.268	**75.561 ab**
Late spring	68.128	71.713	68.458	68.903	**69.300 c**
Mean (N)	**73.966**	**74.364**	**75.030**	**75.147**	***74.625***
LSD (0.05) NxS = NS					
LSD (0.05) S means = 2.7035					
LSD (0.05) N means = NS					
CV = 2.52					
			Year 2001		
Autumn	76.828	77.478	77.258	76.435	**77.000 a**
Early winter	73.835	74.925	77.510	75.523	**75.448 b**
Late winter	75.248	75.555	74.320	75.685	**75.202 b**
Early spring	77.788	77.685	77.490	76.928	**77.473 a**
Late spring	73.595	73.083	73.568	71.288	**72.883 c**
Mean (N)	**75.459**	**75.745**	**76.029**	**75.172**	***75.601***
LSD (0.05) NxS = NS					
LSD (0.05) S means = 1.1597					
LSD (0.05) N means = NS					
CV = 2.36					
			Year 2002		
Autumn	79.850	80.128	82.508	80.818	**80.826 b**
Early winter	80.738	79.625	81.755	80.953	**80.768 b**
Late winter	83.690	84.173	84.268	84.153	**84.071 a**
Early spring	82.487	84.980	84.740	85.943	**84.538 a**
Late spring	78.630	79.440	80.015	79.563	**79.412 b**
Mean (N)	**81.005**	**81.495**	**82.547**	**82.093**	***81.923***
LSD (0.05) NxS = NS					
LSD (0.05) S means = 2.2214					
LSD (0.05) N means = NS					
CV = 2.20					

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

White clover

Highest mean white clover IVOMD of 82.45% was produced in 2002, compared to 77.48% in 2000 and 75.50% in 2001 (Table 9.7).

Table 9.7 In vitro organic matter digestibility (IVOMD) (%) of white clover as influenced by fertiliser N rate (kg N ha^{-1}) and season of fertiliser N application at Elsenburg

	Year 2000				
	Fertiliser N rate (kg ha^{-1})				
Season of application	**0**	**50**	**100**	**150**	**Mean (S)**
Autumn	79.145	78.113	78.060	80.118	**78.859 a****
Early winter	78.940	80.310	79.695	79.338	**79.571 a**
Late winter	79.513	78.890	78.880	78.690	**78.993 a**
Early spring	77.018	76.020	76.855	78.010	**76.907 b**
Late spring	73.625	74.140	72.358	72.503	**73.091 c**
Mean (N)	**77.648**	**77.671**	**77.170**	**77.717**	***77.484***
LSD (0.05) NxS = NS					
LSD (0.05) S means = 1.675					
LSD (0.05) N means = NS					
CV = 1.74					
	Year 2001				
Autumn	81.325	78.197	79.925	80.720	**80.046 a**
Early winter	77.530	77.353	79.098	79.283	**78.315 b**
Late winter	70.680	72.818	72.223	NA	**71.906 d**
Early spring	76.313	76.143	75.765	75.107	**75.880 c**
Late spring	71.123	72.420	71.165	70.710	**71.354 d**
Mean (N)	**75.394**	**75.238**	**75.158**	**75.501**	***75.501***
LSD (0.05) NxS = NS					
LSD (0.05) S means = 1.4409					
LSD (0.05) N means = NS					
CV = 2.91					
	Year 2002				
Autumn	80.558	83.455	83.028	81.280	**82.080 c**
Early winter	81.257	81.155	78.840	77.810	**80.560 d**
Late winter	84.613	83.693	83.745	84.940	**84.201 b**
Early spring	86.587	86.053	85.653	88.587	**86.720 a**
Late spring	77.837	78.980	78.853	78.905	**78.697 e**
Mean (N)	**82.219**	**82.489**	**82.394**	**82.609**	***82.452***
LSD (0.05) NxS = NS					
LSD (0.05) S means = 19.426					
LSD (0.05) N means = NS					
CV = 2.14					

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

The highest IVOMD values were measured for fertiliser N application in autumn and winter of 2000, autumn of 2001 and early spring in 2002 and the lowest during late spring in all years (Table 9.7). The rapid growth rate in late spring (as a result of higher temperatures and increasing photoperiod as shown in Chapters 2 & 4) could promote early plant maturity (both ryegrass and clover) and therefore lowered the digestibility of the forage if harvesting was delayed, especially where a fixed regrowth cycle is practised (Morrison, 1980).

Calcium (Ca)

While the Ca content of all ryegrass samples (Table 9.8) can be regarded as deficient (NRC 2001), values for clover (Table 9.9) over all treatment combinations were much higher than the 0.67 % required by a dairy cow for optimum milk production (NRC 2001). The mean Ca content of the herbage (grass and legume combined), over all treatment combinations, was 0.73 % leaving few treatment combinations in short supply to sustain optimal milk production. These results further emphasise the advantage of growing perennial ryegrass and white clover as companion crops as a pasture. No interaction ($P>0.05$) between season of fertiliser N application and fertiliser N rate on Ca content was recorded (Tables 9.8 & 9.9).

Perennial ryegrass

Mean Ca contents of the ryegrass were 0.477% (2000), 0.425% (2001) and 0.396% in 2002 (Table 9.8). This decrease is not related to climatic conditions. No definite trend in the reaction of Ca content to fertiliser N rate or season of application was found, but in general the highest Ca contents were recorded during early winter. All treatments resulted in Ca levels higher than the 0.25% required for optimum plant productivity (Reuter & Robinson, 1997).

Table 9.8 Perennial ryegrass calcium (Ca) content (%) as influenced by fertiliser N rate (kg N ha^{-1}) and season of fertiliser N application at Elsenburg

Season of application	Fertiliser N rate (kg ha^{-1}) 0	50	100	150	Mean (S)
Year 2000					
Autumn	0.530	0.440	0.440	0.500	**0.483 b****
Early winter	0.535	0.585	0.588	0.620	**0.582 a**
Late winter	0.518	0.498	0.493	0.500	**0.502 b**
Early spring	0.425	0.415	0.395	0.408	**0.411 c**
Late spring	0.443	0.378	0.385	0.430	**0.409 c**
Mean (N)	**0.490**	**0.464**	**0.461**	**0.492**	*0.477*
LSD (0.05) NxS =NS					
LSD (0.05) S means = 0.0424					
LSD (0.05) N means = NS					
CV = 9.48					
Year 2001					
Autumn	0.438	0.378	0.373	0.398	**0.396**
Early winter	0.475	0.420	0.383	0.393	**0.418**
Late winter	0.430	0.423	0.455	0.450	**0.440**
Early spring	0.473	0.440	0.403	0.485	**0.450**
Late spring	0.413	0.413	0.400	0.463	**0.422**
Mean (N)	**0.446 a**	**0.415 bc**	**0.403 c**	**0.438 ab**	*0.425*
LSD (0.05) NxS = NS					
LSD (0.05) S means = NS					
LSD (0.05) N means = 0.0308					
CV = 11.37					
Year 2002					
Autumn	0.268	0.290	0.280	0.338	**0.294 c**
Early winter	0.455	0.448	0.485	0.485	**0.468 a**
Late winter	0.375	0.407	0.370	0.403	**0.389 b**
Early spring	0.440	0.407	0.400	0.433	**0.416 b**
Late spring	0.405	0.375	0.438	0.440	**0.414 b**
Mean (N)	**0.380 b**	**0.383 b**	**0.394 ab**	**0.419 a**	*0.396*
LSD (0.05) NxS = NS					
LSD (0.05) S means = 0.0504					
LSD (0.05) N means = 0.0292					
CV = 10.82					

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

White clover

Mean white clover Ca content showed the same trend as perennial ryegrass namely a gradual decrease from 2000 (1.385%) to 1.208% in 2002 (Table 9.9).

Table 9.9 White clover calcium (Ca) content (%) as influenced by fertiliser N rate (kg N ha^{-1}) and season of fertiliser N application at Elsenburg

	Year 2000				
	Fertiliser N rate (kg ha^{-1})				
Season of application	**0**	**50**	**100**	**150**	**Mean (S)**
Autumn	1.283	0.895	0.765	1.016	**0.990 c****
Early winter	1.810	1.565	1.485	1.535	**1.599 a**
Late winter	1.313	1.428	1.223	1.228	**1.298 b**
Early spring	1.763	1.658	1.573	1.540	**1.639 a**
Late spring	1.358	1.327	1.415	1.478	**1.399 b**
Mean (N)	**1.505**	**1.377**	**1.292**	**1.350**	***1.385***
LSD (0.05) NxS = NS					
LSD (0.05) S means = 0.1643					
LSD (0.05) N means = NS					
CV = 18.58					
	Year 2001				
Autumn	1.263	NA	NA	NA	**1.263**
Early winter	1.435	1.400	1.240	1.250	**1.368**
Late winter	1.380	1.290	1.207	1.100	**1.283**
Early spring	1.453	1.347	1.285	1.520	**1.406**
Late spring	1.350	1.353	0.895	1.123	**1.218**
Mean (N)	**1.382**	**1.343**	**1.153**	**1.251**	***1.308***
LSD (0.05) NxS = NS					
LSD (0.05) S means = NS					
LSD (0.05) N means = NS					
CV = 16.47					
	Year 2002				
Autumn	0.893	0.880	0.905	0.863	**0.885 c**
Early winter	1.583	1.525	1.595	NA	**1.562 a**
Late winter	1.378	1.330	1.340	1.285	**1.341 a**
Early spring	1.147	1.420	1.375	1.400	**1.317 ab**
Late spring	0.845	0.983	0.943	0.963	**0.933 bc**
Mean (N)	**1.170**	**1.211**	**1.168**	**1.025**	***1.208***
LSD (0.05) NxS = NS					
LSD (0.05) S means = 0.3944					
LSD (0.05) N means = NS					
CV = 15.94					

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

Only season of fertiliser N application influenced clover Ca content in 2000 ($P<0.05$) and 2002 ($P<0.05$). There were no definite trends except that the lowest Ca levels were observed in late winter and late spring. Autumn 2000 and 2002 and late spring 2002 resulted in Ca concentrations of between 0.9 and 1.1%, a range regarded by Reuter & Robinson (1997) as marginally deficient.

Phosphorus (P)

Most treatment combinations for ryegrass (Table 9.10) and clover (Table 9.11) resulted in higher values than the 0.38% recommended to maintain optimal dairy production (NRC, 1989). The mean calculated pasture P content of 0.50% should therefore be adequate to maintain optimal dairy production (NRC, 1989).

Perennial ryegrass

Mean P content of the ryegrass decreased from 0.516% in 2001 and 0.508% in 2002 to 0.496% in 2000 (Table 9.10).

P concentration of perennial ryegrass tends to decrease as fertiliser N rate increases. The highest ($P=0.05$) P content in all years covered by the study was recorded where no fertiliser N was applied. No differences between 50 and 100 kg N ha^{-1} were recorded in any year covered by the study. The P concentration of ryegrass was lower in the 150 kg N ha^{-1} treatment than in the 100 kg N ha^{-1} treatment only in 2001.

The effect of season of application on ryegrass P content was inconsistent with the highest ($P=0.05$) values recorded in late winter (2000) and winter as well as early spring (2001) and autumn (2002).

An interaction between treatment combinations in 2002 showed that P content decreased with increased N application rates in autumn and spring, but not in winter. The highest ($P=0.05$) P value was recorded in autumn with no fertiliser N and the lowest ($P=0.05$) in late spring where 100 and 150 kg N ha^{-1} were applied.

The P content of all treatments, excluding treatments receiving fertiliser N in early spring 2000, was higher than the minimum of 0.2 to 0.25% which can be classified as marginal deficient (Reuter & Robinson, 1997).

Table 9.10 Perennial ryegrass phosphorus (P) content (%) as influenced by fertiliser N rate (kg N ha^{-1}) and season of fertiliser N application at Elsenburg

	Year 2000				
	Fertiliser N rate (kg ha^{-1})				
Season of application	**0**	**50**	**100**	**150**	**Mean (S)**
Autumn	0.575	0.300	0.273	0.280	**0.367 c****
Early winter	0.478	0.373	0.385	0.355	**0.398 c**
Late winter	0.988	0.878	0.845	0.778	**0.872 a**
Early spring	0.213	0.173	0.170	0.165	**0.180 d**
Late spring	0.895	0.650	0.570	0.545	**0.665 b**
Mean (N)	**0.630 a**	**0.484 b**	**0.458 bc**	**0.4245 c**	*0.496*
LSD (0.05) NxS = NS					
LSD (0.05) S means = 0.1169					
LSD (0.05) N means = 0.0586					
CV = 18.17					
	Year 2001				
Autumn	0.525	0.468	0.433	0.430	**0.464 b**
Early winter	0.633	0.565	0.535	0.533	**0.566 a**
Late winter	0.598	0.585	0.575	0.548	**0.576 a**
Early spring	0.683	0.528	0.595	0.468	**0.568 a**
Late spring	0.470	0.418	0.380	0.363	**0.408 b**
Mean (N)	**0.582 a**	**0.513 b**	**0.504 b**	**0.468 c**	*0.516*
LSD (0.05) NxS = NS					
LSD (0.05) S means = 0.0922					
LSD (0.05) N means = 0.0319					
CV = 9.71					
	Year 2002				
Autumn	0.750 a*	0.643 b	0.555 c	0.495 cdef	**0.611 a**
Early winter	0.553 c	0.518 cde	0.528 cd	0.515 cde	**0.528 b**
Late winter	0.510 cdef	0.513 cde	0.528 cd	0.515 cde	**0.518 b**
Early spring	0.530 cd	0.397 gh	0.443 fg	0.453 efg	**0.449 c**
Late spring	0.530 cd	0.475 def	0.375 h	0.363 h	**0.436 c**
Mean (N)	**0.588 a**	**0.515 b**	**0.488 bc**	**0.469 c**	*0.508*
LSD (0.05) NxS = 0.0682					
LSD (0.05) S means = 0.0644					
LSD (0.05) N means = 0.0298					
CV = 8.62					

Means in the same year followed by the same letter are not signifficantly different (P=0.05)

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

White clover

Mean white clover P content varied between 0.630% in 2000, 0.417% in 2001 and 0.459% in 2002 (Table 9.11).

Table 9.11 White clover phosphorus (P) content (%) as influenced by fertiliser N rate (kg N ha^{-1}) and season of fertiliser N application at Elsenburg

	Fertiliser N rate (kg ha^{-1})				
Season of application	**0**	**50**	**100**	**150**	**Mean (S)**
Year 2000					
Autumn	1.010	0.690	0.613	0.813	**0.781 b****
Early winter	1.040	1.025	0.928	0.995	**0.997 a**
Late winter	0.828	0.700	0.745	0.725	**0.7494 b**
Early spring	0.498	0.345	0.373	0.487	**0.421 c**
Late spring	0.173	0.180	0.205	0.238	**0.200 d**
Mean (N)	**0.710**	**0.610**	**0.573**	**0.660**	***0.630***
LSD (0.05) NxS = NS					
LSD (0.05) S means = 0.1591					
LSD (0.05) N means =NS					
CV = 32.50					
Year 2001					
Autumn	0.367	NA	NA	NA	**0.367 c**
Early winter	0.490	0.523	0.590	0.515	**0.515 a**
Late winter	0.430	0.423	0.450	0.460	**0.435 b**
Early spring	0.435	0.363	0.395	0.395	**0.401 bc**
Late spring	0.333	0.353	0.390	0.407	**0.366 c**
Mean (N)	**0.413**	**0.416**	**0.439**	**0.438**	***0.417***
LSD (0.05) NxS = NS					
LSD (0.05) S means = 0.0655					
LSD (0.05) N means = NS					
CV = 13.19					
Year 2002					
Autumn	0.518	0.588	0.533	0.640	**0.569 a**
Early winter	0.508	0.515	0.510	NA	**0.511 a**
Late winter	0.388	0.387	0.503	0.460	**0.434 b**
Early spring	0.480	0.390	0.385	0.410	**0.421 b**
Late spring	0.373	0.330	0.360	0.370	**0.358 c**
Mean (N)	**0.452**	**0.448**	**0.461**	**0.488**	***0.459***
LSD (0.05) NxS = NS					
LSD (0.05) S means = 0.0628					
LSD (0.05) N means = NS					
CV = 14.14					

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

Only season of fertiliser N application influenced clover P concentration (Table 9.10). White clover P content decreased as season of fertiliser N application progressed from early winter to late spring (Table 9.11). Clover P contents for all treatments except late spring fertiliser N applications in 2000 were higher than the minimum of 0.25 to 0.3 % recommended by Reuter & Robinson (1997).

Nitrogen yields

Herbage nitrogen yields refer to the total N removed (kg ha^{-1}) by the pasture in the first regrowth cycle after fertiliser N application.

Herbage N yields

Herbage nitrogen yields (kg N ha^{-1}) are presented in Table 9.12. Mean herbage N yield decreased from 73.32 kg N ha^{-1} in 2000 and 64.30 kg N ha^{-1} in 2002 to 57.78 kg N ha^{-1} in 2001. The lower N values in 2001 were most probably the result of the high rainfall as discussed earlier.

Increased fertiliser N rates resulted in increased (P=0.05) herbage N yields. With the exception of autumn 2000, the highest herbage N yields were noted in early and late spring, which may be the result of higher N mineralisation due to increasing soil temperatures.

An interaction between fertiliser N rate and season of application recorded in 2001 showed a clear seasonal response where no nitrogen was applied in contrast to no seasonal response where 150 kg ha^{-1} was applied. The highest herbage N yields were recorded in autumn and spring (early and late) at 150 kg N ha^{-1}, while the lowest herbage N yields were recorded in autumn and winter (early and late) where no fertiliser N was applied.

The mean yields of herbage N, over a single regrowth cycle of 5 weeks, varied between 17.24 kg N ha^{-1} and 119.98 kg N ha^{-1} in the 0 kg N ha^{-1} and 150 kg N ha^{-1} fertiliser N treatments respectively (Table 9.12). Ball *et al*. (1978) recorded an annual turnover of 720 and 920 kg N ha^{-1} by the herbage of a grass-clover pasture with applications of 0 and 448 kg N ha^{-1} respectively, clearly showing the positive effect of the clover fraction on N supply. Whitehead (1970) recorded a nitrogen yield of 293 kg N ha^{-1} in a grass-clover pasture in southern England.

Table 9.12 Herbage nitrogen yields (kg N ha^{-1}) of a perennial ryegrass-white clover pasture as influenced by fertiliser N rate (kg N ha^{-1}) and season of application at Elsenburg

	Fertiliser N rate (kg ha^{-1})				
Season of application	**0**	**50**	**100**	**150**	**Mean (S)**
Year 2000					
Autumn	93.43	92.23	105.86	119.98	**102.88 a****
Early winter	43.49	47.28	58.64	65.76	**53.79 d**
Late winter	46.02	57.96	71.94	78.63	**63.64 c**
Early spring	50.08	70.71	86.48	93.41	**75.17 b**
Late spring	50.61	58.93	71.36	101.70	**70.65 bc**
Mean (N)	**56.73 d**	**65.42 c**	**78.85 b**	**91.90 a**	*73.32*
LSD (0.05) NxS = NS					
LSD (0.05) S means = 9.399					
LSD (0.05) N means = 6.9236					
CV = 14.85					
Year 2001					
Autumn	19.99 j*	47.05 hi	64.63 ef	84.42 ab	**54.02 b**
Early winter	17.24 j	47.57 hi	64.83 ef	70.50 de	**50.03 b**
Late winter	21.45 j	45.63 i	64.91 ef	75.98 cd	**51.99 b**
Early spring	44.05 i	59.80 fg	79.28 bc	84.81 ab	**66.98 a**
Late spring	42.59 i	53.74 gh	70.57 de	88.47 a	**63.84 a**
Mean (N)	**29.06 d**	**50.76 c**	**68.84 b**	**80.84 a**	*57.78*
LSD (0.05) NxS = 7.4319					
LSD (0.05) S means = 7.8632					
LSD (0.05) N means = 3.3237					
CV = 9.10					
Year 2002					
Autumn	55.48	62.71	72.61	82.08	**68.22 b**
Early winter	21.75	34.54	44.56	53.25	**38.53 d**
Late winter	26.64	42.37	58.77	68.19	**48.99 c**
Early spring	64.86	72.47	87.85	99.06	**81.06 a**
Late spring	67.96	73.97	89.99	106.82	**84.68 a**
Mean (N)	**46.42 d**	**56.41 c**	**69.86 b**	**80.98 a**	*64.30*
LSD (0.05) NxS = NS					
LSD (0.05) S means = 7.473					
LSD (0.05) N means = 4.9429					
CV = 11.91					

****Means in the same year followed by the same letter are not signifficantly different (P=0.05)***
*****Means(bold) in the same year for the pasture or specific fraction, column or row, followed by the same letter are not significantly different (P=0.05)***

Perennial ryegrass

Mean ryegrass N yields were found to be 42.02 kg ha^{-1} in 2000, 43.23 kg ha^{-1} in 2001 and 35.70 kg ha^{-1} in 2002 (Table 9.13).

Table 9.13 Nitrogen yields (kg N ha^{-1}) of perennial ryegrass in a perennial ryegrass-white clover pasture as influenced by fertiliser N rate (kg N ha^{-1}) and season of application at Elsenburg

	Year 2000				
	Fertiliser N rate (kg ha^{-1})				
Season of application	**0**	**50**	**100**	**150**	**Mean (S)**
Autumn	28.05	38.56	51.67	69.06	**46.84 a****
Early winter	16.59	23.03	32.59	38.33	**27.64 b**
Late winter	18.44	33.58	45.36	55.74	**38.28 ab**
Early spring	20.41	43.51	63.10	75.76	**50.70 a**
Late spring	19.33	38.00	51.90	77.31	**46.64 a**
Mean (N)	**20.57 d**	**35.34 c**	**48.93 b**	**63.24 a**	***42.02***
LSD (0.05) NxS = NS					
LSD (0.05) S means = 14.854					
LSD (0.05) N means = 6.4148					
CV = 23.97					
	Year 2001				
Autumn	12.77 hi*	42.16 f	61.24 c	81.24 a	**49.35 a**
Early winter	8.34 i	35.45 fg	54.41 de	61.18 cd	**39.85 c**
Late winter	8.59 i	30.52 g	52.57 e	70.60 b	**40.57 c**
Early spring	17.73 h	40.81 f	60.73 cd	70.49 b	**47.44 ab**
Late spring	18.13 h	33.50 g	51.80 e	65.51 bc	**42.23 bc**
Mean (N)	**13.11 d**	**36.49 c**	**56.15 b**	**69.80 a**	***43.89***
LSD (0.05) NxS = 6.776					
LSD (0.05) S means = 5.7551					
LSD (0.05) N means = 3.0303					
CV = 10.84					
	Year 2002				
Autumn	19.99	28.66	43.16	50.19	**35.50 b**
Early winter	9.71	21.50	33.49	42.69	**26.85 c**
Late winter	7.72	19.92	33.16	44.18	**26.25 c**
Early spring	17.15	36.92	52.55	66.87	**43.37 a**
Late spring	20.30	41.41	57.23	67.13	**46.51 a**
Mean (N)	**14.86 d**	**29.30 c**	**43.46 b**	**53.55 a**	***35.70***
LSD (0.05) NxS = NS					
LSD (0.05) S means = 6.7516					
LSD (0.05) N means = 4.8923					
CV = 21.17					

Means in the same year followed by the same letter are not signifficantly different (P=0.05)
*****Means(bold) in the same year for the pasture or specific fraction, column or row, followed by the same letter are not significantly different (P=0.05)***

Increases in fertiliser N rate resulted in increased (P=0.05) N yields emphasising the positive response of perennial ryegrass to fertiliser N applications. Fertiliser N applications in autumn and spring (early and late) tends to result in higher ryegrass N yields compared to winter applications mainly as a result of increased DM production, most probably caused by improved N uptake at higher temperatures and increased soil N mineralisation during these seasons.

A significant interaction recorded in 2001 showed a more prominent response to N rates in autumn compared to other seasons of application. The lowest ryegrass N yields were produced in early and late winter where no fertiliser N was applied and the highest in autumn in combination with 150 kg N ha^{-1}.

White Clover

The highest mean clover N yield was produced in 2000 namely 31.21 kg N ha^{-1} compared to 13.49 kg N ha^{-1} in 2001 and 28.60 kg N ha^{-1} in 2002 (Table 9.14).

Mean clover N yields (kg N ha^{-1}) were higher where no fertiliser N was applied compared to treatments that received fertiliser N. Reaction to season of application was inconsistent although spring applications resulted in higher (P=0.05) clover N yields in 2001 and 2002.

A significant interaction between fertiliser N rate and season of application in 2002 showed that although clover N yield was suppressed by all N applications, suppressions were more severe in spring compared to autumn and early winter. The lowest and highest fertiliser N yields were recorded in early winter and spring respectively.

Apparent recovery of fertiliser nitrogen.

Apparent recovery of fertiliser N was calculated through expressing the total N yield on the fertilised plots (50, 100 and 150 kg N ha^{-1}) minus the N yield on the unfertilised plots (0 kg N ha^{-1}) as a percentage of the fertiliser N applied (Table 9.15).

Mean apparent N recoveries of 20.99% (2000), 50.26% (2001) and 32.43% (2002) were recorded.

Table 9.14 Nitrogen yields (kg N ha^{-1}) of white clover in a perennial ryegrass-white clover pasture as influenced by fertiliser N rate (kg N ha^{-1}) and season of application at Elsenburg

			Year 2000		
	Fertiliser N rate (kg ha^{-1})				
Season of application	**0**	**50**	**100**	**150**	**Mean (S)**
Autumn	65.39	53.67	54.19	50.92	**56.04 a****
Early winter	26.89	24.25	26.05	27.43	**26.16 b**
Late winter	27.57	24.39	26.58	22.89	**25.36 b**
Early spring	29.67	27.19	23.37	17.64	**24.47 b**
Late spring	31.27	20.94	19.45	24.39	**24.01 b**
Mean (N)	**36.16 a**	**30.09 b**	**29.93 b**	**28.66 b**	***31.21***
LSD (0.05) NxS = NS					
LSD (0.05) S means = 8.266					
LSD (0.05) N means = 5.477					
CV = 27.56					
			Year 2001		
Autumn	7.22	4.89	3.39	3.17	**4.67 c**
Early winter	8.90	12.12	13.89	12.42	**11.64 b**
Late winter	12.86	15.11	12.34	10.76	**13.05 b**
Early spring	26.32	18.98	18.55	14.32	**19.54 a**
Late spring	24.46	20.24	18.78	22.97	**21.61 a**
Mean (N)	**15.95**	**14.27**	**13.36**	**12.98**	***13.49***
LSD (0.05) NxS = NS					
LSD (0.05) S means = 2.2392					
LSD (0.05) N means = NS					
CV = 28.00					
			Year 2002		
Autumn	35.49 bc*	34.05 bc	29.45 cdef	31.89 bcde	**32.72 b**
Early winter	12.04 hi	13.04 hi	11.07 hi	10.56 i	**11.68 d**
Late winter	18.92 gh	22.45 fg	25.61 defg	24.01 efg	**22.75 c**
Early spring	47.71 a	35.55 bc	35.30 bc	32.19 bcd	**37.69 a**
Late spring	47.66 a	32.57 bcd	32.76 bcd	39.69 ab	**38.17 a**
Mean (N)	**31.56 a**	**27.11 b**	**26.39 b**	**27.43 b**	***28.60***
LSD (0.05) NxS = 8.1094					
LSD (0.05) S means = 3.9682					
LSD (0.05) N means = 3.6027					
CV = 19.57					

Means in the same year followed by the same letter are not signifficantly different (P=0.05)
*****Means(bold) in the same year for the pasture or specific fraction, column or row, followed by the same letter are not significantly different (P=0.05)***

Table 9.15 Apparent N recovery rates (%) of a perennial ryegrass-white clover pasture as influenced by fertiliser N rate (kg N ha^{-1}) and season of application at Elsenburg

	Year 2000			
	Fertiliser N rate (kg N ha^{-1})			
Season of application	**50**	**100**	**150**	**Mean (S)**
Autumn	-2.41*	12.42	17.70	****9.24 b**
Early winter	7.59	15.15	14.85	**12.53 b**
Late winter	23.89	25.93	21.74	**23.85 ab**
Early spring	41.25	36.39	28.88	**35.51 a**
Late spring	16.65	20.75	34.06	**23.82 ab**
Mean (N)	**17.39**	**22.13**	**23.45**	***20.99***
LSD (0.05) NxS = NS				
LSD (0.05) S means = 18.461				
LSD (0.05) N means = NS				
CV =74.76				
	Year 2001			
Autumn	30.83	42.90	53.75	**42.49 bc**
Early winter	62.81	81.12	81.13	**74.99 a**
Late winter	41.59	60.44	69.53	**57.19 ab**
Early spring	22.33	40.91	44.02	**35.75 c**
Late spring	22.20	55.37	45.12	**40.90 bc**
Mean (N)	**35.95 b**	**56.15 a**	**58.71 a**	***50.26***
LSD (0.05) NxS = NS				
LSD (0.05) S means = 21.303				
LSD (0.05) N means = 9.602				
CV = 29.58				
	Year 2002			
Autumn	15.00	26.71	31.84	**24.52 b**
Early winter	27.83	34.22	44.64	**35.57 ab**
Late winter	34.41	50.85	54.93	**46.73 a**
Early spring	12.94	28.46	39.53	**26.97 b**
Late spring	11.55	30.24	43.31	**28.37 ab**
Mean (N)	**20.35 c**	**34.10 b**	**42.85 a**	***32.43***
LSD (0.05) NxS = NS				
LSD (0.05) S means = 19.459				
LSD (0.05) N means = 7.56				
CV = 37.66				

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

Apparent N recovery rates increased (P=0.05) as fertiliser N rate was increased for all years covered by the study. The increasing clay content (10 to 22.8%) within the top

300 mm soil possibly contributed to the relative high apparent recovery of fertiliser N at the higher N rates as leaching of N was restricted by the higher clay content as soil depth increases.

No definite trend regarding the effect of season of application on apparent N recovery was noted.

Conclusions

Nitrogen application rate and season of application had a significant effect on the quality parameters measured in this study. However the range of values within which the concentration of forage quality parameters varied exceeded the requirements for optimum animal performance. There was a small but significant decrease in pasture DM content as fertiliser N rate increased. A decrease in DM content could result in decreased DM intake relative to ryegrass-clover pastures that receive no N, and therefore reduced production per animal. Decreased production per animal must however be measured against increased availability of forage per hectare and increased stocking rate. However the low herbage availability during the cool winter months is likely to be of more importance to livestock production at a given stocking rate than DM content of the forage on offer. Increasing pasture dry matter production in the cooler months must be weighted against the potential negative effects of decreased DM content of the forage.

Due to above minimum levels found for most parameters tested, it is envisaged that the treatment combinations as used in this study will not be at any major disadvantage to pasture mineral content or quality as well as the grazing animal if grazing management is practised according to established norms.

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Chapter 10

CONCLUSION

General

The study was initiated to develop fertiliser N strategies with the aim of minimizing the negative effect of the winter gap on fodder flow management. Glasshouse and field trials were done to develop a better understanding of the response of perennial ryegrass and white clover plants, either singularly or as companion species in a mixed pasture, to environmental factors likely to restrict plant performance during the cool season in the Western Cape Province. The knowledge obtained will be used to address the winter - or feed gap experienced in perennial ryegrass-white clover pastures, through strategic N fertilisation management. The study comprised of two components. Glasshouse studies were done to evaluate the effect of soil temperature, soil water potential and growing season on the response of perennial ryegrass and white clover to different N rates under controlled conditions. Simultaneously a field study was done to evaluate the effect of 0, 50, 100 and 150 kg N ha^{-1} applied as a single dressing in autumn, early winter, late winter, early spring or late spring on dry matter production, clover content and forage quality. Soil ammonium-N and nitrate-N content were measured in both studies.

Glasshouse studies have shown that:

- White clover dry matter production was not negatively influenced even at application rates of 150 kg N ha^{-1}. These results were obtained under a range of soil temperatures varying between 6 and 18 °C under 18 °C day - and 12 °C night ambient temperature conditions. The reaction of a perennial ryegrass-white clover pasture under field conditions may however differ as day and night temperatures differ with seasons. The competitive effect of the two species growing as companion crops under field conditions may also influence the performance of the ryegrass and clover components.

- Soil water potentials of –10 to – 35 kPa did not negatively influence the primary dry matter production of white clover but ryegrass PDM was significantly reduced by soil water potentials of – 25 and – 35 kPa. The – 25 and – 35 kPa soil water potentials caused lower rygrass TDM yields, a reaction not observed in white clover.

- Increased fertiliser N rates generally caused significant (P=0.05) increases in perennial ryegrass TDM production. The same reaction was found with white clover although not significant.

- The highest root dry mass was measured at the –35 kPa soil water potential but this benefit was not reflected in increased dry matter production in any of the two pasture crops.

- The relatively high –10 kPa water potential treatments were not detrimental to the productivity of both crops as proved by non-significant differences in TDM production.

- Results suggest that N movement to the root surface and subsequent N uptake by perennial ryegrass were restricted at – 25 and – 35 kPa especially at the lower (30 and 60 kg N ha^{-1}) fertiliser rates. White clover was hovever not affected showing its independency on soil N supply.

- The lower fertiliser N rates (30 and 60 kg N ha^{-1}) were not able to maintain prolonged high perennial ryegrass productivity as exposure to increased photoperiod during October/November did not result in significant ryegrass yield increases, a situation not observed for white clover.

- The application of fertiliser N may increase the number of perennial ryegrass tillers and place ryegrass at a competitive advantage relative to white clover especially if applied to pastures under unfavourable environmental conditions.

The application of fertiliser N to manipulate dry matter production under field conditions were tested during autumn, early winter, late winter, early spring and late spring. It is envisaged that fertiliser N strategies will relate to season of application and this discussion will therefore be based on individual seasons within the cool season of the year. This conclusion is further based on the assumption that the pasture continuously grew under optimum environmental and management conditions as were experienced during years 2000 and 2002. The application of fertiliser N on perennial ryegrass-white clover pastures exposed to adverse conditions normally results in the senescence of root nodules and fertiliser N may further suppress white clover growth as was found in 2001. The application of fertiliser N under these conditions are therefore very risky and generally not recommended as only ryegrass tiller development will be stimulated placing white clover under severe competitive disadvantages relative to the companion perennial ryegrass component.

This field study has shown that the application of fertiliser N, in four of the five seasons tested, reduced the negative effect of the cool season (winter gap) on pasture productivity. The results obtained by fertiliser N application during the different seasons were as follows:

- Autumn – Extend pasture productivity later into autumn and thereby effectively shortening the duration of the winter gap.
- Early winter – Increased dry matter production in the season characterized by the most severe fodder shortages.
- Late winter – Advancing the attainment of pasture readiness for grazing sooner in spring and thereby reducing the duration of the winter gap.
- Early spring – Same as late winter although less effective in terms of advancing grazing readiness.
- Late spring – Too late to be of any benefit regarding the management of the winter gap as pasture productivity was at the same - or higher levels of dry matter production compared to dry matter production at the onset of the winter gap.

Calculating the required N input (fertiliser N + recycled N) at a known initial clover percentage is strongly influenced by the productivity of the pasture, the season when applied and the predicted clover percentage at the end of the first regrowth cycle. Multiple regression lines were developed for each of the three years covered by the study. Decreasing and increasing clover contents were observed for years 2000 and 2002 respectively. Year 2001 represents a perennial ryegrass-white clover pasture exposed to adverse environmental conditions causing possible senescence of clover root nodules placing clover at a competitive disadvantage relative to ryegrass with fertiliser N application. Using regression lines as listed in tables 7.2 and 8.2 shows great potential in calculating the desired fertiliser N rate and the resultant expected PDM production and maintaining a clover content of between 30 and 50% as prescribed by Martin (1960). Varifying the regression lines on independent data could result in the development of models instrumental in predicting the effect of fertiliser N rate on dry matter production and expected clover content with a high degree of accuracy.

Regression lines do not include parameters like leaching potential and N response efficiencies and the final N rate should be set after some observations regarding season of application had been taken into consideration.

Autumn application of fertiliser N

In addition to the results obtained through regression lines, the following information will assist in deciding on the fertiliser N rate to be applied. Clover DM production was not influenced by fertiliser N rate, indicating that rygrass dry matter production was stimulated without any negative effects on clover productivity. Although the highest DM production was found with applications of 150 kg N ha^{-1}, the application of 50 kg N ha^{-1} will result in the highest N response efficiencies as applications of 50, 100 and 150 kg N ha^{-1} resulted in N response efficiencies of 9.7, 7.2 and 6.5 kg additional DM produced per kg N applied. Application of 150 kg N ha^{-1} also exceeds the N uptake capacity of the pasture and thereby increases the risk of N leaching.

Early winter application of fertiliser N

Clover DM production was not influenced by fertiliser N rate, the drastic decrease in clover % possibly the result of generally lower clover productivity as temperatures decreased. Although the highest DM production was found with applications of 100 and 150 kg N ha^{-1}, the application of 50 kg N ha^{-1} will result in the highest N response efficiencies as applications of 50, 100 and 150 kg N ha^{-1} resulted in N response efficiencies of 7.6, 6.6 and 5.0 kg additional DM produced per kg N applied. Applications of 150 kg N ha^{-1}, and to a lesser extent 100 kg N ha^{-1}, also exceeded the N uptake capacity of the pasture and thereby increase the risk of N leaching. Low uptake, as shown by low herbage N yield (Table 9.12), and high carry-over of soil N at the 100 and 150 kg N ha^{-1} rates render these rates as risky under environmental conditions prevailing in the Western Cape Province.

Late winter application of fertiliser N

Clover DM production was not influenced by the 50 and 100 kg N ha^{-1} application rates however 150 kg N ha^{-1} significally reduced clover DM content. Although the highest DM production was found with applications of 100 and 150 kg N ha^{-1}, the application of 50 kg N ha^{-1} will result in the highest N response efficiencies as applications of 50, 100 and 150 kg N ha^{-1} resulted in N response efficiecies of 10.3, 9.0 and 7.5 kg additional DM produced per kg N applied. Application of 150 kg N ha^{-1} also exceeds the N uptake capacity of the pasture, as reflected in significantly higher residual dry matter production during the second regrowth cycle. The higher N yield (Table 9.12) shows that the pasture's N uptake capacity increased relative to early winter making the 100 kg N ha^{-1} treatment less risky compared to early winter.

Early spring application of fertiliser N

These results show that the application of 50 kg N ha^{-1} can be recommended as the 29% clover content found in 2000 quickly recovered to 36% at the end of the second regrowth cycle. The application of fertiliser N might be used as management tool to manupulate clover percentage as the clover fraction may benefit from higher temperatures in late spring and summer as optimum temperatures for white clover growth is higher than for perennial ryegrass. Clover DM production was reduced by all fertiliser N rates illustrating the increased sensitivity of the pasture composition as temperatures increase in spring. Although the highest DM production was found with applications of 100 and 150 kg N ha^{-1}, the application of 50 kg N ha^{-1} will result in the highest N response efficiencies as applications of 50, 100 and 150 kg N ha^{-1} caused N response efficiencies of 14.8, 12.1 and 9.0 kg additional DM produced per kg N applied. No increases in RDM production relative to the 0 kg N ha^{-1} treatments showed that the uptake capacity of the pasture increased due to increased pasture productivity as a result of higher temperatures and increased photoperiod, causing a sharp decrease in N leaching potential. The high N yield (Table 9.12) shows that the pasture's N uptake capacity increased by between 27.8 (48%) and 43, 3 kg N ha^{-1} (97%) if 100 kg N ha^{-1} rates in early spring as compared to early winter figures. The same comparison at the 150 kg N ha^{-1} resulted in figures of 27.7 (42%) and 45.8 kg N ha^{-1} (86%). The higher N uptake during the first regrowth cycle therefore clearly reduced the risk of N leaching.

Late spring application of fertiliser N

The effect of the application of fertiliser N in late spring showed the same effects as early spring. The dry matter yield increase was however too late, as the benefit of increased dry matter production occured when early season dry matter yields in unfertilized perennial ryegrass-white clover pastures as found at the 0 kg N ha^{-1} treatments, were at levels comparable with those at the onset of the winter gap.

This study has shown that the effect of the winter feed gap can successfully be addressed through the application of fertiliser N during the cool season. The benefits of strategic fertiliser N applications to perennial ryegrass-white clover pastures during the cool season should however be evaluated in terms of the amount and quality of available stored forage, the price and quality of available off-farm forages and the cost per unit N (R kg^{-1}) of the N fertiliser to be applied. The fertiliser N rate to be considered will be dictated by the productivity of the pasture, clover content and season when dry matter will be needed.

Future research

- Quantifying the recovery of recycled N under grazing is necessary to determine the actual fertiliser N to be applied, as the N inputs in this study refers to fertiliser N only. Under permanent grazing the contribution of recycled N should be included as contributor to total N input and thereby reducing the amount of chemical fertiliser N needed.

- Economic evaluation of the data collected in the field study.